The Evolution of Diversity

Beckley & Bonillas

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From Two Pervasive Atoms to Millions of Different Molecules

From One Cell to Millions of Different Forms of Life

By

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The photosynthesising sea slug Elysia chlorotica.

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Introduction

‘Life is full of surprises’, they say. The same could be said about science. At least that’s what we, the authors, concluded during our research in preparation for this work. We approached the task with attitudes that were shaped by teachings of the Theory of Evolution that had been formulated in the early 20th century. The modern evolutionary synthesis stipulates that evolutionary changes occur gradually over a long period of time by a process of small, accumulative genetic mutations on which natural selection then acts. What we discovered, however, was that there is much more to evolution than just that, and individually we were compelled to adopt a more pluralistic point of view. What began as a modest inquiry into the evidence for two biological processes -*hybridisation* and *symbiosis*- developed into having to reconsider our existing perceptions of how evolution works. We had completely underestimated the amount of empirical data that illustrates and provides evidence for these processes and, as it often happens, the more we looked, the more evidence we found. Although much scientific research has been done to unravel the underlying principles of symbiosis and hybridisation, no one, we believe, has as yet sought to explain them to a wider public in the context of evolutionary change. Furthermore, during our research, it soon became apparent that the evolution of life is embedded in scientific principles that transcend biology and we, therefore, decided that for a better understanding one needs to draw insight from further afield. We suggest that in order to appreciate how the complexity of biological life could evolve, one needs to embrace not only biological sciences but also the sciences of physics and chemistry. We will begin by unveiling the building blocks of life and then take the reader, step by step, from simple origins into the realm of biological diversity.

In this work, we will examine two important processes of evolution -symbiosis and hybridisation. The latter term is employed in the widest sense of the word. Although these processes have contributed in no small measure to the diversity of biological life on our planet, they are so far largely overlooked or considered unimportant. To understand the process of evolution fully, however, one must appreciate that its fundamental principles are embedded within the wider evolution of the universe and are a reflection of these cosmic processes of change. The details are enormously complex and sometimes obscure, yet the underlying trends are easier to understand when we consider that at the beginning of time all matter began its existence in the form of incredibly small particles. Gradually, some of this matter began to increase in mass by combining into new forms. These new forms could then combine anew or re-combine to begin a cascade of varieties, governed, of course, by the laws of physics and chemistry. Eventually, new combinations produced all the materials that are contained in our universe. Billions of years later, they combined into biological life itself, including into us humans; hence the saying that, essentially, we are made of stardust. This is how the late American cosmologist Carl Sagan expressed it: “The cosmos is within us. We are made of star-stuff”.

One of the fundamental questions confronting biologists is how did life begin with a single cell (or perhaps two or more) and evolve to produce the wonderful diversity we notice around us today? To answer this question, or at least to shed some light on it, we must consider and include what it was that preceded biological life. What are the building blocks of life and how did they all come together? Traditionally, biologists have focused on the important study of molecular genetics to provide answers. In this work, however, not just the genetic but all the molecules that define life will be recognised and elucidated for their significance in the evolution of form. This does not mean that we dismiss the work of geneticists as irrelevant but rather that our examination is complimentary. The simple molecules that give rise to biological life are limited in number and invariant and can be seen almost as necessary conditions. At the other end of the scale, the complex combinations of molecules that constitute the larger organisms are much more variable but also vulnerable to the contingencies of the prevailing environments. Consider also that many organisms have preceded contemporary life but are now extinct. Indeed, what one sees today is only a small fraction of what has gone before. Biological organisms have come and gone, but the underlying constituent building blocks always remain the same. In other words, homology underwrites diversity.

We will take a whistle stop tour of the fundamental particles and atoms that constitute matter and, more importantly, form the basis of biological life. Then, we will introduce the biological molecules that are the basic components of life, most notably the ones that contain carbon and are fundamental to the basic animal body form; lipids, the molecules that principally constitute the cell membranes and body structure; carbohydrates that supply energy; DNA and the various forms of RNA that constitute nucleic acids; and amino acids that constitute proteins and enzymes and are important for structure and the immune system. Not all of these building blocks, however, are produced within the organism’s own cellular machinery. Take the essential amino acids, they have to be supplied through food sources. And last but not least there is water, which is vital for life and links up with the building blocks but not with carbohydrates. Together they constitute the fundamental building blocks of life.

Furthermore, we will introduce the importance of biology’s chirality or handedness. This is easier to understand when we consider mirror images and the fact that our left-hand glove does not fit our right hand and vice versa. Asymmetry is just as significant in biology as it is in physics and the origin of matter in the initial evolution of the Universe. Alongside the basic building blocks for life, there is also the occurrence of variety brought about by molecules being either symmetric or asymmetrically left- or right-handed. Two amino acids may be composed of exactly the same atoms, but at the same time, they may be of opposite handedness. This will determine their shape, and shape is crucial to function and interaction.

Another fundamental question that biologists are grappling with is how does one define ‘life’? As a consequence, there is much discussion about viruses and whether they qualify for being alive. Whatever the outcome of that discussion, it is undoubtable that viruses have played an important part in the evolution of organisms. There is much still to learn about their full impact and methods of reproduction, but viruses can evolve rapidly by several diverse methods of combination that yield new hybrid forms. In most instances, these newly formed viruses can themselves combine again to produce an astonishing diversity. The rapid evolution of viruses is not only important to medical science but also, in the long term, viruses interact with biological organisms and in some instances change the organism’s structure. Viruses are a significant vector of genetic exchange between organisms both large and small.

Moving up the scale to the world of microbes, there is another amazing array of diversity, only second to viruses in total biological mass and variety. Microbes have a close relationship not only with viruses but with all other organisms as well. Like the virus, the microbe or the ‘germ’ has received a bad press and are commonly considered pathological. Without their presence, however, no other life could evolve or even exist. Microbes occupy all areas of the Earth, including the most hostile of environments. However, of importance here is the liaisons they form with other organisms and the part this has played in evolution. Indeed, their rapid evolutionary processes of lateral gene transfer have produced such a diversity that classification is very problematic. Moreover, the close relationship that microbes have with other organisms challenges the evolutionary theories of the 20th century. Nevertheless, it will be argued here that evolutionary theory is now much stronger with the recognition of microbial symbiosis.

The discussion of Symbiosis occupies a large part of this work. However, it is not symbiotic behaviour that is examined but the physical symbiosis that leads to alterations in the form and behaviour of an organism or the organisms involved. Consideration is given to the lichen, which boasts over 25,000 species, some of which were originally classified as plants. Looking back, it seems odd that biologists should make such a fundamental error, but that was, unfortunately, the manifestation of prejudice against the concept of symbiosis. It was even said at the time that symbiosis was "too fantastic for present mention in polite biological society". Indeed, many researchers met with such disdain that they failed to get their works recognised. One person who gave up her study of lichens was Beatrix Potter, the famous author of children's books. Her peers refused to read her papers not only on account of the subject matter but also because of her gender. There is often the danger that the conservative mind denies new evidence and in an attempt to fit it to the prevailing theory alters facts rather than change the theory to fit the new facts. We will address this issue in the later stages of this work.

The intimate relationships between organisms belonging to different kingdoms bring to light fundamental problems in the methods of classification and the inherent problem of the traditional concept of 'individuality'. Every human contains, for instance, millions of microorganisms. We acquired our first microbes already before birth, and throughout our lifetime they go on to multiply and live alongside our body cells. Humans are not unique in this; indeed, all animals, plants and fungi are composites of microorganisms. The full implications of this discovery with respect to the immune system and welfare of all living organisms and the science of treating and preventing diseases are largely unknown. In this context, the implications for the traditional theory of 'modification by descent' is deserving of further scrutiny later in this book.

Hybridisation in its widest sense is the second major process of evolutionary change that we examine in this work. Until very recently, hybridisation, if indeed considered at all, was seen only as a minor factor in evolution. The perception of a horse mating with a donkey and producing an infertile mule (or hinny) is prevalent. This outcome seems to be firmly established as being typical of all hybridisation events, to the extent that no consideration is given to the many occurrences that produce fertile offspring. Attitudes toward hybridisation are similar to those toward symbiosis and are reflected in the biological and philosophical literature of the last century by dismissive popular statements against this process as a factor of evolution. 'Only plants hybridise'; 'animals only hybridise under human husbandry and never in a natural setting'; and 'hybridisation reduces biological and genetic diversity'. These and many others are largely myths and will be exposed here. We will describe many examples of viable and fertile hybridisation among 'higher order' organisms, including our own, Homo sapiens. Hybridisation occurs across all levels of life and, indeed, it occurs within the inanimate world of particles, atoms and molecules. Fortunately, there are now several researchers who have realised and demonstrated the evolutionary significance of hybridisation, although, regretfully, their work remains obscure to a wider audience. It is ironic that, while evolutionary theorists ignored hybridisation, horticulturists did not. In fact, many who imitated the lessons of hybridisation in the natural world often became wealthy producing viable plants for ornamentation and human consumption. It is likely that 95% of modern fruit and vegetables are actually hybrids.

Ploidy refers to the number of sets of chromosomes that comprises every organism's genome. In this work, we will also focus our attention on polyploidy and occurrences where whole genomes are multiplied, a condition that is common in plants and less so in animals. And we will pay particular attention to allopolyploidy, where genome multiplication derives from the contribution of two different species. Traditional evolutionary theory faces a further challenge from recent research into 'chimerism' and questions the notion that heredity of DNA is exclusively linear. Chimerism is the condition where a single organism is composed of cells from different *zygotes* (fertilized eggs). This can happen, for example, when non-identical twin zygotes fuse in the womb. In other cases, cells are passed from the embryo to the mother and vice versa. These processes, once considered rarities, may actually be extremely common, and it is now believed that all humans may be chimeric to a lesser or greater extent.

The implications derived from the recognition of hybridisation and symbiosis as major factors of evolutionary diversity will be considered in regard to the prevailing Theory of Evolution. The method of classification derived from the Biological Species Concept is now severely tested in the light of chimerism, hybridisation and symbiosis. Similarly, the famous representation of the tree of life by the German biologist Ernst Haeckel is actually a very poor representation as it grossly oversimplifies the complex nature of increasing diversity. Indeed, some modern biologists now prefer the terms, 'web of life' or 'mosaic of life' in reflection of recent scientific insights. The wealth of evidence of reticulate evolution uncovered during research for this work has caused the authors to question certain metaphors and terminologies in the language that is currently in use in biology. The problem stems from the fact that, in spite of the evidence of a fresh body of scientific discoveries that has accumulated throughout the 21st century, the language employed is still rooted in the nineteenth-century and explains biological phenomena in terms of their apparent purpose or function. We believe that reform is therefore called for to represent a more pluralist approach to evolutionary theory. The proposed reforms here are not meant to be definite answers but are suggestions for opening a door to a wider debate. The authors hope that the Theory of Evolution will eventually be stronger for it in a world of growing hostility and reaction to scientific ideas.

Chapter One

The Principles of Combination

The details of life are vast and complex, but the underlying principles are fewer and easier to understand when one begins with the most simple and then builds from there. But what is the most simple? The Ancient Greek philosopher Democritus (460 – 370 BC) was probably the first person to realise that all objects and substances one is surrounded with are made up of smaller parts. The smallest parts, those that he thought were indivisible, he called *a-tomos,* from which the word 'atom' is derived. The Greek philosopher and scientist Aristotle (384 - 322 BC) however, did not follow this insight and instead believed that the universe was composed of five basic elements: air, earth, fire and water on the planet and aether above the clouds in space. These basic elements, he thought, could be combined in various ways to produce all the substances in nature. In many ways, Aristotle was a brilliant thinker. He laid down the basic principles for logical reasoning, conducted empirical methods of observation and was highly influential and original in the field of virtue ethics. Moreover, his major work, which became known as Aristotle's *Metaphysics*, still has the support of many contemporary philosophers. His views on the basic elements, however, were completely misleading, and it took until the nineteenth century for this to be fully realised.

Although Democritus was indeed correct that all substances were composed of smaller atomic parts, he was incorrect in the details. In successive discoveries between 1897 and 1911, it was discovered that atoms themselves are not indivisible but are in fact composed of smaller parts such as protons, neutrons and electrons. All of the 118 elements, some of which are synthetically produced, are made of combinations of these constituents and one can think of them as building blocks. Starting with the simplest element hydrogen, it is composed of only one proton and one electron, which makes it unique, as all the other elements have also one or more neutrons in their core. Atoms are defined, in part, by the number of protons they possess. Helium, for example, has two protons and uranium has ninety-two; their atomic numbers are therefore 2 and 92 respectively. To discover the mass of an atom, one simply adds the number of protons to the number of neutrons.

Further surprises came in the 1960s with the discovery of the quark, a particle that composes both the proton and the neutron. There are twelve quarks, six of which are anti-quarks. The anti-quarks can inhabit the anti-neutron and anti-proton. 'Anti' means that every particle has a mirror image particle, akin to a left and a right hand. As far as is known, quarks are indivisible and constitute all the matter in the universe.

Quarks – Neutrons, Protons & Electrons – Atoms – Elements

From the late 18th century onward, scientists gradually discovered what elemental substances are composed of. In 1774, the English scientist and philosopher Joseph Priestly (1733 - 1804) isolated oxygen. Shortly after 1776, the great English experimental and theoretical chemist and physicist Henry Cavendish (1731 – 1810) isolated hydrogen, followed by another important discovery in 1789, when the Frenchman Antoine-Laurent de Lavoisier (1743 – 1794), considered the father of modern chemistry, discovered carbon (C). These discoveries were not accepted without controversy because many scientists of the time held alternative ideas and believed in the existence of a substance called ‘phlogiston’. This was, however, only a postulate and eventually, phlogiston was discarded from the list of elements that were to make up what is known today as the periodic table, one of the great achievements of science. In 1869, the Russian chemist Dmitri Mendeleev (1834 – 1902) organised the periodic table into groups of elements with similar properties and ascending atomic masses. At the time, only 63 elements were known, but with the help of the periodic table, Mendeleev was able to predict the existence of the remaining, naturally occurring elements.[1]

Molecules

Atoms are the building blocks that in nature combine in many instances to make more complex structures called molecules. A molecule consists of at least two different atoms joined up and held together by covalent bonds between the electrons of the atoms. These combinations of atoms can be very modest, like when one atom of carbon combines with two atoms of oxygen to form the gas carbon dioxide (CO_2). On the other hand, combinations of atoms can also be more complex and are then called macromolecules, like when many atoms combine to form geometric lattice structures. Generally, the more bonds there are between atoms, the more strength the molecule has. To break the bonds of multiple lattice structures, higher temperatures are required. For example, graphite, a carbon-based macromolecule, has a melting point of more than 3,600°C. Glass manufacturing also requires high temperatures to break the lattice structure of silica, a form of sand, which together with other minerals is the

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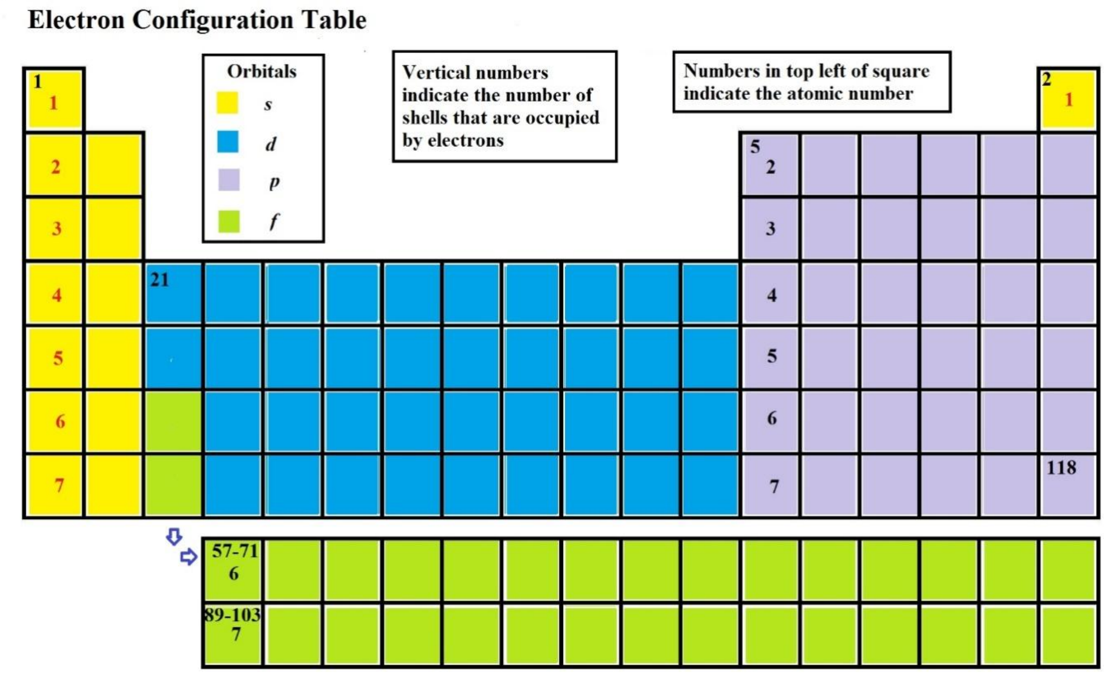
[1] It is also important to note that atoms can vary in composition. 1. If an atom has variation in the number of neutrons then they become isotopes and their mass changes accordingly. 2. If the number of electrons does not match the number of protons then the atom is an ion with either a positive or negative charge.

raw material. After heating, the molten glass has to cool down quickly to prevent the lattice structure from reforming in an opaque manner. Other macromolecules include alloys, which consist of at least one metal element combined with another element, which is often another metal. Brass, for example, is composed of 70% copper and 30% zinc.

Atoms of different size and substance can also combine to produce unexpected molecules, take sodium chloride, for instance. Sodium (Na) is a highly reactive metal that in its powder form can easily explode, and chlorine (Cl) is a dangerous and harmful gas that is used in chemical warfare. However, when combined in proportions of 40% sodium and 60% chlorine, it becomes the edible molecule table salt (NaCl), which is essential for life.

The most common way atoms combine is by before-mentioned covalent bonding, but this bonding comes in different forms and strengths. One of them is the *hybridization of orbitals*, which is important when complex molecules form their eventual topology. The term hybridization is employed as an analogy to what happens in the animal or plant world, where two members from separate species mate and produce an entirely new form of offspring. The term orbitals refers to the electrons that occupy a particular trajectory or shell around the atom's nucleus. There are four types of orbitals, sometimes called *sub-shells s, p, d* and *f* and are illustrated in the electron configuration table below, which is a variation of the periodic table. The *s* subshell can hold no more than 2 electrons, the *p* subshell can hold 6, the *d* subshell can hold 10 and the *f* subshell can hold up to 14 electrons.



Some of the electrons in each of the atom's different orbitals can overlap or 'merge' cloud-like in a strong bond and produce a new form of molecule. These hybrids take a particular geometric form. The gas methane (CH_4), for instance, is a chemical compound consisting of one carbon atom and four hydrogen atoms and forms a molecule in the three-dimensional shape of a tetrahedron (figure 1).

Figure 1. A Tetrahedron. Each of the four faces is of equal proportions.

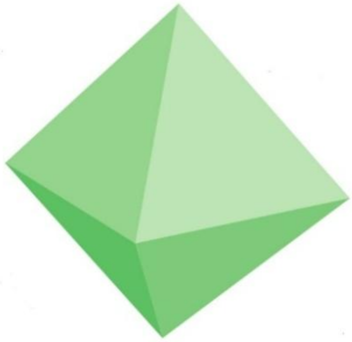


Figure 2. An Octahedron. Each of the 8 faces is of equal proportions. Both, the tetrahedron and the octahedron are Platonic solids.

Sulfur hexafluoride (SF_6) is an extremely potent <u>greenhouse gas</u> that is <u>inorganic</u>, colourless, odourless and non-<u>flammable</u>. It consists of one sulfur and six fluorine atoms that hybridise and form the shape of an octahedron (figure 2). The hybridisation of orbitals can result in five geometric shapes; they are linear, trigonal planar, tetrahedral, trigonal bipyramidal and octahedral.

Allotropes of Carbon

Not only hybridised orbitals determine unusual geometric forms. Carbon, for instance, is a very gregarious element that can form many liaisons with other elements. Even in its singular form without combining with other elements, it can manifest itself in unique ways. The most popular form of carbon is the diamond (figure 3), whose lattice structure is composed of one carbon atom covalently bonded to four other carbon atoms. Each carbon atom is in an inflexible tetrahedral complex, where it is equidistant from its adjoining carbon atoms. The physical unit of diamond consists of eight atoms that are fundamentally arranged in a cube-like form. There are no 'free' atoms, therefore diamonds do not become ionised and are very poor conductors of electricity. Their atomic structure lays the foundation to the hardest known, naturally occurring material with a high melting point and an insoluble surface. Lonsdaleite (figure 3) occurs as a microscopic crystal with a hexagonal lattice structure and, under pressure, it is actually stronger than a diamond. The occurrence of lonsdaleite is rare, however, and is formed when a meteorite strikes Earth and pressurises any graphite present.

The two fullerenes of similarity are the C_{70} and the C_{60}, both of which combine hexagonal with pentagonal structures to make a nearly spherical shape and with the latter looking like a leather football. The C_{70} and C_{60} names refer to the number of carbon atoms that each consists of and are just examples. There are many more fullerenes; the smallest is the C_{20}, and illustrated below is a fullerene composed of 540 carbon atoms. The smaller fullerenes have been discovered in nature, but the larger ones are the result of development within nanotechnology. All are hollow with each atom interconnected to at least three other atoms. This is distinct from the amorphous carbon, where an atom may only bond with a single atom and the allotrope lacks a crystalline structure. This form is most often found in soot or charcoal. These basic atomic structures determine what elements can and cannot do and are of immense importance in the application of material science. None more so than in research into graphite allotropes.

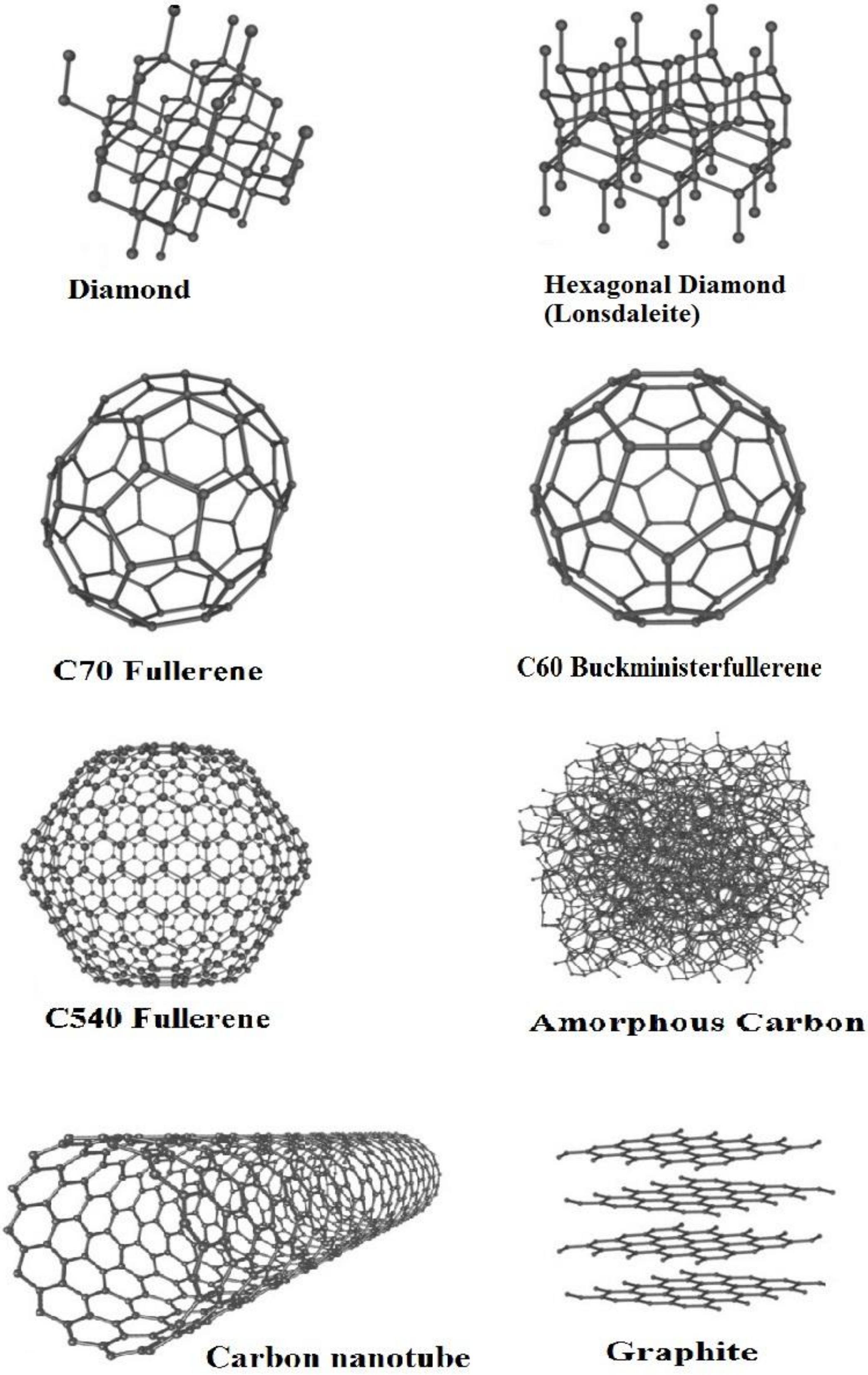


Figure 3. Some examples of carbon allotropes. (Original sketches by Michael Strock)

Graphene

The material of the 21st century is undoubtedly graphene, which is the thinnest form of graphite and only one atom in thickness. It is sometimes referred to as the first known two-dimensional substance. It can be tube-like as a carbon nanotube, or it can be like a single sheet (figure 3). Both are configured as hexagonal grids but without a 3D lattice structure. One might think that being only one atom thick there would be breakages and the application for materials would be therefore very limited. The opposite, however, is true. The electromagnetic interaction that holds together all matter is extremely strong and graphene is actually two hundred times stronger than steel. This is not all; besides graphene's strength, it is very lightweight and malleable, yet harder than a diamond. Furthermore, it is transparent and a good conductor of electricity and heat and it is impermeable to water, fluids, oxygen and even helium.

The potential applications for graphene are enormous and, unlike many materials, it has multi-functional possibilities.[2] Besides being already used in sports racquets, there is a long list of potential uses such as structural components in transport vehicles; as a protective paint; as a filter; as an electrical facilitator; in medicine; as a sensor; and in alternative sustainable energy production. Moreover, several other one-dimensional materials can be derived from graphene. They include boron nitride, graphene[3], Molybdenum disulfide (MoS_2), $NbSe_2$, Magnesium diboride (MgB_2) and fluorographene. According to the principles of combination identified earlier, all can be made to hybridise and form yet more materials.

There are, however, some drawbacks. One of them is the reliable and efficient production of these sheets of graphene, which is proving difficult outside the lab.[4] Another potential problem is the issue of toxicity of nanometre particles. Incidentally, a nanometre is one billionth of a metre, whereas a micron is one millionth of a meter. To give some idea of scale, a small bacterium can be one micron in length, and a million sheets of graphene are equivalent to the thickness of a human hair. Research is ongoing to establish the likely hazards to humans, in particular to those who are involved in the production and employment of graphene.

> Several future applications have been suggested for the nanomaterial graphene and its production is increasing dramatically. This study is a review of risk-related information on graphene with the purpose of outlining potential environmental and health risks and guide future risk-related research. Available information is presented regarding emissions, environmental fate, and toxicity of graphene. The results from this study indicate that graphene could exert a considerable toxicity and that considerable emission of graphene from electronic devices and composites

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[2] Robert Young. 2015. http://www.graphene.manchester.ac.uk/explore/what-can-graphene-do/

[3] Graphane should not be confused with grapheme, graphene is polymer of carbon and hydrogen.

[4] Mark Peplow, 2013. http://www.nature.com/news/graphene-the-quest-for-supercarbon-1.14193

> are possible in the future. It is also suggested that graphene is both persistent and hydrophobic. Although these results indicate that graphene may cause adverse environmental and health effects, the results foremost show that there are many risk-related knowledge gaps to be filled and that the emissions of graphene, the fate of graphene in the environment, and the toxicity of graphene should be further studied. (Ardvisson et al. 2012) [5]

Whatever the outcome of this medical and environmental research, it should not distract from the quest of this current work. Principally, the pursuit is to understand the building blocks of materials and life itself. The evolution of the Universe shows that things begin at their simplest and gradually combine and recombine, producing complexity. However, evolution does not necessarily reflect greater complexity. It should be said that not all particles and atoms have merged together. Some structures liaise with other structures and continue on to form new ones; others might reach a dead end, never interacting or becoming potential building blocks. Occasionally, objects can divest themselves of complexity and take on a simpler form, but this too can add to novel features and greater diversity. Some matter does not combine with other matter; one might think of the noble gases, independent of all else. On the other hand, there is carbon, the great facilitator.

Carbon, the Essential Element

Carbon is not the only essential element but it does pervade life such as is known and provides a building block that combines with other essential elements to make organisms. These combinations of small molecules make up macromolecules or, more technically, links of monomers form polymers. Carbon is not exclusively the basis of life, nevertheless, it is the backbone of organic chemistry. In fact, all organic molecules by definition include carbon. The four electrons that occupy the outer shell of the carbon atom enable such connective potential with many other atoms. One example concerns hydrocarbons, which form such materials as petroleum, octane and methane. Perhaps the simplest combination is where the four carbon electrons of the outer shell bond with a hydrogen atom to form methane, which is expressed as CH_4.

If one looks at life and the human body in particular, oxygen is the dominant element. It makes up approximately 65% of body mass, a high figure because of the water content of the body. Carbon accounts for 18% of human body mass but significantly,

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[5] Arvidsson. R, Molander.S&Sanden.B.A. Review of Potential Environmental and Health Risks of the Nanomaterial Graphene. Abstract. Human and Ecological Risk Assessment (Impact Factor: 1.1). 05/2013; 19(4):873-887. DOI: 10.1080/10807039.2012.702039

this includes the building blocks for carbohydrates, lipids, proteins and nucleic acids. These building blocks, together with water, are the molecules of life. The important point here is that all of these molecules of life are composed of smaller parts that combine to make three-dimensional structures. These structures determine the dynamics and activity of the molecules and how they interact with each other. In other words, to fully understand function, one has to know form.

Carbohydrates

As the name suggests, *carbohydrates* are composed of the combination of carbon, hydrogen and oxygen. Primarily, they provide organisms with energy for quick access or for storage. They are also referred to as sugars and one often hears talk of glucose and sucrose, but it includes lactose and starches as well. First and foremost, they are building blocks that are simple at the outset, but then additional blocks can be added. Take, for instance, the sugars: triose has a basis of three carbon atoms; pentose, which includes ribose, has a basis of five carbon atoms; and hexose, which includes glucose, has a basis of six carbon atoms.

Sometimes the basic building blocks contain exactly the same atoms but can differ in the order these atoms connect to each other. For instance, the molecular formula for both, glucose and fructose, can be written as ($C_6H_{12}O_6$), but the arrangement of the atoms is not the same; there is a difference in shape and hence a difference in the way each molecule interacts with other molecules. These simple sugars are called monosaccharides and cannot be broken down into smaller units of carbohydrates. They can, however, combine with each other to produce disaccharides and larger polymers of polysaccharides. Table sugar or sucrose ($C_{12}H_{22}O_{11}$) is a disaccharide; and starch, whose basic formula is $(C_6H_{10}O_5)_n$, is a polysaccharide. The polymers are more difficult to break down into smaller units and starch is, therefore, good for energy storage and found in plants. Another plant polymer is cellulose, which provides a toughness to the structure of the plant cell. This small change in atomic structure illustrates a fundamental difference between plants and animals.

Lipids

The same fundamental units of carbon, hydrogen and oxygen atoms combine also to form *lipids* that include fats, *steroids* and *phospholipids*, which are all important parts of an animal’s structure. Because of their atomic structure and shape, fats are twice as good for energy storage as carbohydrates, which is also of benefit for insulation. Unlike carbohydrates, fats are hydrophobic, they do not mix with water. One may have noticed in the washing up bowl how fats tend to accumulate in an impervious layer that is not dissolved by hot water. One may have also noticed differences in the types of fat; at room temperature, lard is thick and opaque, whereas table oil is in liquid form (figure 4). Yet, the difference in atomic structure is slight: lard is an example of a saturated fat with the maximum number of bonds between carbon, atoms and hydrogen. On the other hand, table oil is an example of an unsaturated fat, and here one finds that some of the carbon atoms have not bonded with hydrogen atoms. This difference changes the topology of the molecules. The molecules of lard stack well together without gaps, giving a solid appearance, whereas the molecules of table oil do not stack well and therefore are of less viscosity.

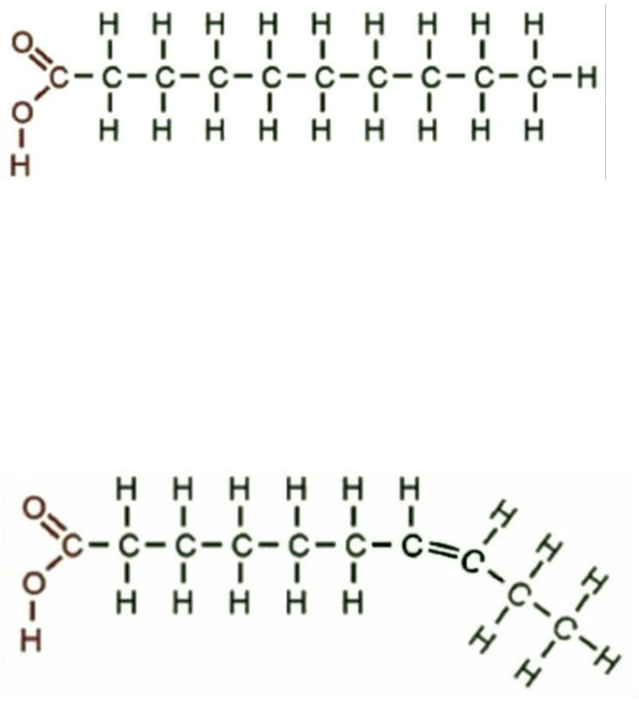

Figure 4. The top formulation shows how saturated fats are composed. The lower formulation shows that not all carbon atoms are bonded with hydrogen atoms. This absence produces unsaturated fat.

A lump of lard.

Vegetable oil.

Another form of lipid is cholesterol, which is the basis of all steroids and is important in the formation of the cell membrane. From cholesterol, as an example, the steroids of gender and growth, *estrogen* and *testosterone* are derived. Estrogen and testosterone are composed of exactly the same atoms, but the ordering is slightly different. Once again, small changes in the arrangement of the same atoms have enormous implications for the development of an organism.

Phospholipids are essential components of the cell membranes of nearly all life forms and some viruses. They can ‘stack’ upon each other to form a bilayer which is impermeable to most water-soluble molecules and act as a barrier to molecules that may interfere with the cells’ normal interactions. The structure consists commonly of two chains of fatty acids bonded by a glycerol molecule to a phosphate and choline molecule. One can see here that the building blocks of life begin to get more complex.

Nucleic Acids

With *nucleic acids*, the complexity of the basic building blocks increases and the participating molecules are carbon, hydrogen, oxygen, nitrogen, phosphorous and sulfur. Here, the two important polymers for the transmission of life are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (figure 5). The atomic difference between these two polymers is slight; DNA has one less oxygen atom, but this small difference completely changes its overall form and dynamics.

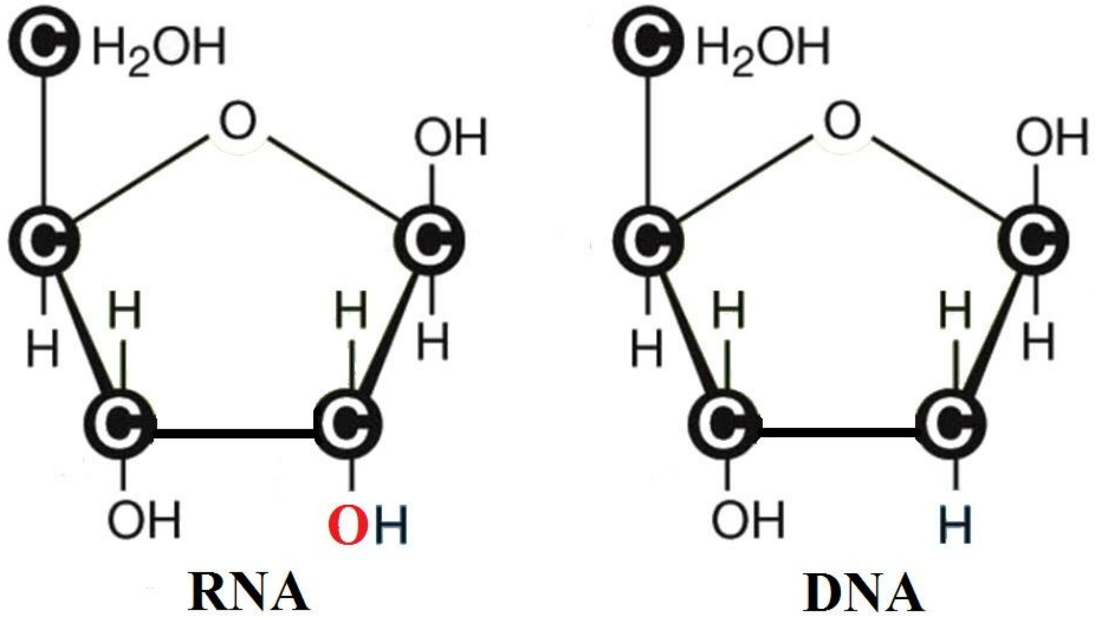

Figure 5. Both of these polymers are ribose sugars. The left side represents ribonucleic acid and the right side deoxyribonucleic acid. The difference is the absence of one oxygen atom in the DNA.

An extension can be seen at this stage where the building blocks discussed above combine with molecules of nitrogen, phosphorous and sulfur. Together, the form takes on a helical shape, which is double-stranded in the case of DNA (sometimes tripled-stranded) and a single strand in the case of RNA. The interactions between these two forms of nucleic acids and the influence of the cell produce amino acids, the next subject of this work.

From Amino Acids to Proteins

The basis of all proteins consists of polymers that are composed of amino acid monomers. They facilitate the transport of smaller molecules; contribute to the overall structure; make movement of the muscle fibres possible; act as storage proteins; are important components in the functioning of the immune system; and are called enzymes, when they control types and rates of chemical reactions. As an example, there are twenty types of amino acids in the human body, nine of which come from food intake, while the remaining eleven are produced by the cells' interactions; all are linked by means of the ribosomes. The amino acids produced by the body can combine or be stitched together by the ribosomes to produce more complex polypeptides. Some of those can be hydrophilic and interact with water, whereas others can be hydrophobic and will not interact with water.

But What are Amino Acids Made of?

It is interesting that all amino acids start with the same basic structure (figure 6) but then vary at point *R* in the diagram, where another particular variant molecule can bind. The topology of the molecule is always three dimensional, which is crucial to its function; however, it is important to note that its shape can be altered by changes in temperature, salt and pH content. When amino acids are joined together into proteins, the way the proteins are folded is again essential to their functions; haemoglobin, for instance, requires a quaternary structure consisting of four polypeptides.

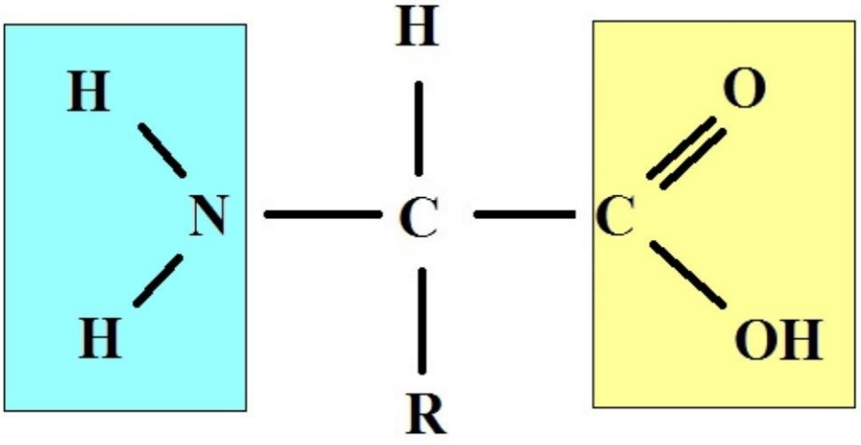

Figure 6. The basic atomic structure of all amino acids. The area shaded blue is known as the amino group and the yellow shaded area opposite is the carboxylic acid group. 'R' refers to the side chain.

Chirality

One of the surprises in biochemistry is the *chirality* or handedness of most of the molecules of life. One is familiar in everyday life that a pair of hands can be drawn to be exactly the same in size and dimension and yet, they differ, depicting a left hand and a right hand. This is important in the interactions of life. Just as the right hand will not fit into a left hand glove, similarly, a left-handed protein will not interact with a cell that only accepts proteins of the opposite hand. Although both handed molecules exist in nature, the molecules of amino acids are almost all left handed, but the sugars of carbohydrates are nearly all right handed. DNA is nearly always of a right hand spiral and RNA seems to be exclusively so. Being left- or right-handed has implications for the interactions of the human body, as Anthony Toole notes-

> Just as glove will only fit the hand for which it is designed, the molecules in the body can only respond to chemicals of the correct orientation. The mirror image form of a chemical will provoke a completely different response. The L-form of limonene, for example, is responsible for the smell of lemons, while the D-form smells of oranges. L-carvone smells of spearmint, D-carvone of caraway. L-nicotine, the natural tobacco product, is toxic; the D-compound much less so. L-adrenaline strongly constricts the blood vessels, while its mirror image has little effect. The mirror image form of the important anti-oxidant, vitamin C, has almost no effect in the body. (Toole 2016) [6]

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[6] Toole. A, 2016, Molecules of the Left and the Right,

Thus, shape is crucial to function.

Summary

Whether one calls it combination or hybridisation, the principle is basically the same; two or more entities that can exist independently come together to produce a new form. As noted before, quarks combine to form protons and neutrons; in turn, protons and neutrons, together with electrons, combine to form atoms. Atoms can combine with other atoms and sometimes do so through the hybridisation of electrons to form molecules or compounds. There is also a chronological order here. When the universe began, entities were of the smallest order, but over time, combinations occurred and entities gradually became more complex. Eventually, one sees carbon combining with hydrogen and oxygen and producing organic life. It should also be noted that there is symmetry that pervades the universe. All matter in the universe is comprised of particles that also have antiparticles, which like molecules can be left- or right-handed. Handedness changes the three-dimensional form and consequently the dynamics of the form when it interacts with other forms.

One may rightly ask why do entities combine in the first instance? What is the cause? Why do atoms have preferred states of existence? Why are the amino acids of life left-handed and not right-handed? Is it all down to chance or probability? The answers are elusive and perhaps one should admit that one simply does not know but can only guess. Some have inferred the hand of some supernatural force, but that suggestion raises many more questions than answers. Concerning the combination of entities, one might consider an analogy with people walking around a busy town. If the individuals walk around the streets and shop for a long time, it is probable that they will bump into someone they know, strike up a conversation or go and have a coffee together. In a similar manner, some particles, traveling in the vastness of space over millions of years, will collide or coalesce eventually. It may prove impossible to predict which particles it will be, but the probability of combination somewhere is perhaps inevitable. Satisfactory answers may still be lacking, but it is known that there is indeed some form of order and regularity that is fundamental to matter and the formation of life. The next chapter will look at the emergence of the simplest forms of life.

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http://myweb.tiscali.co.uk/anthonytoole/Optical.htm

Chapter Two

Viruses, Bacteria and Other Microorganisms

Viruses

Viruses are everywhere. Wherever one looks on earth, whether it is deep in the sea, atop a mountain, in the cold climates of the Artic and Antarctica or the sweltering desert, with the right instruments one can detect the presence of a virus. Altogether, there are estimated to be over 100 million types that make up a much greater mass than the totality of all bacteria. For instance, there are thought to be 10 to the power of 16 human immunodeficiency virus (HIV) genomes on the planet, and an infected whale can release 10 to the power of 13 caliciviruses daily.

Virus is Latin for poison, venom or slimy fluid, and from the earliest of times, viruses have received a bad press. They are perceived as something highly undesirable, dangerous or even fatal to life and a thing of evil in horror and science fiction media. This, however, is a one-sided view, as humans would not be alive today if it were not for the actions of viruses. Viruses can be beneficial in some circumstances, and it is now recognised that they play an important role in the evolution of life.

The term virus was coined in 1898 by the Dutch microbiologist and botanist Martinus Beijerinck (1851 - 1931), but viruses were not actually visualised until 1939 with the advent of the scanning electron microscope. The structure takes several different forms, which are often symmetrical. Some are geometric, sometimes with spacecraft-like legs; others are spherical or cylindrical, sometimes with a spiral of genetic material enclosed. *Retroviruses* have an enzyme called *reverse transcriptase*, which, after entering a cell, enables the transcription of their RNA into DNA. Generally, the genome is composed of either single-stranded or double-stranded RNA or DNA but not both and is enclosed in a protein coat called a *capsid*. Some viruses have a further outer lipid coat called an envelope.

Sizes were originally thought to vary from 20 to 300 nanometres in diameter, which is considerably smaller than the smallest of bacteria. (30nm is one-thousandth the size of a grain of salt). Nevertheless, in 1992, a virus was observed that measured 750nm. At first, it was thought to be another form of bacteria, but subsequent analysis discovered its true nature. It was named *Mimivirus*, short for ‘mimicking microbe’. Recently, other large viruses have been discovered, each containing a surprising amount of genetic material and in some instances out-sizing the smallest of microbes. To be correct, perhaps, one should speak of viruses and *virions*. Virions live outside a biological host cell and make up the greatest mass of what are commonly called viruses. The distinction is, however, a little confused, with biologists and commentators applying contrary definitions and labeling. From here on, the term virus will be employed to cover both concepts.

How does one classify viruses? Viruses on their own accord cannot reproduce or replicate; to do this they require a host cell within a biological organism. Once they enter the host cell they use the cell's reproductive machinery to make copies of themselves.[7] Are they, therefore, alive? This is actually an extremely difficult question to answer. One of the problems is the absence of a consensus of what defines *life*. If one bases the definition on the presence of DNA for instance, then many viruses would qualify as being alive. Yet, fossil remains can contain DNA, though they are not considered to be alive. Moreover, red blood cells may not contain DNA, but they, nevertheless, seem alive and can die. Some have tried to exclude viruses on the grounds that they cannot reproduce independently of another biological organism. However, this would also exclude sterile creatures that can perform all types of behaviour except reproduce. Others have suggested that *adenosine triphosphate* (ATP), an important chemical in metabolism, is a defining characteristic of life and therefore excludes the virus. Once again, however, there are exceptions. Under unusual conditions, some organisms can enter a *cryptobiotic state*, which is a sort of dormancy when metabolism stops and ATP is not produced. Be that as it may, these organisms are not dead and can resume actions when conditions become more favourable.

The difficulty is that if one narrows the definition of life too much, it excludes biological objects that one normally thinks of as alive; but if one generalises the definition, one includes objects that are not normally considered to be alive. Whatever one thinks about this matter, the important point is that viruses interact with biological organisms and that they themselves evolve and change their structures. How is this done?

Viral Evolution

It has become something of a cliché to say that viruses mutate and evolve quickly, but this view is only partially true, for viral transformations can take several forms, including methods of genetic exchange and hybridisation.

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[7] The early study of viruses centred on the relationship with bacteria. The viruses that interact or infect a bacterium are called bacteriophage or phage for short.

Recombination

This occurs when two independent viruses combine to produce a third independent virus (figure 7). For instance, if two strains of the influenza virus enter a host cell, their genetic material can mix freely; chromosomes can cross over, forming a new combination. This recombination is very common with viruses that infect prokaryotes, the single-celled organisms like bacteria.[8]

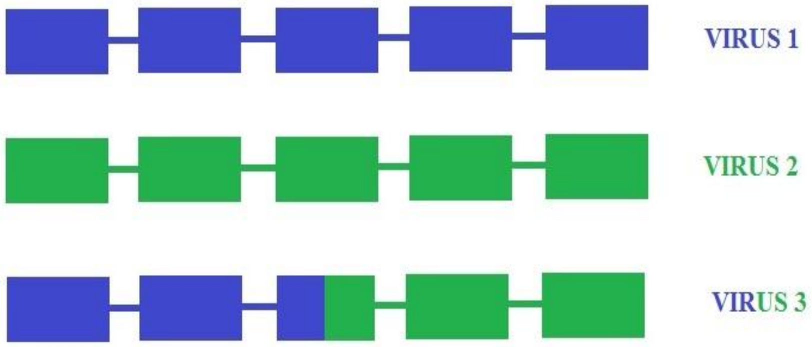

Figure 7. Recombination of 2 viruses to create a third virus

Potentially, the third virus can later recombine with either of the other viruses, creating yet another strain. One can appreciate how these transformations give speed to evolution and diversity.

Reassortment

As with recombination, at least two viruses must infect the host cell at the same time. Within the strains of the influenza virus, for instance, there is segmented RNA, which can mix and form a new assortment within the capsid of the progeny[9] (figure 8).

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[8] http://www.microbiologybook.org/mhunt/genet.htm

[9] Idem

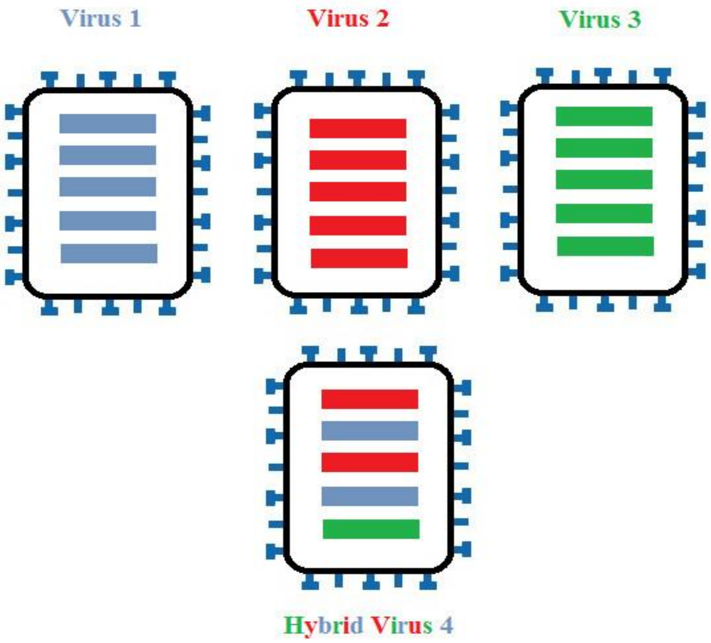

Figure 8. An example of reassortment. The illustrations are figurative only and do not represent actual viruses.

This assortment occurred when an avian virus infected humans in 1918 with what was inappropriately called the 'Spanish' influenza. Later in 1957, a new strain of avian virus emerged, and when it infected humans who were carrying the original 'Spanish' genetic material, a new assortment occurred. Although many may die of influenza, most will survive and carry its genetic material and pass it on to future generations. It was quite a surprise when the first readouts of genome sequencing were achieved and biologists discovered that all organisms carried viral DNA, mostly seemingly inactive but sometimes active.

Complementation

Complementation occurs when either one or both of two viruses that have suffered a mutation and cannot function on their own interact with each other and complement the other virus in its function.[10]

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[10] Idem

Phenotypic Mixing

This occurs when two viruses of a similar nature are able to maintain their original DNA or RNA but incorporate other material, such as the lipid envelope coat, creating a progeny with a new phenotype or outer coat[11] (figure 9).

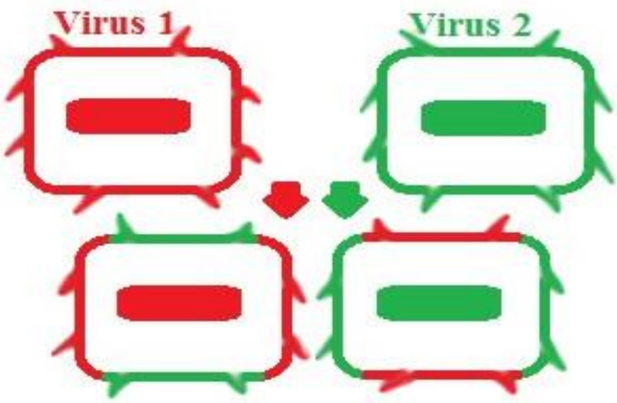

Figure 9. New hybrid viruses are formed; the genetic material remains, but the outer coat or envelope gains novel features.

There is another form of mixing between related viruses in which the progeny maintain their original genome but obtain an entirely new and complete outer envelope by incorporating lipids from the host cell membrane. This is referred to as a *pseudotype*. What is interesting here is that the normal relationship between a genotype and a phenotype, when the genotype inducing or determining the phenotype, is not always followed.

> A possible exception is the little-understood case where apparent heterozygotes of NDV [Newcastle Disease Virus] continue to yield phenotypically mixed progeny of each parental genotype for several generations. (Granoff 1959b, 1962) [12]

Granoff's research of the Newcastle Disease Virus (NDV) highlighted how quickly viruses can evolve. Passing on phenotypes without the corresponding genomes breaks the principles of inheritance as we understand them. This may be a novel spur to the speed of viral evolution, but it creates many problems for those in medical research, as this form of transformation makes a prediction even more difficult.

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[11] Idem

[12] Heterozygosis and Phenotypic Mixing with Newcastle Disease Virus, Allan Granoff 1962 http://symposium.cshlp.org/content/27/319.extract

Viruses and the Evolution of Life

If one were to scan the textbooks and papers of evolutionary biology written in the last century, it would be difficult to find entries about the importance of viruses in the evolution of all life forms. One would, no doubt, discover details concerning the relationships between viruses and bacteria, but one would not learn how bacteria affected higher taxa. It was not until advances in science provided the technology to detail the actual sequences making up the genomes. Once the genomes of several organisms had been mapped, comparisons could be made. There were several surprises: the number of human genes was far less than the 100,000 to 150,000 previously thought with approximations nearer 20,000. Moreover, these genes make up less than 2% of the entire human DNA and the question begs, what is the function of the remaining 98% of DNA? Once perceived to be redundant, non-functional or 'junk' DNA, it is now known that some of this non-coding DNA can influence whether a gene is expressed or not. In fact, this 'dark matter' acts like a switch, turning genes on or off. Another surprising discovery about the human genome was that up to 9% of the 3.25 billion nucleotide bases it contains is derived from viruses, another 34% is derived from virus retrotransposons and 50% from fragments and derivatives of viruses.[13] Fortunately for humans, much of the DNA derived from viruses remains switched off and causes no harm. Chicken pox is an example of a virus that invades a cell and leaves its DNA incorporated into the human DNA, which may in later life be switched on and manifest itself unpleasantly as shingles.

The same viral DNA can be found in all the genomes of every form of life.[14] One incorporation of immense evolutionary significance is thought to be the formation of the earliest placenta. The genes that give rise to this protective membrane are thought to be derived from a retrovirus.[15]

From the perspective of this work, it is important to recognise that viruses can evolve quickly by several different methods of combination that produce new hybrid forms. In most instances, these newly formed viruses can themselves combine again to produce an amazing diversity. In future chapters, it will be seen that these processes of combination and recombination are not exclusive to viruses but are indeed a major part of life's evolutionary processes.

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[13] New Scientist 2100 https://www.newscientist.com/article/mg20527451.200-i-virus-why-youre-only-half-human

Retrotransposons are transposable DNA elements (transposons) that employ retroviral-like reverse transcription during the process of transposition: retrotransposon DNA is first transcribed into an RNA template, then reverse transcribed into DNA, and then inserted into a new genomic site.

[14] Jane B. Reece, Lisa A. Urry, Michael L. Cain , Steven A. Wasserman, Peter V. Minorsky, Robert B. Jackson, Campbell Biology 10th Edition, 2014, Pearson

[15] ShaMi, Xinhua Lee, Xiang-ping Li, Geertruida M. Veldman, Heather Finnerty, Lisa Racie, Edward LaVallie, Xiang-Yang Tang, Philippe Edouard, Steve Howes, James C. Keith, Jr & John M. McCoySyncytin is a captive retroviral envelope protein involved in human placental morphogenesis *Nature*403, 785-789 (17 February 2000) | doi:10.1038/35001608; 1999

Bacteria and other Microorganisms

When in 1674 the Dutch tradesman and scientist Antonie Philips van Leeuwenhoek (1632 - 1723) looked through the microscope that he had crafted himself to investigate a drop of pond water, he had a first glimpse of the amazing microbial world we live in. Today, this unlikely Dutch scientist is regarded as the father of microbiology. The vast history of microbial life spans ecological time into the present. Billions of years ago, when our planet was an inhospitable place for life as we know it, its first and only colonisers were one-celled, simple microorganisms. If one excludes viruses, microbes represent the largest number of organisms on Planet Earth, varying considerably in size, structure, metabolism and habitat. They occupy all three domains in the biological classification of cellular life: 1. archaea and 2. bacteria (both are prokaryotes); and 3. eukarya.[16] The first two domains are made up entirely of microbes, while the third consists of both, microbes and large, multicellular organisms such as fungi, plants, animals and some algae. In 2000, researchers estimated that less than one-half of 1% of the 2 to 3 billion microbial species believed to exist had been identified.[17] They are too many to count and most of them are too small to be seen with the naked eye. They are everywhere; they are in the air we breathe and the soil we tread. A fistful of garden soil contains hundreds or perhaps thousands of different kinds of microbes, and in a single teaspoonful are more than 1,000,000,000 bacteria, 120,000 fungi and 25,000 algae.[18] They exist in the foods we eat and the water we drink; they cover the pages of any book, digital or printed on paper, and the skin of the fingers that are turning the pages; and they live inside plants and animals, including humans. What's more, microbiologists keep discovering them in places one used to think were incompatible with life, such as geothermal springs that harbor temperatures well above boiling point. They also thrive under great pressure in volcanic cracks thousands of metres under the ocean surface; in bitter cold Antarctic brine; inside solid rock and in oil deposits deep within the earth.[19]

One group of microorganisms deserves, perhaps, special mention, they are the *cyanobacteria*. Their precursors were, as are their descendants today, *autotrophs*, which make their own food by photosynthesis and produce oxygen as a byproduct. Cyanobacteria are believed to be responsible for the great oxidation event that shaped

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[16] Prokaryotes and eukaryotes are typically distinguished by their cellular content

[17] Fraser C. M., Eisen J. A. and Salzberg S. L., 2000, Microbial Genome Sequencing, Nature, 406, 799-803, p. 799, accessed 5/9/2015

[18] American Society for Microbiology 2014, Microbe World, available at: http://www.microbeworld.org/what-is-a-microbe/where-they-live accessed 5/9/2015

[19] Montana State online, Unit 1c: Where are Extremophiles found? available at: http://btc.montana.edu/courses/aspx/astro.aspx?ext=3 accessed 8/9/2015

the course of evolution on Earth and provides all complex life forms with the oxygen they need.[20]

Bacterial Evolution

Binary Fission

The most common form of bacterial multiplication is, without a doubt, *binary fission*. Here, a single bacterium can make an identical copy of its DNA within the cell membrane and then divide in two with each half taking one copy of the DNA. The result is two identical bacteria. This is a very rapid form of multiplication or replication without diversification other than when a rare mutation occurs somewhere during the copying process. Binary fission is considered to be a form of *vertical gene transfer* and a type of *asexual cloning*.

Transformation

Bacterial transformation was first discovered by the British bacteriologist Fredrick Griffith (1879 - 1941) in 1928. Nowadays, much more of the process is known. For instance, a bacterium can absorb naked genetic material from the environment. Double-stranded DNA can bind with a receptor cell membrane, but only one strand of the DNA enters the recipient cell and is incorporated into the existing chromosome. This new feature can then be passed on by binary fission.

Conjugation

Bacterial conjugation was first discovered in 1946 by the American molecular biologist Joshua Lederberg (1925 – 2008) and the American geneticist Edward L. Tatum (1909 – 1975) and is often referred to as a form of *horizontal gene transfer*. During bacterial conjugation, two similar bacteria exchange genetic material, and from this combination of genetic materials, a novel form of bacterium emerges. This is how it works: some bacteria contain a *plasmid*, which is a circular piece of DNA that is independent of the chromosome within the cell. The bacterium can physically join alongside another bacterium without a plasmid and make a connection by way of a

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[20] Astrobiology Magazine, 2003, The Rise of Oxygen, available at: http://www.astrobio.net/news-exclusive/the-rise-of-oxygen/ accessed 16/9/2015

conjugation pilus or *conjugation tube.* This tube allows a plasmid copy from the donor bacterium to be transferred to the receptive bacterium (figure 10). Often, though, the tube collapses before the plasmid can be transferred in its entirety, which results in two bacteria with slightly different genetic material. Moreover, in cases where this transfer is incomplete, it can happen that different proteins are conferred on the external wall of the host bacterium.

Effectively, a third bacterium has emerged from the exchanges of genetic material between two similar bacteria, adding to the overall diversity. Bacterial conjugation is not replication, but it is a form of *combinatory hybridism*. It is of particular interest in medical science for understanding how this method of horizontal gene transfer leads to the dissemination of antibiotic resistance.

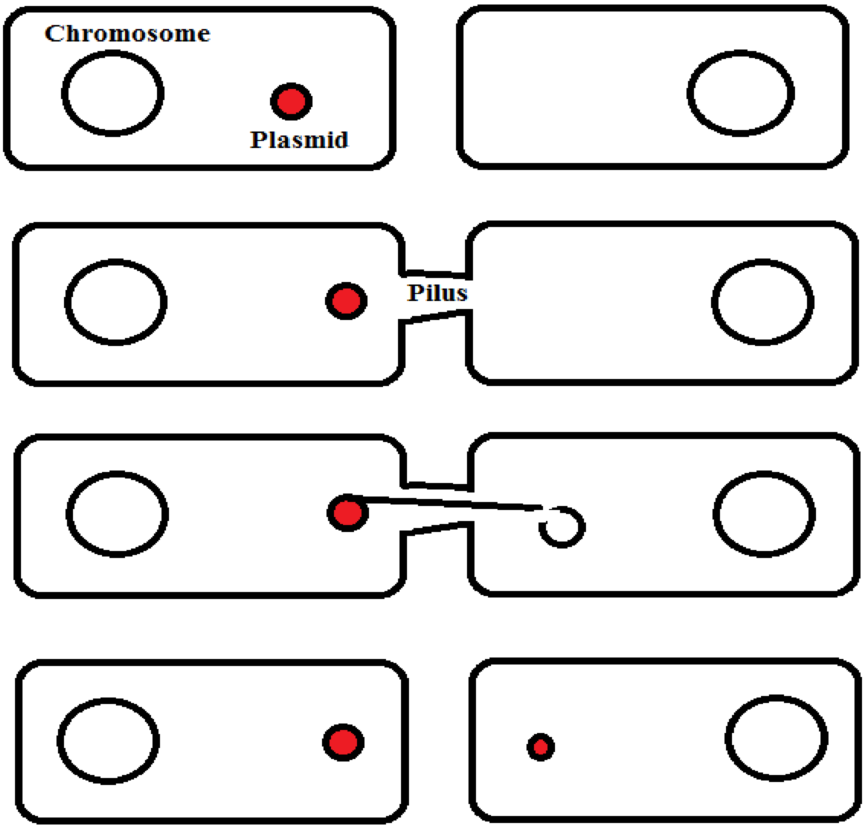

Figure 10. Bacterial Conjugation. A bacterium gains plasmid DNA through a pilus tunnel.

Transduction

In the last section, it was noted that viruses can infect a cell and use the cell's machinery to replicate. Normally, this interaction eventually destroys the infected cell, but sometimes the cell survives. This latter case is called the *lysogenic cycle*. When a bacterial virus or bacteriophage invades a host bacterium, it does not necessarily damage the cell, but its DNA becomes incorporated into the cell’s own DNA. The bacterium, still able to perform binary fission, passes on the new form of the genome through the generations.

Genetic transfer is not the only method of evolution. The symbiotic relationships between different types of microbes have provided a major evolutionary transition to the eukaryote cell, the basic building block for all algae, fungi, plants and animals, and will be discussed in the next chapter.

Summary

Viruses and bacteria are ubiquitous not only with a presence in the most unlikely and hostile places but they are also integrated within all forms of life. Furthermore, both types of organism can evolve quickly by methods of vertical and horizontal transfer of genetic and, in some instances, phenotypic material. Their true influence upon evolution is still not fully known but has certainly been underestimated in the past. In later chapters, it will be explained how important these liaisons and methods of combination are, and that in some cases they are confounding the traditional theories of evolutionary change.

Chapter Three

Symbiosis and Liaisons of Unlike Companions

The Greek word *symbiosis* means ‘living together’, and is commonly interchanged with the term *mutualism.* In biology, symbiosis can refer to mutually beneficial behaviour between organisms. For instance, when the bee collects the nectar from the flowers of a plant, it helps to propagate the plant by spreading its pollen to other plants. The primary concern of this work, however, is the symbiotic relationships among organisms that affect their form and add to the diversity of life – evolution in action.

The biological concept of symbiosis was introduced in 1878 by the German surgeon, botanist, microbiologist and mycologist Anton de Bary (1831 - 1888) in a speech at the Congress of German Naturalists and Medical Practitioners in Kassel, Germany. He defined the term symbiosis as the ‘living together of differently named organisms’ in a variety of cohabitations ranging from parasitic to mutually beneficial.[21]

His own, and the studies of the Swiss botanist Simon Schwendener (1829 - 1919) and the German botanist and mycologist Albert Bernhard Frank (1839 - 1900), had confirmed that lichens were ‘dual’ organisms that consisted of two components, an alga and a fungus. De Bary pointed out that both belonged to distinctly different biological kingdoms, yet, their intimate relationship had resulted in a new species. He took all the evidence into account and suggested that microorganisms that live inside a host could trigger a morphological change of considerable importance for evolution.[22]

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[21] Khakhina L. N., (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concepts of Symbiogenesis, New Haven and London, Yale University Press, p.7

[22] Sapp J., 2002, Paul Buchner (1886-1978) and hereditary symbiosis in insects, International Microbiology 5: 145-50, p.145

The biological phenomenon of symbiosis embraces a broad spectrum of more or less intimate living arrangements between individuals of two or more different species that are described as either *ectosymbiotic* (also *exosymbiotic*) or *endosymbiotic*. These associations can be commensal, meaning they neither harm nor benefit either partner, though they can also be beneficial to one and commensal to the other. In addition, they can be mutually beneficial, and they can be parasitic to the detriment of the host organism. In some cases, the defining lines are obscure, which makes it difficult to clearly determine the type of relationship. Ectosymbiotic partnerships are those, in which one partner inhabits part of the external body surface of the other. A good example is lice that feed on the skin, blood and oil secretion of their host in a parasitic symbiosis. Another example is some barnacle species that attach themselves to their preferred whale species to hitch a ride and feed on the same cloud of plankton like its giant host. In this case, the relationship is described as commensal (or perhaps slightly parasitic).[23] There is also the mutually beneficial symbiosis that exists between the roots of certain vascular plants and certain fungi, a partnership that is called *mycorrhiza*.Equally, there also mycorrhizal fungi that penetrate the roots of their host plants and coexist in endosymbiotic relationships, which will be discussed in more detail further on.

This work concerns itself with endosymbiotic associations, in which the smaller organism, the symbiont, penetrates a cell or other host tissue and both organisms cohabit in a protracted and intimate association. The ensuing relationship functions as a single unit and is generally obligate for both partners but can also be obligate for one and facultative for the other, though in some cases, it can be parasitic as well. When this merger results in the formation of new tissue, a new organ or even an entirely new organism, the process is termed *symbiogenesis*, which will be discussed later in more detail.

Nitrogen - Fixing Bacteria

Terrestrial plants have liaised and evolved with different fungi and also with different bacteria. The host plants comprise mainly the legume family Fabaceae , which include well-known plants such as the soybean (*Glycine max*), the cowpea (*Vigna unguiculata*), common bean (*Phaseolus vulgaris*), alfalfa (*Medicago sativa*) and clover (genus *Trifolium*); trees such as the giraffe’s favourite, the acacia (*Acacia nigrescens*) and also various vines and shrubs. Once again, one can witness mutualistic relationships between members of two different domains, the bacteria and the eukarya. On this occasion, the bacteria live within the roots of the plants. They gain protection and resources they cannot provide for themselves and likewise, the plant gains proteins

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[23] Professional blog about biology, 2015, Symbiosis: relationships between living beings

from the actions of the bacteria. In this association, both participants are affected in their growth and form.

Thousands of years ago, farmers noticed that if they planted the same kind of plant in the same field year after year, there would eventually be a sharp decline in their yields, but then they realised that they could improve the productivity of their fields by a system of plant rotation. All plants require proteins and to make protein, nitrogen is required in the soil. With continuous use, nitrogen (N) becomes depleted and has to be supplemented with nitrogen fertilisers. Although nitrogen is the most abundant gas in the atmosphere, making up about 80% of it, it is unavailable to animals, plants and fungi because of its inert nature. Without any knowledge of science, farmers were able to improve their yields by planting legumes every other or every third year in a particular field. As long as 8,000 years ago, farmers in the Middle East are known to have rotated their crops by planting one year cereal and the following year legumes. The British Agricultural Revolution of the 18th century was based on a four-field crop rotation of wheat, turnips, barley and clover. Today, modern farming in the developed countries is dominated by the use of chemical fertilisers, much to the dismay of those who argue for organic farming methods. Nevertheless, there has been a realisation that nitrogen-fixing plants can play an important role in yield performance combined with a reduced use of chemical fertilisers and pesticides.

Rice farmers in developing countries plant an aquatic fern (genus *Azolla*) in the paddy fields alongside the rice. The *Azolla* plant acts as a biofertiliser by fixing the nitrogen through its association with *Anabaena,* a bacterium that lives in its roots. This process increases the rice yield by 50%. One might wonder, does the *Azolla* plant not compete with the rice plant? Actually, the rice plant grows higher than the *Azolla* and eventually prevents sunlight from reaching it. When the *Azolla* can no longer photosynthesise, the plant dies and decomposes, providing nutrients, including nitrogen, for the rice.

> Less that 5% of the nitrogen sequestered by *Azolla* is available immediately to the growing rice plants. The remaining 95% remains in the *Azolla's* biomass until the plant dies. As the plant decomposes, its organic nitrogen is rapidly mineralized and released as ammonia, which then becomes available as a biofertilizer for the growing rice plants. Various techniques have therefore been developed to maximize *Azolla's* nitrogen fertilization, with the result that *Azolla* now has enormous potential to increase rice production worldwide and hence alleviate food shortages.[24]

Azolla has an unusual, if not unique method of heredity and association with its symbiont cyanobacterium *Anabaena.* In 100 million years, the two organisms have never been apart. In most associations, the two partners are separated at the

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[24] Rice Production. http://theazollafoundation.org/azollas-uses/in-rice-production/2015

reproductive stage and then rejoin but here, the bacteria *Anabaena* are permanently incorporated within the *Azolla* plant. They are located “either in the dorsal lobe leaf cavities or in the sexual structures (sporocarps)”.[25] The presence of the bacteria changes the structure of the plant and improves its ability to garner carbon dioxide. Researchers have referred to the *Azolla* plant as a superorganism because, within these cavities, other bacteria reside as well, but the nature of their interactions with the plant is still to be determined.

Nitrogen - Fixing in Legumes

If one examines the roots of a plant of the legume family Fabaceae, one might notice small nodules and assume there is something wrong with the plant. This, however, would be a mistake, as the nodules are the result of a symbiotic relationship between plant and bacteria. *Rhizobia,* meaning ‘root living’, are the soil bacteria that are most commonly associated with legume symbiosis, but there are other liaisons as well. The soybean, for instance, forms a relationship with the bacterium *Sinorhizobium fredii.*

Rhizobia bacteria can be poisoned by too much oxygen, but living in the roots of a plant protects them. How does this association come about? To begin with, the plant’s root hairs grow and exude sugars and amino acids into the soil, which attract the bacteria. They then attach to the root hairs, which cause the hairs to curl over, trapping the bacteria. Once they are encased, they eat into the cell wall of the hair and reproduce. Inside the cell wall, the bacteria can expand further into the root cortex and eventually, they become intracellular to the root, sealing off the tube to the external environment. This prevents other bacteria from infesting the plant. This process changes the form of the bacterium; it becomes rod-like in shape and gains a further membrane from interactions with the plant cells. After this process is completed, one can observe the external nodules that have formed on the plant root (figure 11).

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[25] Carrapiço, F. 2002. The *Azolla-Anabaena*-Bacteria System as a Natural Microcosm. Proceedings of SPIE, no. 4495, pp. 261-265.

Figure 11. Plant root with nodules

Once the bacteria are within the cortex cell, they convert the inert atmospheric nitrogen (N_2) to ammonia (NH_3), which is biologically as essential to the plant as it is to all organisms. Nodulated plant roots can increase their lengths substantially, thus giving the plant better access to water and minerals. This, in turn, facilitates superior growth above the soil surface compared with those plants without nodulation. Over time, the nodules will grow, sometimes to the size of a large pea, and their colour will also change with plant activity. When young, they are white or grey inside but with the presence of more $NH_{3,}$ they change to pink or a reddish colour. Not all nodules take the form pictured above (figure 11); some can be rod-shaped and some can even resemble a human hand with fingers. *Rhizobia* bacteria further benefit the plant by protecting it from other harmful bacteria and nematodes.

This mutualistic liaison confers resources from the plant to the bacteria within. From photosynthesis, the plant can derive sugars in the form of photosynthates, which, together with other nutritional factors, feed the bacteria and allow them to process nitrogen fixation.

There are some plants other than legumes, which also have a symbiotic relationship of nitrogen fixation in return for photosynthate. These include some tropical plants of the genus *Parasponia* and *Actinorhizal* plants. The latter enjoy a symbiotic relationship with the bacteria *Frankia.* All of these follow a path of reproduction that is different to that of the *Azolla* plant and its symbiont the bacterium *Anabaena.* The seeds of those plants do not contain mutualistic bacteria, the process of entry begins only after germination. Heredity works in many ways.

The Endosymbiotic Theory

From the late 1960s onward, the eminent American biologist and evolutionary theorist Lynn Margulis (1938 – 2011) became the promoter of the symbiotic theory, also known as the endosymbiotic theory of evolution. It posits that *eukaryote cells*, those cells that possess a *nucleus*[26] and other organelles, had evolved into a permanent endosymbiotic partnership through the merger of once free-living, non-nucleated *prokaryote* bacteria.

In Chapter two of her book *The Symbiotic Planet* (Margulis 1999), she tells the story about how, in the 1960s, she came to challenge, the theory of evolution that is based on the all-pervasive orthodoxy of Mendelian nuclear heredity. She had begun to question this doctrine, which, in her opinion, was critically narrowing the perspective of evolutionary scientists. The plot begins with a unicellular, nucleated organism of the genus *Paramecium.* This *protist* is uniformly covered with hair-like structures or *cilia* that propel it through its aquatic habitat in search of food. In a lab experiment, researchers had surgically removed a clump of cilia and replaced it in reversed position. When many generations of its descendants displayed this artificially generated feature, their lab experiment had established two facts: 1) an organism could acquire a new characteristic by other means than through the genetic material within its nucleus and 2) it could pass this new trait on to its descendants. This was an important discovery which, at the time, was simply dismissed as 'Lamarckianism'[27]. Undeterred, Margulis proceeded to collect more evidence for non-nuclear heredity, which she found in a variety of plant species such as *Eupatorium* (a genus in the aster family), *Zea mays* (maize), *Mirabilis jalapa* (four o'clock flower or marvel of Peru), *Oenothera* (a genus of herbaceous flowering plants) and in the genus of green algae such as *Chlamydomonas.*[28]

Margulis's interest in cell inheritance grew further when she came across scientific papers that mentioned the existence of mysterious genes, located outside the nucleus. It led her to review disparate sources of information, including literature discussing *cytoplasmic DNA*. This is DNA that is found not in the nucleus but in other membrane-bound structures that inhabit the cell plasma of eukaryote cells. After much reasoning, she came to an uncompromising conclusion: because these structures resembled present-day bacteria in behaviour as well as in metabolism, the precursors of these bodies had been bacteria. Furthermore, she predicted that one of them, the chloroplast, had retained some of the DNA of its bacterial predecessor. She even went

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[26] The nucleus envelopes the cell's nucleic genetic material. Procaryotes lack a nucleus.

[27] Lamarck proposed that traits could be acquired or lost by use or disuse and acquired traits were inheritable. Later however, the theory conflicted with Mendelian genetics.

[28] Margulis L., 1999, The Symbiotic Planet, London, Phoenix, p.p. 35-36

one step further by proposing that ciliates, a group of *protists* that were formerly classified as protozoans, should be considered to belong to this group. The bodies in question are *mitochondria* and *chloroplasts*, the organelles or organs, in which the biochemical reactions that are essential for the cell's survival are compartmentalised. In the larger organelles, the mitochondria that are found in all eukaryote cells, carbohydrates from food are converted into chemical energy by an oxygen-using process that is called oxidative metabolism. Chloroplasts, on the other hand, are not found in animal cells but are in nearly all plant cells and some algae. They capture sunlight and, together with chlorophyll pigments, convert it into chemical energy in a process that is called photosynthesis.[29]

Lynn Margulis not only embraced, promoted and defended the endosymbiotic theory against the peremptory paradigm of the neo-Darwinian theory of evolution, she also proposed how and in which order these symbiotic mergers had occurred. It was the biologist J.F.R. (Max) Taylor, who in 1974 gave the hypothesis the acronym *SET* for serial endosymbiotic theory. In the third chapter of her book, Margulis outlines how, back in time, when all life on Earth consisted of single-celled organisms, several kinds of independently living cells merged into one symbiotic, interdependent life form. With the green algal cell serving as an example, her rendition of a four-way union is as follows: First, there was the *thermoacidophile*, a sulfur- and heat-loving *archaebacterium*, which merged with another, swimming bacterium to become the *nucleocytoplasm*, the material body that all nucleated cells are made of. The products of this merger were the earliest swimming protists, anaerobic organisms that lived in sludge, muddy pools and rock fissures; oxygen would have poisoned them. Eventually, they encountered and engulfed oxygen-breathing bacteria, which gave them the ability to cope with the free oxygen that was accumulating in the atmosphere. These larger, more complex cells were capable of consuming food by ingesting smaller cells. Some of their 'prey', green photosynthetic bacteria, were first digested, eventually, though, they were 'incorporated', as Margulis calls it, and became the predecessors of chloroplasts. Individuals that had belonged to four groups of free-living, single-celled organisms like the acid heat lovers, the swimmers, the breathers and the photosynthesizers had joined into an interdependent endosymbiotic relationship and become large, complex eukaryotic cells. The cells in Margulis's account would have given rise to swimming green algae that eventually became the ancestors of today's plant cells.[30]

Margulis's scientific model succeeded over others. Subsequently, painstaking research using ribosomal RNA (rRNA) in cutting-edge rRNA-based techniques provided clear

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[29] Nature Education website Scitable by nature EDUCATION, Eukaryotic Cells, available at: http://www.nature.com/scitable/topicpage/eukaryotic-cells-14023963

[30] Margulis L., 1999, The Symbiotic Planet, London, Phoenix, pp. 44-48

evidence that mitochondria and chloroplasts had their origin in an endosymbiotic merger of bacterial cells.[31] A high percentage of genetic material of those bacterial precursors can still be found in their present-day descendants, the free-living oxygen-breathing bacteria and cyanobacteria respectively. Margulis is deservedly credited for having collected and researched an abundance of scientific material that was scattered across various disciplines and written sources; for having synthesised it into the hypothesis of endosymbiosis; and for having brought it to the scientific forefront in the late 1960s. Regrettably, there were many authors, whose writings on the subject received little or no recognition. The American geneticist Ruth Sager (1928 - 1997) comes to mind, who discovered the transmission of genetic traits through chloroplasts as the first known example of genetics that doesn't involve the cell nucleus. It would take a decade and a half and numerous dedicated and inventive researchers, above all Linda Bonen and her team at Dalhousie University in Nova Scotia, Canada, to advance the veracity of the endosymbiotic theory, which is now accepted by most scientists.[32] However, some important questions remain to be answered and the jury is still out on the exact sequence of these original mergers.[33]

Symbiosis and Lichens

Lichens, the ‘dual organisms’ de Bary discussed when he addressed German naturalists, are often confused with mosses. The word derives from Greek ‘to lick’, a reference, perhaps, to their ability for absorbing large amounts of water in a short time. Occurring in a variety of colors, they are found in almost every part of the world, especially where few other plants can survive. Lichens will grow on a wide variety of surfaces such as soil, rock, roof tiles (figure 12) and branches of trees, however, few are seen in polluted areas. Industrial and vehicle pollution affect their health and growth, which makes some types ideal for monitoring air quality in cities. Lichens appear in several shapes that largely correlate with the habitat they live in. Some are leafy, while others are free-standing (figure 13), crustlike, filamentous or tightly clustered. Regardless of their different growth forms, all lichens have a similar internal morphology. Millions of microscopic photosynthesising algal cells or, in some cases, cyanobacteria are embedded in a matrix of fungal filaments that gives the lichen its physical bulk and shape. One of the lichens that grows in the desert and is distributed by wind is *Lecanora esculenta*. It is reputed to be the biblical manna that fell from

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[31] Carl Woese (1928-2012) pioneered the use of ribosomal RNA as probe of phylogenetic relationships and developed the ‘Three Domain model’ classification system, that is now under serious challenge, see Williams T.A. and Embley T.M., 2014, Archaeal “Dark Matter” and the Origin of Eukaryotes, Genome Biology and Evolution, Volume 6, Issue 3, pp. 474-481

[32] Archibald J., 2014, One Plus One Equals One, Oxford University Press, Oxford, pp. 71-82

[33] Martin W. et al, 2012, Modern endosymbiotic theory: Getting lateral gene transfer into the equation, Journal of Endocytobiosis and Cell Research, Volume 23, p. 1

heaven and served as food for man and beast. Other lichens are an important food source for reindeer during the harsh winters in the tundra.

Figure 12. Unidentified lichen on a roof in the UK.

On first sight, they may appear to be single plants, but Schwendener discovered what others had missed when he investigated them through his light microscope. What he saw were two distinct components, an alga and a fungus, both joined in one single organism and in 1867, he published his hypothesis of the lichen's 'dual nature'. Initially, the finding was vigorously contested. The idea of one organism that was composed of several organisms belonging to different kingdoms did not conform to the widely accepted view that all creatures were autonomous beings[34]. It took more than seven decades until Swiss lichenologist Eugen A. Thomas (1912 - 1986) delivered evidence that proved Schwendener's theory to be correct when he succeeded to reconstitute the lichen *Cladonia pyxidata* from its two previously isolated symbionts, an alga and a fungus[35]. Publication of de Bary's lecture in 1879 aroused renewed interest in the phenomenon of symbiosis among many biologists and consequently, lichens became again the objects of intense studies.

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[34] Archibald J., 2014, p. 35-36. *One Plus One Equals One,* Oxford University Press, Oxford

[35] https://en.wikipedia.org/wiki/Lichenology

Figure 13. Cladonia cristatella, often known as the British soldier lichen, is a fruticose lichen belonging to the family Cladoniaceae. Some lichen were misclassified as plants. (Courtesy Barbara Page)

In Czarist Russia, lichens had already been well studied for their ecological and economical value in the Siberian tundra and northern taiga. Meanwhile, like in continental Europe, biological symbiosis had become the focus of scientific investigations, and lichens were the ideal organisms to experiment with. At the time, most biology teaching at Russian universities was conducted by scholars adhering to Darwinian ideas. However, some botanists were troubled with the concept of 'struggle for existence' and competition between individuals, which is the mechanism underpinning natural selection. While they considered the idea credible for the animal world, they did not accept its universal validity for the plant kingdom.[36]

Andrei Sergeyevich Famintsyn (1835 - 1918) was a Russian plant physiologist and teacher at the University of St. Petersburg. In 1890 he supervised the creation of the first laboratory for research in plant anatomy and physiology at the Academy of Sciences. With regard to Darwin's theory of evolution, Famintsyn's position was quite a critical one. In later years he would say that already early in his career he had begun to

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[36] Khakhina L.N. (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concept of Symbiogenesis, New Haven and London, Yale University Press, p.vii-viii

consider the role of symbiosis in evolution. Many years of experimental research on lichens and related studies would lead him to conclude that symbiosis could provide an additional factor to Darwin's mechanism of natural selection as a cause of evolutionary change from simple to complex forms.[37]

In order to prove the symbiotic nature of lichens, Famintsyn concentrated his efforts on investigating the green cell component of diverse lichens and experimental studies thereof. In the 1860s, green cells were considered to be reproductive organs and were therefore called 'gonidia'. One day, he and his student O. V. Baranetsky made a curious discovery when they dissected the *thallus* or body of a lichen under the microscope. While many of the 'gonidia' were entangled with and embedded in the thread-like *hyphae* of the fungus component, the majority were unattached and simply dropped out of the *medulla*, the inner region. This incident inspired their research to cultivate them outside the thallus, which they achieved by various methods. In their experiments, 'gonidia' reproduced by forming *zoospores* (asexual reproductive cells). The zoospores were typical of many algae, which, according to both researchers "completely resemble the form Cystococcus[38] described by Nägeli [Swiss botanist Karl Wilhelm von Nägeli 1817 – 1891]"[39]. In 1868, Baranetsky published *Independently living gonidia of lichens*, in which he confirmed that the 'gonidia' of the lichens they had experimented on were "'physiologically independent organisms"; capable of living "independently of the colourless tissue of the thallus and outside it as independent organisms'".[40] The conclusion of this work was manifold; green cells can exist for an extended period in culture outside the thallus; like algae and fungi they can develop zoospores; they are similar to single-celled algae, especially to the representatives of the genus *Cystococcus*. The experiments had served to prove that two heterogeneous organisms had joined and formed a morphologically unlike union.

This has been the accepted wisdom in lichenology until an article in the journal Science in July of 2016 revealed that there are lichens which are a union of three: an ascomycetous fungus, a photosynthetic alga and -that was the surprise- a basidiomycetous yeast. While a postdoc at the University of Montana, USA, the lichenologist Toby Spribillie and his team had made this discovery when working on two filamentous lichens, the dark brown, edible *Bryoria fremontii* and the toxic *Bryoria tortuosa.* During the course of the investigations, 52 genera of lichens from six continents were found to also contain this yeast in addition to the fungus and alga. Furthermore, it could be determined that the yeast was not a contamination but had

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[37] Idem, p.20

[38] *Cystococcus*, a former genus of algae that is now regarded as a synonym for the genus C*hlorococcum*.

[39] Carl Wilhelm von Naegeli (1817-1891), Swiss botanist, is also known for rejecting Gregor Mendel's paper on the laws of inheritance that the monk had sent him.

[40] Khakhina L.N. (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concept of Symbiogenesis, New Haven and London, Yale University Press, p. 22

evolved alongside its symbiotic partners for more than 200 million years.[41]

Symbionts in Animals and More Conclusive Results

After having experimentally proven the symbiotic partnership of the green cells, the algal component in lichens, Famintsyn now turned his attention to symbiosis in the animal world. As his experimental subjects he chose *radiolaria*[42], which is an abundant life form and part of marine plankton and began by first extracting and then culturing the microscopic *zooxanthellae* (yellow cells) that are the photosynthesising algae living in their tissue. His aim was to study their structure, their function and whether they had the ability to maintain an independent existence outside their hosts. Results of his experiments led Famintsyn to conclude "The major role of the yellow cells consists in the fact that, like other algae, they are able to grow, to multiply, and to build their bodies from inorganic matter. During this, they can serve as food for the radiolaria and support their lives during times of food shortage"[43]. Thus, Famintsyn settled the question about the existence of an endosymbiotic relationship of the zooxanthellae with their animal host.

It is interesting to know that besides residing in the radiolaria, the single-celled zooxanthellae live in the tissues of many more marine animals. Their hosts are among the *foraminifera*, which are single-celled protists with shells; sea anemones; jellyfishes; sponges; flatworms; and molluscs. One of the molluscs to be discussed further on is the sea slug *Elysia chlorotica, which lives* in a symbiotic relationship with the filamentous alga *Vaucheria litorea*. However, the endosymbiotic relationship of zooxanthellae with reef-building coral polyps, which are related to sea anemones, is arguably the association that is most important for humans (figure 14). Corals in tropical shallow waters are a 'symbiotic consortium', or *holobiont*[44] of two endosymbionts: animals (the polyps); protists (the algae); and the mineral calcium carbonate; and, in addition, the countless species that take part in ectosymbiotic associations with the reef. Just like their 'cousins' in lichens, zooxanthellae capture sunlight, which they convert into essential nutrients for themselves and their polyp hosts. In return, they receive compounds they need for photosynthesis and they get to live in a protected environment. They also provide much of the colour that corals display.

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[41] Gies E. 2017 The Meaning of Lichen, Scientific American June 2017, pp 50-51

[42] Radiolaria were formerly classified as protozoa or single-celled animals, today they are recognised to be a group of protists..

[43] Khakhina L.N. (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concept of Symbiogenesis, New Haven and London, Yale University Press, pp24-26

[44] term coined by Lynn Margulis to describe micro organisms living in and around macro organisms in endo- and ecto-symbiotic partnerships

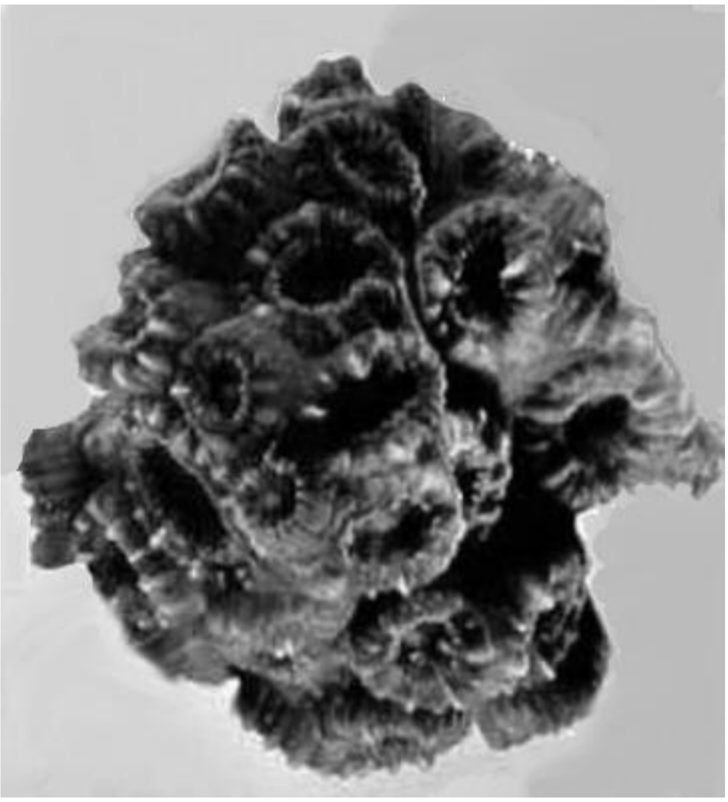

Figure 14. Coral with incorporated zooxanthellae. Without this liaison, the coral would have a bleached and smaller form.

Because of their biodiversity, coral reefs are sometimes called the ‘rainforests of the sea’. Although they cover an area that is less than 2% of the ocean bottom, it is estimated that one-quarter of all marine life depends on them for food and shelter. Their robust condition is not only vital for the entire marine ecosystem around them, they are also of enormous environmental and economic importance for humans. Tropical coral reefs protect shorelines from erosion. They are not only the habitat for the fish that feeds more than a billion people worldwide, they also harbour many species that are the sources for new medicines. In addition, they provide millions of jobs in the fishing and tourism industries. In spite of their enormous value to humans, it is man’s activities that are presenting the greatest threat to coral reefs. Destructive fishing practices, chemically polluted runoff from agricultural land, sewage, oil spills, deforestation, all are having a devastating impact, even when occurring far from the reef. Rising sea surface temperatures and increasing ocean acidification through rising carbon dioxide levels in the atmosphere are manifestations of global warming. Tropical corals prefer a water temperature between 22°C and 29°C. Even a 1% increase above the normal seasonal maximum can cause the photosynthetic symbiont, the zooxanthellae, to abandon their host. With the algae gone, nutrition needed for healthy growth is lost and the corals turn translucent to the extent that their interior white mineral skeletons become visible, a process that is referred to as ‘coral bleaching'. Eventually, if no new algae return, the corals will die either from starvation or other causes. Furthermore, an increase in sea water acidification would make it more difficult for them to build their calcareous skeletons. Eventually, the reefs would weaken and break apart and an entire symbiotic consortium would be lost.[45]

It was believed that in the future corals would only maintain their integrity if they were inhabited by a mixture of zooxanthellae that thrive in different thermal conditions. Recent studies, however, have shown that two populations of a single type of zooxanthellae displayed different thermal tolerances according to the environments they were living in. This is an important finding as most corals are inhabited by only one type of zooxanthellae.[46]

Zoochlorellae (green cells) were the focus of Famintsyn's subsequent studies. Zoochlorellae are unicellular protists that inhabit many aquatic ciliates and invertebrates. Famintsyn studied them in a sponge and in several ciliates, one of which was the previously discussed Paramecium, which in later years would be instrumental in proving that an inheritable trait could be generated by other means than genetics alone. This time, his objectives were to learn about their morphological traits, their physiological features and their ability to maintain an independent existence in their hosts. What he discovered was that zoochlorellae in ciliates are enclosed in a membrane; they possess a nucleus; and they contain a green chromatophore (a photosynthetic organelle), all clearly visible during cell division. Furthermore, he could establish that they were able to divide inside their host. Regarding their morphology, they were typical single-celled algae. In addition, Famintsyn succeeded in separating the zoochlorellae from their ciliate host tissue and cultivating them in isolation. Over the course of several weeks, they grew and divided just as they did inside the ciliate. This work demonstrated that, like zooxanthellae, the zoochlorellae were symbiotic single-celled algae that provide photosynthetic food for their host and in return gain protection and motility. The various experiments Famintsyn had conducted so far were stepping stones toward the study of the green inclusions of plant cells, the chloroplasts.[47]

Chloroplasts are the photosynthesising organelles in plants and the reader will remember the discussion of their symbiotic origin in the context of Lynn Margulis's endosymbiotic theory earlier in this chapter. When Famintsyn began to study them in 1912, their phylogenetic origin was still a matter of speculation. The subject of his

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[45] Smithsonian Ocean Portal, Corals and Coral Reefs, available at: http://ocean.si.edu/corals-and-corals-reefs#section_Coral_Bleaching

[46] ARC Centre of Excellence in Coral Reef Studies (2012), Multiple partners not the only way for corals to stay cool, available at: http://www.sciencedaily.com/releases/2012/01/120120184231.htm

[47] Khakhina L.N. (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concept of Symbiogenesis, New Haven and London, Yale University Press, p.26.

inquiry was whether the bodies in seedlings that contain photosynthetic pigments form from colourless chloroplasts already present in the seed or from the cytoplasm of the cells in the seed. It was an important question because the answer could potentially support the hypothesis of plastid[48] continuity and, by extension, would solve the more general question of their phylogenetic origin. Both views had their proponents among botanists. With his experimental work with seeds from sunflowers, Famintsyn was, in fact, able to confirm plastid continuity from seed to plant cell. He declared that "'first, the chromatophores [cells that contain or produce pigments] are found in the form of colourless leucoplasts in the mature seed, and that, second, the chromatophores of the seedlings form from them exclusively'"[49]. It was now clear beyond the shadow of a doubt that chloroplasts do not arise from the cytoplasm of the seed cell but rather develop from the colourless leucoplasts in the seed.[50]

A former student of de Bary, the German biologist Andreas Schimper[51] (1856 - 1901), had already expressed his thought that chloroplasts might have an endosymbiotic origin in a footnote of a paper published in 1883 -

> If it can be conclusively confirmed that plastids [chloroplasts] do not arise de novo in egg cells, the relationship between plastids and organisms within which they are contained would be somewhat reminiscent of a symbiosis. Green plants may, in fact, owe their origin to the unification of a colourless organism with one uniformly tinged with chlorophyll. (Archibald 2014)[52]

Famintsyn had proven that plastids do not arise "de novo" in egg cells. Now, his quest was to deliver experimental proof for the hypothesis that the plant cell had originated by symbiosis. "The next problem to be addressed is to find methods for culturing outside of the cell those of its constituent parts that appear to be the centres of its life activity",[53] he wrote and chose the chloroplast for his experiment. He decided that it was an essential part of his investigation to separate the plastid from the cell and cultivate it artificially, though he anticipated that it might be impossible to do so. It was to be expected that a protracted and intimate symbiotic relationship with the host cell had led to the loss of the chloroplast's autonomy to exist outside the host. Anyhow, on account of the fact that there was no suitable culture medium available at that time,

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[48] plastids are any of several cytoplasmic organelles such as chloroplasts.
[49] Khakhina L.N. (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concept of Symbiogenesis, New Haven and London, Yale University Press, p.27
[50] Idem, p.p. 26-27
[51] Schimper, besides being the first to have speculated on chloroplasts' symbiotic origin, also coined the terms 'chlorophyll' and 'pastid'
[52] Archibald J., 2014, One Plus One Equals One, Oxford, Oxford University Press, p.39
[53] Khakhina L.N. (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concept of Symbiogenesis, New Haven and London, Yale University Press, p.27

neither Famintsyn nor other researchers were able to culture chloroplasts in the laboratory. [54]

Through his experimental work, which he had begun in the mid-1860s and carried out over the period of half a century, Famintsyn had always aimed to demonstrate that complex organisms are composed of simpler ones of unlike phylogenetic origin. Although he had recognised the importance of Darwin's theory of evolution, he had, from the beginning, identified distinct shortcomings in his work. For example, there was, as he saw it, the problem of the disparity of evolutionary changes, which led him to formulate a theory that was based on two independent processes. The one concerned changes that provide the best adaptation of an organism to environmental changes without affecting the organism's structural organisation. Those he called 'changes of plasticity of organisms' or Darwinian changes. The other he called 'evolutionary changes', changes that were underlying the development of complex organisms from simpler forms, evidenced in an alteration of the complexity of organisation. In his view, Darwin's principal of natural selection provided a perfectly satisfactory explanation for the causes of adaptation, but concerning the causes of evolutionary complexity of form they "are not in the least elucidated by Darwin and remain, as before, an unresolved question"[55]. Meanwhile, based on the results of his many experiments with composite organisms, he proposed that they might arise "through the unification of elementary organisms into colonies, and the transformation of the aggregate of them into an entity of higher order...living, so to say, a sum of the lives of the many thousands of elementary organisms that constitute it"[56]. As undeniable proof for his theory of the evolution of complex organisms, he pointed to lichens and their composite structure, brought about by the merger and interaction of simpler entities. Famintsyn's aspiration to clarify and supplement Darwin's theory of evolution had inspired his line of inquiry throughout the many years of his professional life. Extensive observations and copious amounts of data that he had collected over time led him to the formation of, what he called, "'a new scientific theory," capable of explaining "the phenomena of life on Earth and the basic laws of its further development'"[57]. This was the basis for the early concept of symbiogenesis, which will be discussed later in the chapter.

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[54] Idem, p.27
[55] Idem, p.31
[56] Idem, p.33
[57] Idem, p.33

Figure 15. The rich green colour of the photosynthesising sea slug Elysia chlorotica helps to camouflage it on the ocean floor. Credit: Patrick Krug

> “Leaves that crawl” is a term that Robert Trench (1975) aptly used to characterize the species of sacoglossan [sap-sucking] sea slugs that sequester plastids from their algal food source and maintain them in an active photosynthetic state, effectively allowing the slugs to grow on CO2 and light. (Wägele H., Martin W. F. 2013)[58]

The biologist Robert K. Trench earned his reputation as the world’s leading expert on corals and their symbiotic algae. In 1994, he was awarded the Miescher-Ishida Prize for his contribution to the field of endocytobiology with his ground-breaking paper on the

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[58] Wägele H., Martin W. F., 2014, Endosymbioses in Sacoglossan Seaslugs: Plastid-Bearing Animals that Keep Photosynthetic Organelles Without Borrowing Genes, p.292 https://www.zfmk.de/dateien/atoms/files/waegele_martin_2013_endosymbiosis_chapter_14.pdf

rate of turnover of molecules through a metabolic pathway from *kleptochloroplasts*[59] to their host.

Sea slugs are marine gastropod molluscs and are found in shallow coastal waters around the world. There are thousands of species of this invertebrate animal that appear in a variety of colours, shapes and sizes from just a few millimetres to more than 30 centimetres in length. The herbivorous sea slug *Elysia chlorotica* (figure 15) can grow up to 60 mm in length but is mostly only 20 - 30 mm long. It is also known as sea cucumber, mainly because of its bright green colour and the fact that it looks like an aquatic vegetable when it sits motionless on corals or rocks while grazing on algae. Sea slugs are in fact snails that have experienced the reduction, internalisation or entire loss of their shells over evolutionary time and are therefore vulnerable to predators. However, the vegetable-like appearance of *E. chloritica* serves as a good camouflage.[60]

Since the 1970s, it is known that *E. chlorotica* is sustained by sunlight, just like a plant. This is made possible by *phagocytosis* or ingestion of particles such as bacteria and the sequestration and utilisation of algal plastids. In the process, this particular sea slug sucks up the cellular content of the intertidal filamentous alga *Vaucheria litorea* and retains only the chloroplasts, which become embedded throughout its digestive system; hence its green colour. Since *E. chlorotica* 'steals' the plastids from the alga on which it feeds, the organelles are therefore called *kleptoplasts* once they are part of the slug's organism. Both partners exist in an obligate endosymbiotic association. Inside the slug, the kleptoplasts continue to photosynthesise, providing thus nourishment for their symbiotic host when algal food sources are scarce. Symbiotic chloroplasts function for up to nine months, which is considerably longer than they would normally exist inside the alga. Considering that chloroplasts are without algal nucleocytoplasm and therefore without the algal genome once they are residing in the mollusc host, one wonders how the necessary proteins for their metabolism are produced. It is a question that has occupied researchers for several decades and has been the subject of intense study and discussion. One of two postulates hypothesises that the mollusc provides the essential plastid proteins through genes that were acquired from the algal food source by horizontal gene transfer.

In 2014, a research team led by Julie A. Schwartz from the Department of Integrative Biology at the University of South Florida at Tampa, USA, were able to confirm this hypothesis. By using an advanced imaging technique, they showed that the chromosome of the sea slug *E. chlorotica* contains genes from the alga *V. litorea*, on

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[59] Kleptochloroplasts are chloroplasts from algae that are sequestered or 'stolen' by symbiotic host organisms. Klepto derives from Greek for thief. It was Robert K. Trench who first defined the kleptoplastic character of sap-sucking sea slugs in an article that appeared in Nature in 1969. The term kleptoplasty describing this symbiotic phenomenon was coined in 1990 by Clark K. B. Et al.

[60] http://a-z-animals.com/animals/sea-slug/

which it feeds. One of these genes is critical for maintaining the process of photosynthesis and the repair of damage to chloroplasts. Being part of the slug's genome, the genes are vertically transmitted through the germ line to the next generation. However, chloroplasts are not reproduced inside the sea slug, which means that each new generation of *E. chlorotica* has to harvest them anew from algae, while the genes that are necessary to maintain them are already present in its genome.

The presence of *V. litorea* genes in the *E. chlorotica* genome demonstrates the only known case, so far, of a naturally occurring, horizontally transferred, functional nuclear gene from one multicellular species to another and between different taxa. This finding is important and could be instructive to genetic engineering technology, which uses nuclear gene transfer in the development of gene therapies for the treatment of various genetic disorders in humans.[61]

Important in the context of this work is the fact that horizontal gene transfer is a mechanism for rapid evolution. The research study's co-author Sidney K. Pierce, emeritus professor at the University of South Florida and at the University of Maryland, College Park, both USA, declared: "When a successful transfer of genes between species occurs, evolution can basically happen from one generation to the next.[62]"

Symbiogenesis

Roughly at the time when Famintsyn was building his case for an evolutionary concept of *symbiogenesis*, another Russian scientist was carrying out his own investigations and was compiling a body of work that would lead him to a comparable conclusion. The zoologist and botanist Konstantin S. Mereshkowsky[63] (1855 - 1921) began his studies in natural sciences at the University of St. Petersburg in 1875. Two years later, he started to publish papers on his examinations of marine invertebrates and microscopic, mostly unicellular protists, then called 'infusoria'. At the time, he developed a special interest in *diatoms*, which are a group of algae. After he graduated, he travelled to Germany and then lived in France for a few years. He became a member of the *Société Zoologique de France* and published several papers on his observations of animal pigments. Back in Russia, he was accepted as a lecturer at his former university, but after only three years, he was discharged under dubious circumstances. He moved to the Crimea and worked for more than a decade as a

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[61] Kenney D., 2015, The blog of the Marine Biology Laboratory, Sea Slug has Taken Genes from the Algae it Eats, Allowing it to Photosynthesize Like a Plant, Study Reports, http://blog.mbl.edu/?p=3285,

[62] Idem

[42] Mereshkowsky is the spelling he himself used in 19th century British publications.

pomologist in the gardens of the Livadia and Massandra Palaces near Yalta. In 1898, he travelled to the United States, where he remained for four years doing research work at the Biological Station in Los Angeles and at Berkeley University. Mereshkowsky had found the classification of diatoms, based on their silicified cell walls, insufficient and began reclassifying them according to their internal structure and whether they were mobile or immobile. During this time, together with the distinguished Swedish chemist and biologist Per Theodor Cleve (1840 - 1905), he published several papers on some common diatoms' internal organisational structure. As a result of his observations, emphasis was given to the number and special features of their pigmented bodies, the *chromatophores*. He would later use these detailed studies to support his argument for their symbiotic origin.[64]

In 1902, Mereshkowsky returned to Russia, and while working at Kazan University, he began to link his own detailed studies of diatoms with the body of other extensive research that dealt with the physiology of cells and their internal components. In a letter to Famintsyn, he expressed his interest in chloroplasts. However, his request to share his knowledge with him was met with contempt and ridicule in a paper on chloroplasts as symbionts, which Famintsyn published in 1906. A year earlier, Mereshkowsky had published in both Russian and German *On the Nature and Origin of Chromatophores in the Plant Kingdom*. It was a masterpiece of logic. Notwithstanding the denunciation of his elder rival, his name became intimately linked with the idea that chloroplasts had a symbiotic origin, to the extent that he was regarded by many as the 'founder' of the theory of symbiosis. During the next fifteen years, he kept refining his arguments and adding evidence for his theory that chloroplasts had once been free-living 'cyanophyceae' (cyanobacteria). During this time, he also introduced the concept of symbiogenesis, "the origin of organisms by the combination or by the association of two or several beings, which enter into symbiosis"[65]. His final and all-inclusive work *The Plant Considered as a Symbiotic Complex*, in which he extended his theory to include his thoughts on the origin of life on Earth, was published in French a year before he committed suicide in a hotel room in Geneva, Switzerland. Mereshkowsky had been a man of many conflicts. He had held extreme right-wing socio-political views; he had been a member of an anti-Semitic organisation and an informant to the secret police; and he was a convicted paedophile, which had forced him to flee Russia in 1914. He felt that his life's work, the theory of symbiogenesis and, in particular, his theory on the symbiotic nature of chloroplasts, had been ignored or

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[64] Sapp J., Carrapiço F., Zolotonozov M., 2002, Symbiogenesis: The Hidden Face of Constantin Merezhkowsky, available at: azolla.fc.ul.pt/documents/Mrezhkowsky.pdf, pp. 416-417

[65] Idem, p. 425

rejected by his scientific peers. In spite of his productive and fruitful scholarly endeavours he died a penniless and bitter man.[66]

What were Mereshkowsky's major points in defence of his theory of symbiosis and, in particular, the chloroplasts' origin from cyanobacteria?

- Chloroplasts reproduce by division in complete independence from the cell nucleus. He argued that he had shown this to be true for diatoms "and that Schimper and his successors had demonstrated that plastids of plants exist in the egg in the form of colourless leucoplasts and exist in the spores of a plant in the form of chloroplasts. When the eggs or spores of a plant divide to form tissues of the new plant, the chloroplasts also divide and distribute themselves in the new cells"[67]. It was proof for the uninterrupted continuity of chloroplasts, which, by extension, was proof for their symbiotic origin.
- Chloroplasts resemble 'cyanophytes' (today they are called cyanobacteria) morphologically and physiologically. Because the microscopes of the time did not show these minute organisms in complete detail, there was no consensus about their structure and evolutionary relationships. Mereshkowsky pointed to their shared physiological characteristics of being able to transform inorganic substances into carbohydrates and to synthesise proteins out of inorganic materials.
- Chloroplasts demonstrate functional analogy with algal symbiosis in animals. Mereshkowsky mentioned the well-established cases of algae such as zoochlorellae and zooxanthellae that live as symbionts in protists and most 'lower' invertebrates and function as oxygen suppliers and producers of food for their hosts.
- Last, he advanced a theory for the evolutionary origin of plants, for which he used the lichen as an example. Lichens are of polyphyletic origin. Each one of the lines in existence had its own, independent origin, because each resulted from the symbiotic merger of different algae with different fungi and cyanobacteria. Correspondingly, plants, he argued, were symbiotic organisms that had evolved many times in a similar manner.[68]

While in California, he had studied extremophile cyanophytes (cyanobacteria living in extreme environments) and knew that they could thrive in temperatures near boiling point. In one of his papers, Mereshkowsky speculated about the conditions from which life on Earth had arisen. As he saw it, Earth had passed through four stages, which he called epochs. During the first epoch, the earth was a vaporous ball of fire. In the second, a solid crust formed. Then, ultramicroscopic particles of protoplasm that he

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[61] Idem, pp 433-434

[67] Idem, p. 420

[68] Idem, p.p. 420-423

called ‘mycoplasm’ (extremely small bacteria that lack cell walls) and that were able to exist without oxygen and in temperatures close to boiling point, appeared in the third epoch. He named these organisms ‘biococci’. They were able to build proteins and carbohydrates from inorganic substances and were not only resistant to heat but also to strong mineral salts, acids and some poisons. And finally, in the fourth epoch, after terrestrial waters had cooled to below 50°C and food in form of bacteria was in abundance, ‘amoeboplasm’ (today they would be called prokaryotic organisms) emerged and fed on those bacteria. In some instances, ingested bacteria resisted digestion and instead entered into a symbiosis with the amoeboplasm. At first, they moved around freely, and then they became concentrated in one spot and enclosed by a membrane. According to Mereshkowsky, this is how the cell nucleus came into existence. This, he claimed, was an important step in evolution, because, on account of the symbiotic bacteria, the nucleated organism was now able to produce many different enzymes. Furthermore, he suggested that at the same time, other free-living bacteria evolved and gave rise to cyanobacteria and the diverse group of fungi. Plant cells, he declared, emerged by another process of symbiosis. Red, brown and green cyanophytes became symbionts as chromatophores or chlorophyll corpuscles in nucleated cells that were mostly *flagellates* (organisms that possess one or many whip-like structures for locomotion).[69]

Some of these visionary ideas will sound familiar to the reader, who will recall the endosymbiotic theory and its proponent, Lynn Margulis. When Mereshkowsky published his final work, Europe was seeing the end of WWI and Russia was becoming engulfed in the October Revolution. An extensive body of work, compiled by a dispersed group of scientists from many countries, had been masterfully synthesised by Mereshkowsky into a theory of symbiosis and a concept of symbiogenesis, but would then linger in obscurity for some fifty years. After the rediscovery of the Mendelian law of inheritance and the subsequent formation of the modern evolutionary synthesis, commonly known as neo-Darwinism, genetics began to dominate the science of evolution. Lynn Margulis is not only recognised for her own important achievements in science, but also for her major role in bringing back into the forefront of scientific investigation the concept of symbiogenesis as an important process of evolution. In Russia, Mereshkowsky’s work was criticised as being unscientific. However, Famintsyn’s and Mereshkowsky’s younger colleague, Boris M. Kozo-Polyansky (1890 - 1957), a botanist-theoretician, accepted the evolutionary importance of symbiogenesis and strove to combine the new concept with Darwin’s teachings.

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[69] Idem, pp 426-427

Insects and Microbial Symbionts

> There is nothing in a caterpillar that tells you it's going to be a butterfly.
> Buckminster Fuller

It is estimated that insects, the most abundant animal class in the phylum *Arthropoda* (meaning segmented feet in reference to their jointed legs) represent approximately 90% of all animal life forms on Earth with around 10 quintillions or 10^{30} (in Great Britain that is a ten followed by 30 zeros) alive at any time[70]. The name *insect* derives from Latin and was given to small animals with bodies that appear to be notched into segments that constitute the head, the thorax and the abdomen. These six-legged invertebrates have varied life cycles, but most develop through several stages from the egg to a larva and a pupa on to the adult form. This process is the metamorphosis that Buckminster Fuller referred to. The group includes butterflies, grasshoppers, ants, bees, flies, mosquitoes, cockroaches and beetles among others, but it does not include spiders and centipedes, nor worms. Their biological success is believed to be due to their small size and low energy requirements; an enormous variety in their way of life and sources of food; a protective exoskeleton; wings (that most of them have), which allow them to explore a large habitat in search of food and mates; and last, but not least their immense fecundity.

The following give some little-known facts about some of Great Britain's insects, which demonstrate their great versatility and considerable range of diversity:

Biggest is the emperor's dragonfly with a wingspan of 12 cm
Heaviest is the great silver water beetle at about 25-30 grams
Longest is the stag beetle at 2.5 cm
Tiniest at 0.25 mm is the aquatic fairy-fly, an internal parasite of water beetle eggs
Most travelled are the painted lady butterflies. They fly each year from North Africa to the UK. Occasionally, monarch butterflies and American painted ladies make it across the Atlantic to the UK. Billions of ladybirds cross the English Channel and the North Sea from France and Holland to the UK. In some years, the shoreline is made up of millions of dead ladybirds that didn't quite make it.
Most sophisticated communicators are the honeybees. Returning from a good patch of nectar flowers, they can communicate the distance and direction mainly by the way they waggle their abdomens.
Sexiest are the female water snipe flies. They clasp each other and cluster in big round aggregations on the end of a branch overhanging a river. Males entering the swarms

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[70] http://extension.illinois.edu/insects/06.html

are mated repeatedly until they die. The females then drop their eggs into the water and die, still in their tight aggregations.

Hottest and most explosive are the bombardier beetles. They can produce sprays of boiling phenolic liquid – several squirts per second- into the face of predators such as shrews.

Craftiest: some moths can evade the bats' echolocation by dropping out of the sky as soon as they hear the bat, while others 'shout' back at them.

Best camouflaged: there are many to choose from. Cramp-ball weevils and comma caterpillars are disguised as bird poo, some moths camouflage as lichen on bark, some caterpillars are disguised as twigs complete with last year's bud scars.

Most unappetising: all insects! Nearly all cultures of the world, except those originating from North Western Europe, are happy to eat delicacies such as deep-fried cockroaches, mole crickets and water beetles, midge pâté and grilled palm weevil grubs. Aversion to eating insects is an illogical taboo.[71]

In addition, here is one more little-known fact: symbiotic relationships are widespread between insects and the microbial world. It has been shown that several insect taxa depend entirely on their mutualistic bacteria for successful growth and reproduction and allows, therefore, the assumption that the association with bacterial symbionts is another reason for their enormous success. It is worth noting that around a billion years ago, microbes took up permanent residence in primordial cells to develop into mitochondria and chloroplasts inside of what became the eukaryotic cell. In the same way, fungi were instrumental in the evolution of the first plants that colonised dry land and are living on and in them in intimate relationships ever since. Therefore, it should not come as a surprise to learn that microbes took up residence also in organisms that belong to the animal kingdom. In fact, with the appearance of multicellular eukaryote organisms, microbes found ever more new habitats and myriad ways to interact with their symbiotic hosts.

Some of the earliest work on insect symbionts was done by two German scientists who, at the time, were studying insect development. In 1854, Franz Leydig (1821 – 1908), a German zoologist and comparative anatomist described fungal symbionts in scale insects, and in 1884, the German zoologist Friedrich Blochmann (1858 – 1931) observed the presence of bacteria in some ants, though he was hesitant to call them thus.[72] But it is the German zoologist and cell biologist Paul Buchner (1886 - 1978) who is today considered to be the founder of systematic symbiosis research. He became particularly known for his studies of hereditary symbiosis in insects. Buchner wrote his dissertation under the guidance of Professor Richard Goldschmidt (1878 - 1958), the German-born American geneticist, who is today remembered for his rejection of the neo-Darwinian theory of small and incremental gene changes to be the basis for speciation. In 1909, Buchner earned his doctorate in Munich and shortly thereafter, he heard lectures on microbial symbiosis in insects, given by the professor of genetics

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[71] http://www.nationalinsectweek.co.uk/insect_info/fascinating-facts.htm

[72] Gibson C.M., Hunter M.S., Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. ECOLOGY LETTERS, (2010) 13: 223-234

Umberto Pierantoni (1876 - 1959) at the University of Naples in Italy. Buchner was captivated by this line of study and began to direct his interest toward this novel subject. Some work had been done on algal endosymbionts in hydra and sea anemones, but to most scientists, it seemed unlikely that microbes, which were mainly associated with disease, could possibly be beneficial symbionts living inside 'higher' organisms. Independently from each other, Pierantoni and Karel Šulc (1872 - 1852), a Moravian embryologist at the University of Brno in what is today the Czech Republic, had published observations on what was then considered to be a primitive gland organ in the gut tube of some insects. They suggested that it was a symbiotic organ that contained intracellular microbes, which they called 'yeast fungi'. Šulc named this organ *mycetome* or fungus organ. During his studies, Pierantoni also discovered luminescent bacteria in the cells of light organs in certain beetles and in addition, he found that bacteria, living inside the cells of certain insects, were in some cases transmitted from generation to generation. He called this occurrence 'hereditary symbiosis', a subject that attracted Buchner's interest. Subsequently, Buchner began to direct his investigations toward intracellular symbionts in plant sap-sucking insects such as aphids, scale insects and cicadas. Over the course of a decade, he established that the so-called 'gland' cells contain endosymbiotic bacteria. He also demonstrated in a number of insect species how symbionts are inherited through the egg cytoplasm. He also discovered that a number of aphid species had two different symbionts and that cicadas had two to three symbionts. Moreover, he suggested that some symbionts produce enzymes that help their hosts to digest their food. In 1921, he published his first book *Tier und Pflanze in intrazellularer Symbiose* (*Animals and Plants in Intracellular Symbiosis*), which grew during the ensuing years and subsequent editions significantly in size.

After the end of WWII, no books on hereditary symbiosis existed in the English language. Overshadowed by the discovery of chromosomal genes in the 1930s, most scientific attention had focused exclusively on population genetics and chromosomal inheritance. This changed, however, when genetic studies of bacteria proved that transfer of genetic material was also possible by non-Mendelian inheritance. In the United States, several scientists, among them the American microbiologist and Nobel laureate Joshua Lederberg (1925 – 2008), who in 1952 had defended the origin of mitochondria and chloroplasts by symbiogenesis, knew Buchner's work and were instrumental in getting its fourth edition translated into English. It was published in 1965 under the title *Endosymbiosis of Animals with Plant Microorganisms.* One might wonder what was meant by plant organisms. It can be explained by the fact that at the time Buchner compiled his book, the science of biology was still divided into only two categories, botany and zoology, with microbes lacking their own natural history. Upon publication, Buchner's treatise, however, was met with some resistance. At the time, biological symbiosis was considered to be a rare phenomenon, and furthermore, most researchers were unwilling to accept that microorganisms such as bacteria and fungi, which were understood to be mostly pathogens, could be beneficial to more complex organisms. Besides, it was in many cases difficult to ascertain whether the relationship between microbe and host was mutualistic or parasitic. Nevertheless, Buchner was certain that the animal host was the dominant partner and in charge of the microbe's destiny. Buchner, like several other scientists at the time, recognised the potential of symbiosis as a source for evolutionary novelty and wrote in the English version of his book that he was anticipating the day "when professional bacteriologists will no longer be able to ignore the new findings brought to light by endosymbiosis research"(Sapp 2002)[73].

During the intervening time, it has been well documented that all aphids live in a mutually beneficial nutritional relationship with intracellular bacteria of the genus *Buchnera* (named in honour of Paul Buchner) and that they are inherited through the host egg. Buchner remained a lifelong sceptic of the belief that natural selection, functioning on random genetic variations, is the only mechanism for evolutionary change.[74]

Since Buchner's and the earlier pioneers' achievements, considerable progress has been made in the field of microbial endosymbiosis. There is now among entomologists growing recognition of the widespread existence of this biological phenomenon in arthropods and the impact it has on the biology of hosts and symbionts. Because of molecular tools such as 16S rDNA sequence comparison, bacterial symbionts are now to some extent easy to research and have therefore been the subject of most studies so far. Best understood are the bacteria that provide their hosts with an advantage for survival and/or reproduction by allowing for specialised feeding habits, thus ameliorating a diet that would otherwise be nutrient deficient. Buchner had discussed such particular symbiont/host relationship in his book. In addition, some bacteria are found to provide heat tolerance and protection against pathogens and parasites. On the other hand, fungal symbionts are hyperdiverse and less researched, to the extent that their presence is often ignored. There are some well-studied cases of ectosymbiotic fungi associated with leafcutter ants and termites, for instance but until recently, there was little investigation of fungal endosymbiosis.[75]

Like all animals, insects need amino acids, B vitamins, specific fatty acids and sterols in their diet. Many of these nutritional requirements are supplied by their microbial symbionts and it appears that fungi and bacteria specialise in what they offer their respective hosts. Free-living bacteria display diverse nutritional strategies; however, bacteria associated with insects receive organic materials mostly from their hosts. Fungi, on the other hand, have the ability to get nutrients from a wide range of carbon sources by secreting enzymes into their surroundings and extracting the nutrients they need. In the process, both types of microorganisms produce dietary compounds as well as certain by-products that are valuable or even essential for their hosts and pass them on. In general, research into microbial mutualism among diverse insects has found that fungi produce for their hosts sugar and glycerol, the latter is a component of a type of fat, while bacteria produce amino acids for their hosts. In blood-sucking

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[73] Sapp J., Paul Buchner (1886-1978) and hereditary symbiosis in insects, International Microbiology, (2002) 5: 149

[74] Idem, pp.145-150

[75] Gibson C.M., Hunter S.M., Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects, ECOLOGY LETTERS, (2010) 13: 223

insects such as the bed bug and the tsetse-fly, bacterial and fungal symbionts also produce vitamins, which are a vital component in the insects' diet but are rare or absent in the blood of vertebrates. In addition, for certain insects, sterols are synthesised by their yeast symbionts.[76]

The pattern of relationships between the two major microbial symbiont groups and their hosts and the modes of symbiont transmission show distinct differences as well. Again, the interrelationships between insects and bacteria are best understood. Of those, the partnership between the aphid and its primary obligate, maternally transmitted intracellular symbiont *Buchnera aphidicola*, an association that goes back 150 - 250 million years, is best documented. As a general rule with few exceptions, it can be said that bacterial relationships are mutual and obligate. In the case of *B. aphidicola* in aphids, the intracellular symbiont allows a deficient diet of phloem sap by supplying the essential amino acids that would otherwise be lacking. The host, in turn, has developed bacteriocyte cells as a safe habitat for its bacterial partners and transmits its endosymbionts vertically through the maternal line to the next generation. As a consequence of such intimate lifestyle inside a eukaryote host, the bacteria have shed over time nonessential genes that their free-living cousins have retained and have thus become totally dependent on their host. Moreover, host and symbionts have co-diversified over time. Molecular techniques have shown that some hosts and several obligate, matrilineally transmitted, intracellular symbionts have gone through *co-cladogenesis*. This is a process of evolutionary change in which both, host and symbiont, have branched off in parallel from their common ancestral types into new species.[77]

Fungi, being heterotrophic organisms with external digestion, don't lend to an intracellular symbiosis with a host in the same way as bacteria do. In general, fungal symbioses are extracellular and facultative and symbionts are primarily transmitted horizontally. By infecting many unrelated hosts and having many opportunities for exchange with free-living microbes, they are often associated with pathogenesis. It is intriguing, though, that a few intracellular, matrilineally transmitted fungal symbionts exist that have neither lost genes nor co-diversified with their hosts.[78] Fungal symbionts appear to be extraordinarily diverse and much more research is needed to investigate their roles in insect-fungal endosymbioses. Researchers have suggested that because of enhanced nutrition and diminished susceptibility to parasites and pathogens, insects living with bacterial symbionts have greater advantages in respect to survival and/or reproduction than their conspecifics that live without them.[79]

If bacteria of the genus *Buchnera* (type species *aphidicola*) are the last word in microbial symbiont in insect species of the sap-sucking kind, bacteria of the genus *Wolbachia* (type species *pipientis*) set the record among arthropod species in general. With an estimated distribution of up to 70%[80] among insect species alone, they are the

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[76] Ibid, p.p. 227-228
[77] Ibid, p. 225
[78] Ibid, p. 232
[79] Ibid, p. 232
[80] Moran N.A., Symbiosis, Current Biology Vol 16 No 20, page R870

most abundant intracellular bacteria known so far. Both genera are endosymbionts and both are passed on vertically through the host egg, although *Wolbachia* bacteria also undergo extensive lateral transfer among unrelated insect species. However, what distinguishes the two genera most is the fact that *Buchnera* bacteria are living in mutually obligate partnerships with their hosts, while *Wolbachia* bacteria*,* or rather the strains that are associated with insects, are largely reproductive parasites. *Wolbachia* bacteria spread very successfully by infecting their hosts' reproductive systems, a characteristic that has earned them the inappropriate sobriquet 'selfish symbionts'. Within filarial nematodes (threadlike worms), they live not only in a mutualistic partnership with their hosts, they also are essential for their existence. One is reminded of the wide range of symbiotic associations, which in this case is exhibited in one and the same *Wolbachia* endosymbiont. Of particular interest here are the diverse ways by which *Wolbachia* bacteria influence their hosts' reproductive systems and by doing so alter their hosts' biology. As a consequence, it raises the important question whether microbial symbionts can influence the process of speciation.

Wolbachia bacterial infection takes place in the cytoplasm of the host's egg and, as was mentioned earlier, transmission to the next generation is mainly vertical from the infected female to the next generation. Males are also infected but cannot transmit the bacterial parasite through the sperm (figure 16).

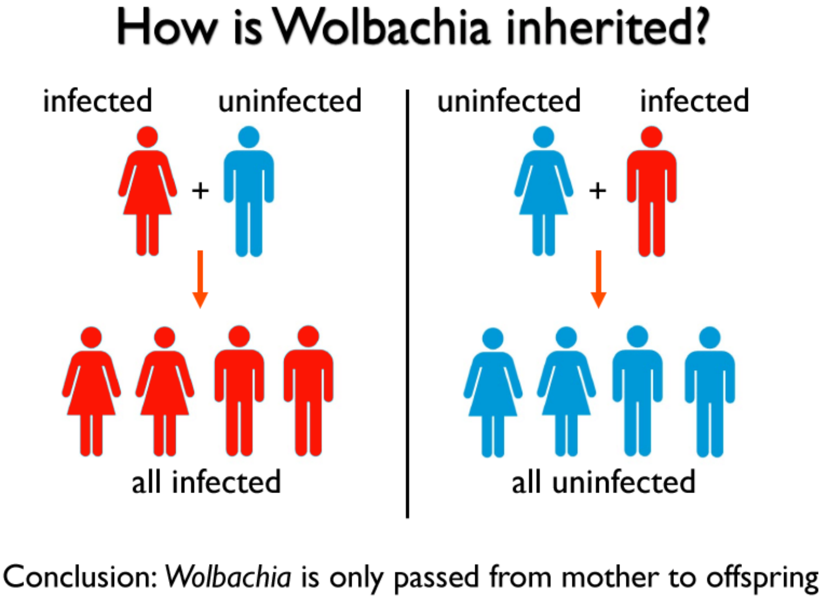

Figure 16. Source: http://www.rochester.edu/college/BIO/labs/WerrenLab/WerrenLab-WolbachiaBiology.html

There are four main modes of reproductive manipulation that infection by *Wolbachia* bacteria can induce:

1) feminisation of infected males (figure 17)
2) killing of infected males (figure 18)
3) *parthenogenesis* (development of an egg without fertilisation) (figure 19)
4) sperm incompatibility with the egg, which is termed *cytoplasmic incompatibility* (CI) (figure 20).

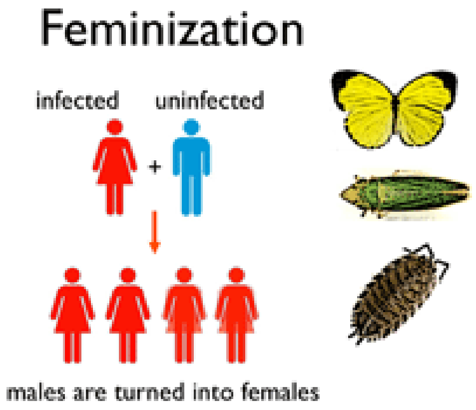

Figure 17.

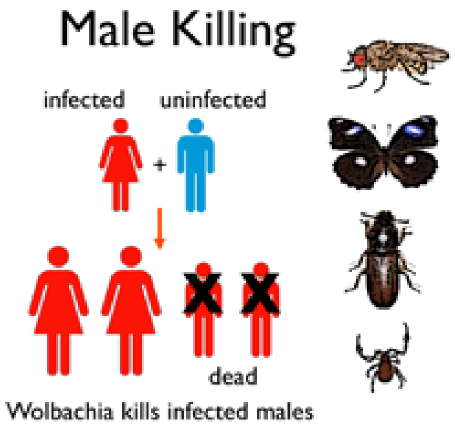

Figure 18.

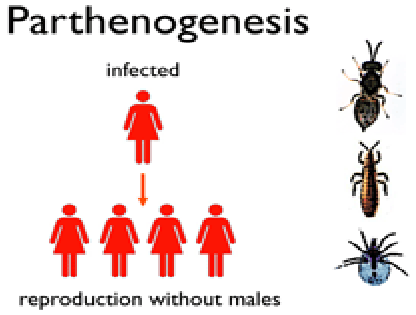

Figure 19.

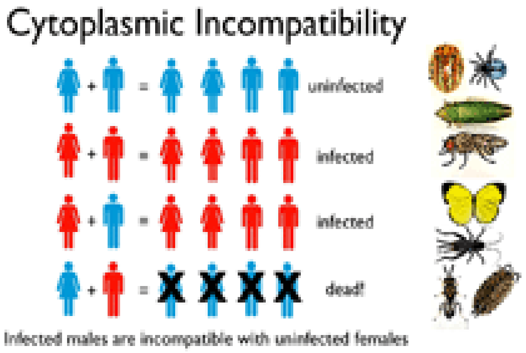

Figure 20.

Figures 17., 18., 19., 20. Different modes of infection.
Source: http://www.rochester.edu/college/BIO/labs/WerrenLab/WerrenLab-WolbachiaBiology.html

Each one of these modes leads to a sex ratio distortion in the host population in favour of females that spread the parasitic symbiont. Feminisation, the conversion of infected genetic male offspring into genetic females is least common and in insects so far only found in the Asian corn borer (*Ostrinia furnacalis*), which is a kind of moth and a grass-dwelling leafhopper. Male-killing has been found among flies, beetles, butterflies and moths. *Wolbachia* endosymbiont-induced *parthenogenesis* is most frequently found among insects such as thrips and wasps and produces female offspring without fertilisation by sperm. Among these different modes, cytoplasmic incompatibility (CI) is the most frequently found *Wolbachia* bacteria-induced manipulation among arthropods and works with two components: 1) bacterial modification of the sperm and 2) bacterial rescue of this modification in the egg. *Wolbachia* bacteria in the testes modify the sperm and, if the same bacterial strain is present in the egg, the modification is rescued; otherwise there is incompatibility and the result is F_1[81] non-viability, or simply, the embryo will not live. Other instances of CI occur when infected males mate with females that are either uninfected or are infected with a different strain of *Wolbachia* bacteria. All other combinations of crosses are compatible, which means that as long as the female is infected with the matching strain, the bacterial symbiont is passed on to the next host generation. The processes by which these different modes of manipulation work are still not understood.

Among arthropods in general and insects in particular, *Wolbachia* bacterial host infection functions like a global pandemic that is comparable with a human pandemic infection. When *Wolbachia* bacteria spread through a host population that consists of infected and uninfected individuals, the number of infected females passing on the parasitic symbiont is increased by the number of infected males that cannot produce offspring with uninfected females or females that carry a different strain of *Wolbachia* bacteria.[82] Also noteworthy is the fact that, depending on the host species they inhabit, some *Wolbachia* strains are able to induce different reproductive manipulations. For example, the same strain of *Wolbachia* causes CI in the almond moth (*Cadra cautella*), while in the Mediterranean flour moth (*Anagasta kuehniella*) it causes male killing. The equivalent observation was reported in the fruit fly species *Drosophila recens* and *Drosophila subquinaria* respectively.[83] In addition, different *Wolbachia* strains can be found in a single host species. Furthermore, it has become clear that extensive recombination has taken place between the various *Wolbachia* strains, which manifests itself in a considerable genetic diversity between the strains.[84]

Endosymbiont - Assisted Speciation?

As was mentioned earlier, *Wolbachia* bacteria infect not only vertically but also unrelated insect species horizontally. Approximately one-third of sequenced invertebrate genomes show genes that stem from *Wolbachia* bacteria, which allows the statement that gene insertions of *Wolbachia* bacteria are common and

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[81] F_1 is the first filial generation resulting from a cross of the first set of parents

[82] Wolbachia Biology, Werren Lab, https://rochester.edu/college/BIO/labs/WerrenLab/WerrenLab-WolbachiaBiology.html

[83] http://courses.umass.edu/mic590s/2009/Reading/Werren2008.pdf, Werren J.H. et al, Wolbachia: master manipulators of invertebrate biology, Nature Reviews Microbiology, Vol 6, October 2008 p. 747

[84] Idem p. 744

widespread. These insertions can be as small as less than 600 bp (base pair of DNA) as in *Nasonia* species (parasitoid wasps) or as large as more than 1 Mbp (megabase pair of DNA). The latter is nearly the size of the entire genome of the *Wolbachia* bacterium, as is also the case in the tropical fruit fly (*Drosophila ananassae*). Whether these gene insertions can result in novel functions has yet to be demonstrated but considering the high frequency of infection and the rather common occurrence of lateral gene transfer, some new traits can be expected. In addition, the insertion events may cause chromosomal rearrangements that by themselves have the potential for playing a part in reproductive isolation and speciation.[85]

The existence of bacterial intracellular symbionts in eukaryote organisms is ubiquitous. Combined with the growing evidence that inheritable microorganisms are important factors in the process of evolution, the assumption can be made that *Wolbachia* bacteria-induced CI has great potential to be a major factor in rapid speciation. The idea is not new. In the 1920s, Ivan E. Wallin (1883 - 1969), a virtually unknown American biologist and anatomist, held the then-unorthodox view that intracellular symbiosis played a crucial role in evolution. At the time, his Russian counterparts Mereshkowsky and Famyntsin and their pioneering works on symbiogenesis, which were discussed earlier, were virtually unknown in the western world. Wallin's conviction was based on his own research and observations of the cell organelles mitochondria and chloroplasts and had led him to conclude that they had evolved from bacterial symbiosis at the cellular level, a process he termed 'symbionticism'. Subsequently, he wrote a series of nine papers entitled *On the Nature of Mitochondria*, which appeared in the *American Journal of Anatomy* between 1922 and 1925.

In 1927, Wallin published a book titled *Symbionticism and the Origin of Species*, in which he elaborated on his theory and proposed that microbial symbiosis was not only responsible for the origin of mitochondria, but it was also a mechanism for evolutionary novelty and the origin of new species. At the time, the new discipline of genetics and the discovery that heredity was transmitted through the chromosomes in the nucleus dominated cell biology. While geneticists believed that rearrangements and mutations of genetic material might provide the mechanism for speciation, they rejected Wallin's suggestion that gene transfer from the symbiont to the nucleus of a host species was possible. In their view, this was outright heresy. It provoked strong reactions in the scientific community and earned him criticism and disdain. It is unfortunate that in the 1920s the technical tools necessary to empirically defend his hypothesis were not yet available to him. After this rejection by his peers, Wallin did not participate in scientific research any further and 'symbionticism' became a forgotten subject.[86]

Added to the methodologies of cell biology and molecular biology, the new field of *genomics*, the study of an organism's genome, has been instrumental in considerably advancing research. Results obtained so far support the hypothesis for symbiont-

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[85] Idem p. 749

[86] Mehos D.C., Appendix: Ivan E. Wallin and his Theory of Symbionticism, Khakhina L. N., (originally published in Russian in 1979) Margulis L., McMenamin M., eds., 1992, Concepts of Symbiogenesis, New Haven and London, Yale University Press, pp 149-161

induced speciation. The arguments are: 1) Microbial symbionts are pervasive in eukaryote organisms, in which they comprise major parts of the genetic and cellular material. 2) Host species commonly display strong specificity for their microbial symbionts that play significant roles in their nutrition, reproduction and development and immunity. These interactions may be obligate for either the host or the symbiont or for both to the extent that parallel divergence between host genes and microbial symbionts appear to be common. 3) Immune genes play a disproportionate role in evolution relative to the rest of the genome. Parts of the host's immune genes are ceaselessly either managing beneficial symbionts or turning their defences toward intruding pathogens. These conflicting interactions between host genes and microbes can generate rapid co-evolutionary changes in either of the organisms and lead to reproductive barriers or incompatibility. When two host species hybridise, immune-related incompatibilities are commonly a factor in hybrid incompatibility[87], as will be evidenced in the next chapter.

Speciation, the evolutionary process by which new biological species arise, can come to pass under different circumstances. The occurrence that is considered to be most common is the separation of a population by a geographical or external barrier, resulting in the isolation of an incipient group. Reproductive isolation (RI) may eventually lead to the loss of the ability to interbreed with the original group. Speciation can also occur through intrinsic barriers that result from changes in behaviour such as different usage of the same habitat, changes in the timing of courtship or changes in courtship or mating rituals.

In addition, compelling evidence exists for the occurrence of microbial symbiont-induced changes of behaviour that result in RI. For instance, in an experiment, genetically identical fruit flies (*Drosophila melanogaster*) that were brought up on diets of either molasses or starch acquired different microbial symbionts, which led to pronounced mate discrimination between them. Continuing with the same diet, the intolerance was maintained for dozens of generations but when the flies were treated with antibiotics, mate discrimination was instantly 'cured'. Studies with another fruit fly, *Drosophila paulistorum*, showed that increased mate discrimination was related to *Wolbachia* endosymbionts, while its association with the parasitic wasp of the genus *Nasonia* decreased mate discrimination. The exact mechanisms underlying mate preferences are not yet known.

Then there is the aforementioned incidence of bacteria-induced parthenogenesis, another behaviour-related RI that results in an asexual population splitting from a sexual population. Asexual reproduction becomes established because asexual females suffer frequently from an accumulation of mutations that have a detrimental effect on traits involved in sexual reproduction. The result is a form of *cladogenesis*, the branching off of a new species from a common ancestral type. In the case of ecological isolation, one is reminded of *Buchnera aphidicola* and their particular hosts, the aphid species. Metabolic interdependence between them allows for exploration of nutrient-

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[87] Brucker R.M., Bordenstein S.R., Speciation by symbiosis, Trends in Ecology and Evolution, Vol. 27, No. 8, pp 444

deficient habitats and therefore expansion into a new niche environment where other species that lack the symbiont would not follow. Geographic and behavioural isolation could lead to RI, and speciation could be the outcome. Accordingly, variation in plants that aphids, aided by their *Buchnera* endosymbionts, feed on is shown to be correlated with some cases of aphid speciation. Analyses of host organisms' habitat specificity have mostly focused on nuclear genes and less on the crucial role that bacterial symbionts play in their nutrition and overall way of life. Buchner had described the symbiotic association between aphid and microbe as an extension of the heritable genetic variation present in the host species. It is now clear that the microbial symbiont augments the host genome with functional genes that open up nutritional opportunities for the host.[88]

In the above-mentioned cases, one speaks of pre-mating isolation. The classic example of post-mating isolation is the reproductive manipulation of its host induced by *Wolbachia* bacteria. When, for instance, the male has a bacterial infection that is not rescued by the female for the restoration of normal development, there is no compatibility and the embryo is not viable. This outcome is termed unidirectional cytoplasmic incompatibility (CI). Likewise, incompatibility results when male and female hosts within one and the same population are infected with different strains of *Wolbachia* bacteria. Crosses between individuals carrying the different microbial symbionts are incompatible in both directions, an outcome that is termed bi-directional CI. *Wolbachia* bacteria-induced CI can lead to RI between incipient species and can potentially be followed by a speciation event.[89] Infection with more than one strain of *Wolbachia* bacteria would be an additional factor to CI.

It is prudent to remember at this point that many different concepts are in use to define a species, the biological species concept (BSC) being one of them. All are arbitrary constructs that were devised for biologists' conveniences but don't necessarily consider the fact that all eukaryotic organisms are communities of hosts and their endosymbiotic microbes.

The mechanisms of CI remain a key focus of *Wolbachia* endosymbiont research. The question what role bacterial symbionts such as *Wolbachia* play in inhibiting or promoting the formation of new invertebrate species is the focus of a research project at Vanderbilt University in Tennessee, USA. Vanderbilt University was awarded a grant of more than $1,270,000.00 to investigate the microbial basis of animal speciation. Results of this research are expected to be published in 2017.

At the same time, research in arthropod-borne diseases that affect crop plants, domestic animals and humans is continuing. Devastating infectious diseases such as malaria, dengue fever, yellow fever and filariasis are transmitted by mosquitoes; zika can be added to the list. Taking advantage of the prevalence of symbiotic bacteria in

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[88] Brucker R.M., Bordenstein S.R., Speciation by symbiosis, Trends in Ecology and Evolution, August 2012, Vol. 27, No. 8, pp 445-446

[89] http://courses.umass.edu/mic590s/2009/Reading/Werren2008.pdf, Werren J.H. et al, Wolbachia: master manipulators of invertebrate biology, Nature Reviews Microbiology, Vol 6, October 2008 p.749

disease-transmitting insect vectors in general and of *Wolbachia* bacteria, the reproductive parasites in particular, efforts are being made in developing symbiotic controls for insect pests and the reduction of vector competence.

Fungi, Toadstools and Mycorrhizae

When in 1753 the great Swedish botanist Carolus Linnaeus (1707 - 1778), also known as Carl von Linné, introduced in his botanical work *Species Plantarum* his signature binomial classification system, he listed fungi together with plants. Considered by many to be of an inferior kind, they did not get the attention they deserved until pioneer mycologist A. De Bary, who, 25 years later would introduce the concept of biological symbiosis to a wider audience, began to investigate them. Plant diseases, especially potato blight, had become a scourge and were causing devastating famines and widespread crop failures with severe economic consequences. In order to understand the origin of different plant pathologies, De Bary investigated the life histories of various fungi, and in 1853 he published a book about fungi that cause rusts and smuts in cereals and other plants. On a request in 1861 from the Royal Agricultural Society of England, he studied the potato fungus and, after a decade and a half of conducting experiments, he succeeded in describing the course of the blight. Furthermore, his studies had led him to conclude that fungi did not belong with plants but instead should be classified with the lower animals; he, therefore, referred to them as 'mycetozoa'. According to Linnaeus, the natural world was divided into three kingdoms, one for minerals, one for vegetables and one for animals. If it wasn't a mineral, it had to be either a plant or an animal; there was evidently no arrangement for anything else. Between 1866 and 1884, de Bary contributed extensively to the classification and systematisation of the botanical knowledge of the time and in the process, he established mycology as a science.[90] Today, Fungi, like Protista, Plantae and Animalia occupy their own biological kingdoms in the domain of the mainly multicellular Eukaryota. It is now acknowledged that, along with bacteria, fungi play a hugely important role in the ecosystems of all living organisms with immense ecological and economic consequences. Life on this planet, it can be said, would not be what it is without fungi.

Fungi exist either as unicellular, non-filamentous yeasts or multicellular, filamentous moulds, though the vast majority of them cannot be seen with the naked eye. It is not known, how many fungi species exist, notwithstanding, 1.5 million was a conservative estimate in 2001. At that time 99,000 species had been described and new ones were being named at the rate of 1,200 per year. However, at this speed, it will take another 1,100 years to describe and catalogue all remaining species, although, in the

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[90] http://www.encyclopedia.com/topic/Heinrich_Anton_De_Bary.aspx

meantime, many will have become extinct due to loss of habitats and hosts.[91] Yeasts, of which currently only some 1,500 species are identified, are widely dispersed in nature. They are single-celled organisms that normally replicate asexually by budding into colonies. Some also multiply by fission but when under stress, they reproduce sexually by means of producing spores. One of the many beneficial species is the yeast *Saccharomyces cerevisiae*, which is commonly used in baking, brewing and wine making by way of a complex process that is called fermentation. When, for instance, the yeast is added to flour or cereal grains as in baking and beer brewing, several enzymes in the yeast cell activate chemical reactions that break down the carbohydrates into starches and sugars. The yeast feeds on some of the sugar, while the remainder is converted into carbon dioxide (CO_2), the gas that expands dough while it is baking; and, in the absence of oxygen, it produces alcohol. The same yeast strain is also used in wine making, because the natural yeast coating on the berries would give only unpredictable results. Another commercial use of yeast on an industrial scale is the production of ethyl alcohol, also known as ethanol fuel, which is mixed with gasoline in an attempt to extend the availability of a non-renewable resource.[92] In recent years, some car models were built to run on an ever higher percentage of ethanol fuel, drastically increasing demand for it. Regrettably, in countries that are pursuing this policy, valuable farmland and precious water are used to grow maize, soybeans, sugar cane and other crops for fuel production with government subsidies. In the process, food security for the world's poor is threatened and the environment is damaged through extensive use of fertilizers.[93]

Besides the numerous useful fungi, there are also many parasitic fungi in existence. For instance, some live between peoples' toes as part of their skin micro 'flora' and others are opportunistic pathogens like *Candida albicans,* which cause oral and genital infections[94]. The yeasts that live in the guts of mammals and various insects should also not be forgotten and will be discussed in more detail later.

Moulds, the multicellular fungal species, reproduce sometimes sexually but mostly asexually by forming spores. Their body is the *mycelium*, which consists of *hyphae*, the very fine, tubular, branching filaments through which they absorb moisture and nutrients. A typical hypha might be no more than a hundredth of a millimetre in diameter. Among the abundant species of moulds that exist, many are beneficial and desired. For example, *Penicillium notatum* became a life saver. It brought the Scottish bacteriologist Sir Alex Fleming (1881 – 1955) fame when he accidentally discovered in 1928 that it can kill a large number of bacteria. His finding led to the development of

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[91] http://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/IntroFungi.aspx

[92] http://www.scienceclarified.com/Ex-Ga/Fermentation.html

[93] Ford Runge C.,2010, The Case Against Biofuels: Probing Ethanol's Hidden Cost, posted in: Biodiversity Energy Policy & Politics Science & Technology Asia, available at: http://e360.yale.edu/feature/the_case_against_biofuels_probing_ethanols_hidden_costs/2251/

[94] http://www.cdc.gov/fungal/diseases/candidiasis/index.html

the first and most widely used antibiotic Penicillin, for which he and his colleagues, the British biochemist Ernst Chain (1906 – 1979) and the Australian pathologist Sir Howard Florey (1898 – 1968) received the Nobel Prize in 1945.[95]

Several other strains of *Penicillium* and also *Geotrichum candidum* are added in the process of cheese making to attain, for instance, the distinctive appearances and flavours of blue cheese and Camembert, respectively.[96] Plenty of obnoxious or even harmful moulds exist as well. There are those that grow on shower curtains and many others that spoil one's foods. But worse still, there are several pathogenic moulds that cause allergies and diseases in humans and other organisms. For instance, some of the parasitic moulds of the genus *Trichophyton* cause the contagious skin disease known as athlete's foot. Fortunately, only comparatively few pathogenic fungi harm humans grievously. On the other hand, a number of them cause extensive damage to a wide range of agricultural crops. In light of future challenges in the form of increasing population numbers and global warming, they could potentially pose a considerable threat to global food security.[97] *Penicillium notatum* is only one of many fungal species that are used for medicinal purposes. In their environments, fungi face competition from an abundance of microbes and many are equipped with a defence mechanism that produces substances to fend them off. The pharmaceutical industry has turned some of these metabolites not only into antibiotics but also cholesterol inhibitors, immunosuppressants and even cancer drugs, as well as insecticides and pesticides for use in agriculture. Finally, the use of fungi (and of bacteria) in bioremediation, the cleaning up of contaminated soil and groundwater, needs also mentioning. Some enzymes that are extracted from fungal metabolisms are used for degrading dangerous substances that contaminate land and water in an environmentally friendly way. These treatments would otherwise be carried out with hazardous chemicals.[98]

Now, where do the toadstools fit in? They are the fungi one finds on supermarket shelves, or the seasonal wild varieties some people gather in the woods and fields. The latter have a reputation to be either delicious, deadly or intoxicating. According to 4,600-year-old hieroglyphics, the ancient Egyptians believed mushrooms to be the plant of immortality and on account of their exquisite taste, pharaohs decreed them to be food exclusively reserved for royalty. Louis XIV of France is said to have enjoyed his button mushrooms so much that on his orders, their cultivation began in caves outside

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[95] http://www.biography.com/people/alexander-fleming-9296894#the-road-to-penicillin

[96] https://cultivationofacheesemonger.wordpress.com/2012/11/16/the-ripening-of-a-camembert/

[97] Berber D.P., Gurr S.J. 2015, Crop-destroying fungal and oomycete pathogens challenge food security, Fungal Genetics and Biology, Volume 74, 2015, p.p. 62-64, available at: http://www.sciencedirect.com/science/article/pii/S1087184514001972

[98] http://www.aber.ac.uk/fungi/fungi/importance/biotechnology.htm Fungal Biotechnology, ii) Bioremediation

of Paris. A deadly variety, according to Roman opinion, was served to Claudius, Emperor of Rome in 54 CE by his fourth wife Agrippina in order to seize the throne for her son Nero. There are also hallucinogenic mushrooms or *psilocybin* with psychedelic properties, so named for the psilocybin and psilocin compounds they contain. When eaten, they can alter one's senses or produce false perceptions. Archaeological and historical evidence testifies to their use already in ancient times. Historians believe that Aztec spiritual leaders exploited their hallucinogenic properties to induce an altered state of consciousness that allowed them to communicate with their gods.

However, what may not be commonly known is that mushrooms are the ephemeral *sporocarps* or fruitbodies of the sometimes very long-lived *mycelia*, which are the fungal organisms that grow hidden from sight in the forest floor around wooded plants. These fruitbodies develop and grow from hyphae, organise underground into stem and cap, for example, and then push up above ground to appear in a variety of shapes and colours. Their seemingly sole activity is to carry the reproductive bodies, the spores. On the underside of the caps are usually either gills or pores from which the spores are released into the environment to sexually reproduce into new mycelia.[99] In addition, there are fruitbodies that do not conform to this description. Some grow like shelves from tree trunks and logs, while others include the highly priced culinary delights, the truffle fungi. Truffles are *hypogeous* (underground) tubers, which grow on the roots of certain trees and shrubs and, when ready to release their spores, are highly aromatic. Because they remain hidden underground or in leaf mould, they depend on fungivores for their reproduction. Detected by their strong odour, a variety of creatures dig them up, eat them and deposit the spores somewhere else in their faeces.[100]

Soil fungi are in permanent search for nutrients and moisture and by sending out their mycelia, permeate the forest floor in all directions. By doing so, they form what British biologist Tom Wakeford in his book *Liaisons of Life* (2001) calls so appropriately the 'wood wide web'.[101] One of these 'webs' is one of the largest and oldest living organisms in existence. Inconceivable as it may seem, one individual fungus of the species *Armillaria bulbosa* (now renamed *Armillaria gallica*) lives as a subterranean mat under a forested area of approximately 15 hectares near Crystal Falls in Michigan, U.S.A. DNA fingerprinting techniques found the mushrooms collected throughout this area to be mostly produced by a single clone of this fungus. Its interconnected *rhizomorph*, the system of root-like structures specific to this fungus and consisting of bundled mycelial hyphae, was estimated to weigh a minimum of 9,700 kg and to have

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[99] http://www.herbarium.usu.edu/fungi/funfacts/mushroom.htm

[100] Molina R., Smith J.E., 2009, Ties That Bind: Pacific Northwest Truffels, Trees, and Animals In Symbiosis, Science Findings of USDA, Issue 118, 2009

[101] Wakeford T., 2001, Liaisons of Life, New York, Chichester, Weinheim, Brisbane, Singapore, Toronto, John Wiley & Sons, Inc. p. 35

an approximate age of 1,500 years.[102] Since the discovery of this humongous fungus, even bigger ones have been reported. The largest to date is an *Armillaria ostoyae* that is growing in eastern Oregon's Blue Mountains under an area extending over 900 hectares and is estimated to be 2,400 years old.[103] Unfortunately, both species are *necrotrophic* parasites and are slowly killing off their host forests to then feed off the lifeless remains. By good fortune, the majority of soil fungi are mutualists and happen to be the largely unappreciated guarantors of this planet's ecological sustainability.

Although fungi are diverse in their morphology, lifestyles, reproductive organisation and nutritional strategy, which depends on their habitat, they all have one thing in common, they are *heterotrophs.* Unlike *phototrophic* plants, they acquire their nutrients like animals from the surrounding environment, may that be detritus, live plants, cheese, soil or flesh. However, they can't ingest their food like animals. Instead, they secrete digestive enzymes into the substrate on or through which they are growing. These enzymes break down the substrate into small enough molecules, from which the fungi then absorb the nutrients they need.[104] Many are *saprotrophs*, the decomposers and never-tiring garbage disposers of this planet. They obtain their nutrients from decaying organic material such as wood, plants or the remains of animals. Saprotrophs recycle 85% of the carbon that is sequestered in dead organic matter and release it back into the environment for reuse by animals and other organisms. Shiitake and Portobello mushrooms, two of the edible fungi one finds readily on supermarket shelves, are saprotrophic; they live and feed on an organic substrate and can, therefore, be easily grown in cultivation.[105] In addition, there are the *biotrophs*. These fungi live in close symbiotic partnerships with their host plants or with animals or with algae as in lichens, the 'dual organisms' that were discussed earlier. Biotrophs get some of their nutrition from live hosts and in return, they provide their hosts also with some benefit.

In the forest, these intimate symbiotic partnerships between members of the two distinct kingdoms of Plantae and Fungi are forged by the mycelia of the soil fungi. They are in search of nutrients and, sustained by the plants that host them, they benefit from an either endo- or ectosymbiotic association. One might ask how this process gets started and how it works. An international team of scientists from INRA (French National Institute for Agricultural Research), Europe's top agricultural research institute) and Lorraine University in France have been able to decipher the molecular 'dialogue' exchanged between some plant roots and the fungus *Laccaria bicolor.* They

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[102] The mushroom forming fungus, *Armillaria bulbosa*, is one of the oldest and largest living organisms, ADAPTED PEER REVIEWED LITERATURE, available at: http://www.clarku.edu/faculty/dhibbett/TFTOL/APRL/APRL/APRL%20Armillaria%20bulbosa.pdf

[103] Volk T., 2002, The Humongous Fungus – Ten Years Later

[104] Life History and Ecology, Fungi are heterotrophic, available at: http://www.ucmp.berkeley.edu/fungi/fungilh.html

[105] https://faculty.unlv.edu/landau/fungi.htm

found that the fungus on the 'prowl' is attracted by and grows toward the signal molecules that plant roots constantly release into the *rhizosphere*, the surrounding soil. In the fungus, the presence of the roots brings about the release of small proteins, called effectors, which condition the host-to-be for a symbiotic relationship. Normally, when a plant is attacked by one of the many fungi and bacteria that are competing for resources in the rhizosphere, its defense mechanism is triggered. This study showed that one of the effector proteins of *Laccaria bicolor* neutralises this defense mechanism and allows the fungus to invade and establish itself in the host.[106] This case reminds one that among fungi and plant roots only a thin line exists between a parasitic invasion and a welcome partnership.

The symbiotic entanglement of fungal hyphae with plant roots results in a 'dual organ' that is called *mycorrhiza.* The word, which is a combination of Greek and Latin and means 'fungus root' was coined by Albert Frank, the botanist who had undertaken the pioneering work on lichens with de Bary and Schwendener. In a paper in 1885, he introduced the term to describe the symbiotic association of a fungus with a root. In this article he argued that what are now called *ectomycorrhizae* were wide-spread phenomena, affecting fungi and woody plants. Based on his observations, he hypothesised that through mycorrhizae both partners were depending on each other for nutrition in a mutualistic symbiosis. At the time, his thesis was rejected by most of his peers, but in recent years, nearly all of his major hypotheses have shown to be correct.[107] Two types of mycorrhizae are generally recognised: *ectotrophic* and *endotrophic*. Ectomycorrhizal fungi (EM) associate mostly with shrubs and trees and belong to two major groups, the Basidiomycota and Ascomycota, which are distinguished by the way they produce spores. EMs enter only between and not into the cortical cells of the host root, before they enclose the root tips with a hyphen mantle, through which moisture and nutrients are exchanged. *Boletus edulis,* the red-topped toadstool and the truffle tuber are two of a myriad of few fruitbodies that testify to this type of partnership between fungi and wooded plants. The vast majority, however, are *endomycorrhizal* or *arbuscular mycorrhizal* fungi (AM) that partner with all plant species, except mosses, and belong to the Glomeromycota, a third group of strictly biotrophic fungi. Their hyphae grow into the cortical cells of the host root but, unlike parasitic fungi, remain outside the vascular tissue. As a reminder that diverse types of symbioses can exist without strictly defined boundaries, there is a third model, the *ectendomycorrhizae.* They show both characteristics at once and are predominantly found in plants of the genera *Pinus* (pine) and *Picea* (spruce) and, to a lesser extent, in *Larix* (larch).[108]

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[106] INRA/Martin F., 2014, Symbiosis in Fungi: Enforced surrender?

[107] Trappe J.M., 2005, Abstract, A.B. Frank and mycorrhizae: the challenge to evolutionary and ecologic theory, Mycorrhiza 2005, 15(4):277-81

[108] http://www.davidmoore.org.uk/assets/mostly_mycology/diane_howarth/mycorrhizal%20types.htm

More than 90% of plants live in endomycorrhizal relationships with fungi, which makes this type of fungus the most abundant on the planet. Besides carbon dioxide (CO_2), sunlight and water, plants need many soil nutrients that are, however, unevenly distributed. One of them is phosphorus (P), an element that is essential for all forms of life but is not available to plants in sufficient amounts. Extending for dozens of metres through the rhizosphere, the fungal web of very fine hyphae enters the tiniest of spaces where plant rootlets can't penetrate. An array of enzymes, secreted by the hyphae, dissolve and free various soil nutrients and minerals encountered and transfer them to the mycorrhizae, where they are then available for absorption by the plant. Fungi not only ensure their host's enhanced nutrition and the supply of moisture during dry spells, they also protect the plant root tissue from (other) parasitic invaders. In exchange, the fungal symbionts draw nutrients from the plant and absorb between 10 - 30% of the sugars the plant has produced by photosynthesis and channelled through the vascular system to the roots. Some fungi are tree-specific, others partner with various tree species or genera, and frequently, interconnected mycorrhizal networks are shared between several plants.[109] More than half a century ago, the Swedish botanist Erik Björkman provided the first evidence for the latter when he injected a Norway spruce with radioactive glucose, which later was received by a plant nearby. At the time, his experiment suggested that, aided by their mycorrhizal symbionts, trees could possibly overcome the varying availability of resources over space, time and species. In the early 1990s, mycologist Suzanne Simard, then at Oregon State University, and her team were able to demonstrate the accuracy of this implication. They found resource transfers not only between trees of the same but also between different species, which were sometimes connected by up to ten fungal symbionts. But what was completely unexpected was the presence of a distribution process, whereby taller trees, whose canopies were bathed in sun and trapping its energy, shared nutrients with smaller, shaded trees. Björkman's and Simard's observations demonstrated not only that reality differs from accepted evolutionary theory, which asserts that individual organisms compete for the same resource by depriving others, it also suggests that the sharing of fungal symbionts could stimulate new ecological and evolutionary processes.[110]

It is generally agreed by many scientists that the ancestors of today's Embryophyta (land plants) were phototrophic aquatic green algae, yet, algae would have been poorly equipped for life on dry land. Desiccation and the inability to exploit nutrients from the substrate would have been two major constraints. Notwithstanding, initial land colonisation was in the form of biofilms consisting of complex microbial communities of phototrophs living on superficial water and heterotrophs feeding on phototrophs. Evidence suggests that land was once dominated by these

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[109] Wakeford T., 2001, Liaisons of Life, New York, Toronto, John Wiley & Sons, Inc., pp. 42-44

[110] Idem, pp. 48-49

microorganisms. Unlike biofilms, land plants have a lower tolerance for lack of moisture and nutrients and therefore, microbial terrestrial algae would only have been able to inhabit land by associating with filamentous fungi providing those resources. Thus, it is hypothesised that colonisation of dry land and eventual evolution of Embryophyta began with lichens, the symbiotic partnerships of phototrophic algae with heterotrophic fungi. Fungal mycelia supplied the moisture and resources contained in the substrate, and in return were nourished by photosynthates produced by the algae. Although fossilised lichens are scarce, some possible specimens in South China date to less than 551 million years ago (mya). More convincing fossils are reported from the early Devonian Rhynie Chert in Scotland (dated ca. 407 mya) where several early Embryophyta were also found. In addition, some lichens are dated to the late Silurian period (415 mya) and display a more modern structure. This serves to prove that lichens were already diverse by the Siluro–Devonian period, nonetheless, in age they don’t exceed the most ancient Embryophyta.

As was mentioned earlier, with few exceptions, all extant land plants live in symbiotic partnerships with a variety of soil fungi. Through morphologically different mycorrhizae, they exchange mostly nutrients and water but also substances for defence against toxins and parasites. As was also mentioned earlier, the vast majority among the fungal symbionts today are endomycorrhizal (AM) fungi of the phylum Glomeromycota. Over the past twenty years, palaeontology and molecular biology have both supported the understanding that Glomeromycota were associated with the precursors of land plants. Their existence is well established in the Rhynie Chert ca. 407 mya. Furthermore, their colonisation is also seen in the aerial photosynthetic axes of some of the oldest observed plants, showing intercellular hyphae, arbuscules and *vesicles* the fluid-containing structures within the cell. The same colonisations can be seen in the thalli of extant, non-vascular, rootless plants such as liverworts and hornworts and in vascular clubmosses, the *lycophytes*. When the fungal association is not in direct connection with a root, it is called mycorrhizal-like or ‘paramycorrhiza’. Liverworts and hornworts are held to be most closely related to the precursors of ancestral land plants. Therefore, the conclusion suggests itself that an arbuscular paramycorrhiza is the ancestral type of all mycorrhizae. This hypothesis is further supported by results from molecular analyses. Before mycorrhizae can form, genes have to become active in the plant to make a colonisation by a fungus possible. In all modern plants, including basal lineages like hornworts and liverworts, the genes that are involved in the transduction of signals emitted by Glomeromycota are SYM genes (symbiosis genes). Moreover, genetic mutants of alfalfa (*Medicago sativa*), which suffer from a deficiency of SYM genes (symbiosis-related genes), can be recovered by introducing gene copies from these lineages.[111]

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[111] Selosse M-A, Strullu-Derrien C., 2015, Origins of the terrestrial flora: A symbiosis with fungi?

Nonetheless, the consensus for Glomeromycota's exclusive role in having facilitated colonisation of the terrestrial environment by the earliest plants is being contested. Evidence was presented "that several species, representing the earliest groups of land plants, are symbiotic with fungi of the [subphylum] Mucoromycotina [which are predating its basal or sister lineage Glomeromycota]. This finding brings up the possibility that terrestrialisation was facilitated by these fungi rather than, as conventionally proposed, by members of the Glomeromycota.[112]" No matter which fungal phylum eventually will get the prize for being first, diverse research reinforces the hypothesis that without mycorrhizal fungi, the greening of the Earth would not have begun, nor would it be what it is today.

Based on scant fossil evidence, the appearance of real land plants on terra firma is thought to have occurred between 480 and 460 million years ago. However, according to a genetic study carried out by a research team at Pennsylvania University, USA, this happened much earlier than previously assumed. The team studied the mutations of 119 genes that are common to hundreds of species of extant fungi, plants and animals and calibrated them against known evolutionary events in the history of animals. The result is a 'molecular clock'[113], by which it can be estimated how long ago each species came into existence. The research team found that land fungi had appeared by roughly 1.3 billion years ago, while the age for plants on land was about 700 million years. From these data, they inferred that an increase in land plant abundance was the cause for two major climate events Earth has experienced in the last three-quarter to half billion years, known as 'Snowball Earth' and the Cambrian 'explosion'. Snowball Earth occurred between 750 and 580 mya and were periods when the entire globe was covered in ice. The Cambrian explosion was an evolutionary event of major diversification of organisms. In a relatively short time, beginning roughly 530 mya, a large variety of multicellular animals appeared in the fossil record from which most of the animal groups evolved that are in existence today.

The possible scenario is as follows: lichens, the first colonisers of the terrestrial environment, moisturised and nourished organisms like cyanobacteria and green algae. As a consequence, oxygen (O_2), the byproduct of their photosynthesising way of life, was continually released into the atmosphere. At the same time, lichens contributed to reducing atmospheric carbon dioxide (CO_2) levels by producing acids that dissolve rocks. As the calcium (C) released from rocks was washed by rain into the oceans, it formed eventually into sedimentary limestone of mostly calcium carbonate ($Ca\ CO_3$). Thus, carbon atoms were not released into the atmosphere but trapped in the rock. As land plants evolved and expanded in range, more carbon dioxide from the

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[112] Bidartondo M.I. et al, 2011, The dawn of symbiosis between plants and fungi, Biology Letters 2011 Aug 23; 7(4): 574–577

[113] Although the concept of 'molecular clock' is widely accepted, there is some controversy. See: http://www.as.wvu.edu/~kgarbutt/QuantGen/Gen535Papers2/Molecular_Clocks.htm

atmosphere was converted into carbon by photosynthesis. One-third of the biomass of woody plants, for instance, is a polymer called *lignin*, which contains carbon. When plants died, geological processes buried them and carbon atoms were sequestered in the substrate. More carbon from plants was taken from the atmosphere and buried in swamps millions of years ago, resulting in hydrocarbon and carbon deposits of oil and coal. The authors of the study suggest that, notwithstanding additional factors, the biggest cooling effect resulting in a series of Snowball Earth events was the consequence of a reduction of CO_2 levels that was caused by fungi and plants inhabiting the land at that time. Plants did not only use up CO_2 from the atmosphere, thus cooling Earth's temperature, they simultaneously also increased the percentage of oxygen in the atmosphere. The authors believe that when another occurrence of a Snowball Earth event was due, surface temperatures did not cool sufficiently. This coincided with an increase in the abundance of land plants and, with a further increase in oxygen levels in the atmosphere, triggered the evolution of land animals in the Cambrian period.[114]

Since the beginning of the Industrial Revolution, fossil fuels in the form of oil and coal have been burned in ever increasing quantities. According to many scientific studies, releasing their carbon atoms back into the atmosphere is contributing to the warming of the planet. Calculations predict a mean annual temperature increase of 2-5°C by 2100, with the greatest warming expected in northern Europe in winter and in southern Europe in summer. This will put the elderly and people suffering from respiratory and cardiovascular disease at greatest health risk. Such outcome was witnessed in 2003, when a severe heat wave claimed the lives of more than seventy thousand people across thirteen European countries. Currently, about 75% of the European Union population is living in urban areas and their numbers are rising. Scientific modelling studies that were based on a green cover reduction of 10% forecast a rise of temperatures by up to 8.2°C over the next seventy years. Urban areas are notoriously lacking in green spaces, although it is well documented that trees and shrubs can have a considerable cooling effect. They reflect light and heat, and transpiration of water from plant leaves results in lowering temperature, not to mention plants' contribution to flood management by facilitating the absorption of rainwater. Other modelling studies have shown that a 10% increase of urban green cover could reduce the temperature increase to only 1%. Furthermore, living near green spaces brings immediate benefits for mental and physical health. Statistics for the entire population of England disclose that people who live near vegetated areas

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[114] Hedges B. et al, 2001, First Land Plants and Fungi Changed Earth's Climate, Paving the Way for Explosive Evolution of Land Animals, New Gene Study Suggests, Penn State Science, available at: http://science.psu.edu/news-and-events/2001-news/Hedges8-2001.htm

show a 25% lower death rate from all causes, even after adjustment for impacts on health caused by poverty.[115]

On a global scale, research suggests that forests and climate change are inherently linked since hundreds of millions of years and remain so in the modern era. Changes in global climate, accompanied by extreme weather events, are having a negative impact on the world's forests. At the same time, forests have the capacity to mitigate this negative impact by removing greenhouse gas emissions from the atmosphere and as a result of photosynthesis, sequester carbon atoms in their biomass. The world's forests are not only essential in safeguarding the climate, they also sustain more than 1.6 billion people by providing them with food, water, fuel, medicines and income from employment. In addition, forests support up to 80% of terrestrial biodiversity.[116]

> Forests and the services they offer society are crucial for sustainable development and human well-being. While many efforts to slow or halt deforestation have been successful, approximately 13 million hectares of forests continue to be lost each year, contributing up to 20 per cent of annual global greenhouse gas emissions. The clearing of land linked to the development of agricultural commodities is a key factor behind deforestation. At the same time, about 2 billion hectares of degraded forests and other lands – all around the globe – need to be restored. Actions to combat deforestation and speed up the restoration of degraded lands will contribute to economic growth, poverty reduction and greater food security as well as help communities adapt to climate change and secure the rights and livelihoods of indigenous peoples and local communities.[117]

As an example, the clearing of trees over extended areas in the rainforest of the Amazon basin is brought about by logging, burning and mining and for agricultural purposes. Devoid of trees, soils dry out. The top layer of humus becomes airborne or gets washed away by rain and flooding and microbial life dies because there is no leaf litter to feed on. These processes result in vast tracks of land without trees and their symbiotic mycorrhizal fungi, indeed, without much life at all; it is land that is largely biologically inert. Reforestation of such land or any other degraded land, for that matter, or its use for farming is difficult and left unproductive. In the Amazon basin, 75% of the soil is deficient in nutrients and new growth succumbs easily without their attendant networks of mycorrhizal fungi.[118]

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[115] http://www.eea.europa.eu/articles/forests-health-and-climate-change/ European Environment Agency, Forests, health and climate change

[116] http://www.un.org/climatechange/summit/wp-content/uploads/sites/2/2014/07/New-York-Declaration-on-Forests_8-Oct-2015.pdf New York declaration on forests, p3

[117] http://www.un.org/climatechange/summit/action-areas/Forests

[118] http://www.theguardians.com/Microbiology/gm_mbr10.htm

The importance of mycorrhizal symbionts to all plant life and to reforestation efforts, in particular, is now well understood. In recent years, experimental research has shown that seedlings that are inoculated with mycorrhizal fungi not only get a better start but the chemical and biological properties of the soil improve also after planting.

In Morocco, for instance, a group of scientists with a multidisciplinary background have used this insight and have carried out field experiments with seedlings of the argan tree (*Argania spinosa*).[119] These trees are endemic to the semi-desert of the Sous Valley in southwestern Morocco, where they grow on poor, chalky soils, withstanding extended periods of drought and heat. For centuries, they have provided local Berber communities with dietary oil, firewood, timber and forage for sheep and goats. During the last decade or so, argan oil has gained fame beyond Moroccan borders. Extracting the oil from the nuts has provided women, who are organised into small-scale cooperatives, with an opportunity for economic and social freedom. However, due to overgrazing, overexploitation and aridity, argan forests have decreased drastically in area and density. With this downturn, whole ecosystems are being lost with consequential degradation of the soil, which is accompanied by a decline of invaluable microbial activity. Replanting efforts began in 2000, but regeneration programs of argan forests have not produced expected results.

In order to assess the influence of mycorrhizae on the establishment and development of argan seedlings in reforestation projects, the team, led by Said El Mrabet, started their experiment under controlled greenhouse conditions. Argan seedlings were raised in soil that had been collected from the rhizosphere of argan trees in the wild. The soil contained naturally occurring argan-specific endomycorrhizal fungi (AMF) and was then mixed with a substrate of organic vegetable compost in a 5% proportion of AMF. An equal number of control seedlings were planted in organic compost without inoculation of AMF. After four months, inoculated and non-inoculated seedlings were planted out in an experimental plot in the Admine Forest at Agadir, while other seedlings were kept in the greenhouse for an additional two months before evaluation. Regular watering and monitoring followed, accompanied by comprehensive studies of plant tissues and soil samples using physical and chemical analyses. After six months under greenhouse conditions and compared with control plants, AMF inoculation had improved growth of argan seedlings by 51%, while basal diameter had increased by an average of 29%. In respect to biomass, root production was 66% higher and shoot production was 60% higher than in non-inoculated seedlings. The mycorrhizal effect on nitrogen (N) and phosphorus (P) content in foliar

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[119] El Mrabet S. et al, 2014, The Effectiveness of Arbuscular Mycorrhizal Inoculation and Bio-Compost Addition for Enhancing Reforestation with *Argania spinosa* in Morocco, Open Journal of Forestry, Vol.4, No.1

tissue was significant at 185% for nitrogen and 118% for phosphorus. Mycorrhizal fungi had colonised at least 54% of roots.

At the time of transplanting into field conditions, inoculated and non-inoculated argan seedlings were planted with and without the addition of bio-compost. Twelve months after planting, soil analyses showed that the addition of the soil amendment significantly improved soil fertility by decreasing values for soil pH and increasing values for total nitrogen (N), total organic carbon (C), extractable potassium (K) and available phosphorus (P). The addition had also improved the biological quality of the soil in the rhizosphere and at the same time increased the biomass of young argan plants by about 84% compared to control plants.

During the first year of transplanting, which is the most critical period in reforestation, inoculation of *Argania spinosa* seedlings with mycorrhizal fungi significantly stimulated the production of biomass. At the end of the growth period, shoot biomass was increased by about 169% compared to control plants. This correlates with efficient nutrient uptake from the soil, observable in the highest levels of phosphorus and nitrogen in leaf tissue of mycorrhizae-supported plants. Interestingly, although soil that was amended with organic compost was four times higher in available phosphorus, mycorrhizal inoculation alone was more effective on phosphorus content in leaf and root tissue and affirmed the primary role of AMF in phosphorus uptake. It is believed that increased nitrogen content in plant tissue may also be due to AMF. It may improve decomposition of organic matter and nitrogen capture and increase absorption of phosphorus, which in turn favours atmospheric nitrogen (N2) fixation, a process that was discussed earlier in the context of legumes and their symbiotic soil bacteria. The study also registered improved survival rates for inoculated seedlings in a degraded environment. The experiment demonstrated that inoculation with a mycorrhizal symbiont facilitates reforestation on degraded soil by significantly improving the quality of the soil in the rhizosphere. Furthermore, the study showed that planting *Argania spinosa* seedlings with plant-specific mycorrhizal partners enhanced their capacity for absorption of nutrients from the soil. Uptake of phosphorus and nitrogen was especially boosted and water stress was mitigated, yielding stronger, more vigorous plants.[120] Without a doubt, by planting *Argania spinosa* with a symbiotic mycorrhizal fungus partner, forests and their biological communities can be successfully restored to health and productivity for the immediate benefit of the Berber population. On a larger scale, replanted argan forests will serve as carbon storage for the planet.

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[120] idem p.p.14-23, available at: http://dx.doi.org/10.4236/ojf.2014.41003

Orchids And Their Enablers

Darwin held a fascination for orchids. In 1862 he published a volume entitled *On the various contrivances by which British and foreign orchids are fertilised by insects,* in which he wrote-

> In my examination of Orchids, hardly any fact has so much struck me as the endless diversity of structure...for gaining the very same end, namely, the fertilisation of one flower by the pollen of another. (Darwin 1862)[121]

The following year, in a letter to his close friend, the eminent British botanist J.D. Hooker he wrote-

> I have not a fact to go on, but have a notion (no, I have firm conviction!) that they [orchids] are parasites in early youth on cryptogams[122]! (Darwin, 1863)[123]

In the ensuing years, Darwin's conviction was proven to be factual. In addition to their symbiotic association with insect pollinators, orchids require an additional partnership that is vital to their existence; they need mycorrhizal fungi. Until the French botanist Noël Bernard (1874 – 1911) discovered in 1903 that a fungus on their seeds was needed for germination and a fungus in their roots to continue growing, horticulturists and hobby gardeners were rarely successful with raising orchids from seeds. Bernard died at the early age of 37 but the year before his death, he received the Prix Saintour from the Académie Française for an impressive body of work that was dedicated mainly to plant symbioses.[124]

Orchidaceae, the family of orchids, is one of the largest families of flowering plants on Earth and comprises an estimated 26,000 species worldwide, which is as many as all the known animal species of mammals, birds, reptiles and amphibians combined. Orchids go back to the Late Cretaceous (76 - 84 mya), which coincides with the

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[121] http://naturalhistory.si.edu/exhibits/orchids/orchids-2009.html

[122] Cryptogam means 'hidden reproduction' and refers to plants and fungi that reproduce by spores, not seeds

[123] https://www.darwinproject.ac.uk/letter/DCP-LETT-4061.xml

[124] http://isyeb.mnhn.fr/IMG/pdf/symbiosis_bernard_paper.pdf Selosse M.-A. et al, 2011, Noël Bernard (1874–1911): orchids to symbiosis in a dozen years, one century ago, Symbiosis DOI 10.1007/s13199-011-0131-5, p.p. 5-6

evolution of bees and grow in all sorts of habitats on every continent, except Antarctica.[125]

According to their growing habits, orchids can be divided into three groups. Terrestrials grow in soil, while *epiphytes* grow upon trunks and branches of live plants and are therefore often called ‘air plants’. Epiphytic species are most numerous in the tropics and are found in dense forests high up on tall trees in the light that does not reach the forest floor. Their thick, spongy roots that cling harmlessly to the bark for support, are well suited for absorbing and storing water from rain and mist. A subset of the epiphytes are the *lithophytes* (from Greek for ‘upon stone’); they too grow mostly in the tropics. Lithophytic orchids cling to rock faces, though some also grow among pebbles, and support themselves with strong roots that bury into crevices, where they find the moisture and nutrients they need. Finally, there are also some species that are parasitic *mycoheterotrophs*, orchids that obtain their nutrients through mycorrhizal fungi.[126]

Another superlative worth mentioning is the amount and size of their seeds. Orchids produce the smallest seeds by size (0,05-6 mm) or weight (0.32 - 24 µg) of all seed-bearing plants with up to 4 million seeds in a single seed pod. Darwin calculated that within three generations -

> ....a single plant would nearly clothe with one uniform green carpet the entire surface of the land throughout the globe. (Darwin 1877)[127]

Evidently, this is not what happens. Although the number of seeds produced is enormous, only a tiny fraction will meet stringent biological and environmental conditions that are required for survival. From the moment onward when a seed alights on a specific substrate and at least at some subsequent stage in their lives, orchids rely to varying degrees on their symbiotic partners. A very precise fungus is necessary for germination and growth and a specific insect is required for pollination. In addition to their precarious needs to get started and grow, poaching and increased habitat loss have made orchids the most threatened of all flowering plants and have, therefore, found their place in Appendix II or higher of the Convention on International Trade in Endangered Species (CITES).

As their seeds are minute, there is no room for the *endosperm*, the nutritive tissue that normally surrounds the embryo and assists germination by providing the necessary nutrition. Therefore, in the wild, the embryo will only germinate when the right kind of

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[125] http://www.sciencedirect.com/science/article/pii/S0960982208001723, Roberts D.L., Dixon K. W., 2008, Orchids, Current Biology, Vol. 18, Issue 8, p.p. R325-R329

[126] http://www.gardens.si.edu/come-learn/docs/Template_Orchid_Where%20is%20my%20home.edit.pdf

[127] https://www.kent.ac.uk/sac/scholarships/nerc-roberts.html

fungus has entered the seed and provided the sustenance needed for the initial stage of the orchid's life. Thereafter, the fungus will live in the emerging root system in a mycorrhizal association and enhance the plant's needs, although orchids' nutritional dependencies are highly variable. Terrestrial orchids, for instance, show differing degrees of continued reliance on mycorrhizal fungi with some species being critically dependent for transfer of water, minerals and up to 85% of their carbon needs. In addition, they may also receive some protection against soil pathogens. In return, the orchids supply the fungi with sugar, some vitamins and a safe place to be. In contrast, epiphytes and lithophytes will depend on their fungi for the germination process only. Once fully grown, they may live independently by photosynthesis and intake of water and minerals directly from rainfall or runoff from bark and rock. Because of their independence from mycorrhizal fungi, these types of orchids are much easier to grow and are therefore preferred by horticulturists. True to the existence of a symbiotic continuum that ranges from mutualistic to parasitic, there are also orchid species that lack any ability whatsoever to photosynthesise for their nutritional needs and consequently rely entirely on their fungal partners. Some of these mycoheterotrophs live in a peculiar parasitic, three-way symbiotic mycorrhizal relationship. While the fungus resides in the roots of the orchid, it maintains at the same time a mycorrhizal relationship with a shrub or tree growing nearby. Studies using radioactive tracing have shown that in these cases a carbon transfer is taking place from the shrub or tree to the orchid via the 'fungal bridge'.

Investigations into orchids' associations with their hidden mycorrhizal fungi have lagged behind studies of their varied and very specialised pollination systems that take place in plain sight. Pollination is carried out with varying degree of effectiveness by insects and rarely also by birds, who visit the flowers for their nectar, oils, fragrances, pollen, or to seek shelter. Reduced numbers of pollinating species per orchid species have been recorded, which is resulting in orchids increasingly relying on specific pollinators, a trend that is not seen vice versa. About a third of orchid species attract pollinators with food mimicry, sexual mimicry or brood-site mimicry without any benefit for the visitor. However, when the pollinator benefits, fruit set is on average twofold in all orchid species, whether temperate or tropical.[128]

The lack of an endosperm may be a disadvantage when it comes to germination, but in regard to pollination, it is beneficial. In many higher plants, the endosperm hinders the growth of hybrid seeds that are produced by cross-pollination between species. It has been suggested that unrestricted sharing of genes has resulted in the astonishing

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[128] http://www.sciencedirect.com/science/article/pii/S0960982208001723, Roberts D.L., Dixon K. W., 2008, Orchids, Current Biology, Vol. 18, Issue 8, p.p. R325-R329

diversity of orchids in a wide variety of habitats.[129] This may explain the success of orchid hybridisation that is achieved by commercial horticulturists. During the past twenty years, scientific research and propagation by tissue culture have turned horticulturists' fickle endeavours into a secure and growing commercial success. On the other hand, conserving orchids in the wild presents considerable and daunting challenges.

At the beginning of this century, a third of Britain's fifty native orchid species was under threat from covert collectors who were ignorant of wild orchids' special fungal needs. Habitat destruction from increased livestock grazing was another threat. The lady's slipper orchid (*Cypripedium calceolus*) was once widespread in localised areas of northern England but suffered a severe decline. At the beginning of this century, its numbers were reduced to a single, carefully guarded population. This orchid species is thought to be one of the slowest growing plants in the world, taking 6 - 11 years to produce flowers. It was classified as critically endangered in the *Red List of Great Britain* and among other listings, it is also recorded under Appendix II of CITES.[130] In 1983, *C. Calceolus* became a target of the Sainsbury Orchid Conservation Project in the Micropropagation Laboratory at the Royal Botanic Gardens Kew. The aim was to develop techniques for *in vitro* mycorrhizal-assisted germination, asymbiotic propagation and re-introduction of seedlings into the wild.[131] Kew's propagating effort started with one protected plant in the wild that was hand-pollinated when it bloomed, and the resulting seeds were then sent to the micropropagation laboratory at Kew. Finding which one of the hundreds of fungi, most of which are not even named yet, will form a symbiosis with a particularly endangered orchid species is a task that may take years. Instead, the seeds were sown on an agar medium containing a mixture of amino acids, vitamins and pineapple juice that all together mimic the chemicals that orchids normally receive from their fungal symbionts.[132] Eventually, several thousand seedlings of *C. Calceolus* grown in the laboratory were planted out in secret locations in a re-introduction effort as part of the Species Recovery Programme. By 2002, first flowerings were reported and in 2009, seed pods had formed after natural pollination by insects. The know-how of the Royal Botanic Garden Kew for growing British orchids

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[129] Meisel J.E., Kaufmann R.S., Pupulin F., 2014, Orchids of Tropical America: An Introduction and Guide, Cornell University Press, Ithaca and London, p.10

[130] Lady's slipper orchid, http://www.arkive.org/ladys-slipper-orchid/cypripedium-calceolus/

[131] Sainsbury Orchid Conservation: *In Vitro* Propagation and Re-Establishment of UK Terrestrial Orchids, http://www.kew.org/science/directory/projects/SainsburyOrchCons.html

[132] *Cypripedium calceolus* (lady's slipper orchid), http://www.kew.org/science-conservation/plants-fungi/cypripedium-calceolus-ladys-slipper-orchid

has been applied to conservation and re-introduction of other European species and along with their expertise and techniques, they have shared their orchids with a number of European countries.[133]

Most scientists agree that the symbiosis of an aquatic alga with a fungus, both belonging to different biological kingdoms, lies at the evolutionary basis of terrestrial plants and their eventual colonisation of the land surface of the Earth. Initially, fungi turned the poor substrate into a medium in which the evolving plants anchored their roots and then took up residence in and later on these same roots where they still live in mostly mutualistic symbioses. Over the aeons, their saprotrophic cousins and other microorganisms collectively improved the earth by decomposing all dead organic matter that accumulated. Along with these activities above and below ground, new habitats came into existence, were occupied by new life forms and became part of distinct ecosystems. Although the coevolutionary process of plants, the *phytobionts,* with mycorrhizal fungi, the *mycobionts,* is still poorly understood, the fossil record attests to their coexistence from the outset. Over hundreds of millions of years, this association between algal and fungal components has evolved into various degrees of symbioses that affect virtually all terrestrial vegetation. Most plants need fungal partners to thrive, others need them to survive and, in the case of the orchid family, they are even required for seeds to germinate. Besides enabling the host plants to exploit the soil for water and nutrients, which is reflected in the plants' vigorous growth or survival, mycorrhizal fungi have the capability to sustain entire ecosystems. On account of their non-specific nature, endomycorrhizal fungi form at times simultaneous mycelial networks with plants of not only different species but also different genera and across a considerable distance. In times of adverse conditions, such an interconnectedness among plants presents a 'communal security system' and assures the conservation of the group and by extension the whole ecosystem. Last, but not least, fungi in general and mycorrhizae, in particular, have not only influenced the evolution of Earth's vegetation and the animals that depend on it but as a consequence, it appears that they also had an impact on shaping Earth's climate. As was discussed in detail in the first chapter, carbon is the foundation and also the sustenance of life on planet Earth. It is continually cycled from the atmosphere through the life cycles of plants, sea and land animals, through the soil and back into the atmosphere in what is called the carbon cycle. All soil fungi, but especially mycorrhizal fungi, play an important role in sequestering carbon and thus they are bringing their influence to bear on the carbon dioxide content of the Earth's atmosphere and its climate.

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[133] Sainsbury Orchid Conservation: *In Vitro* Propagation and Re-Establishment of UK Terrestrial Orchids, http://www.kew.org/science/directory/projects/SainsburyOrchCons.html

Summary

Symbiosis, the merger of unlike, single-celled organisms is at the root of evolutionary change from simple to complex. For multicellular life to evolve, the single, most important evolutionary event after the occurrence of life itself was the formation of the eukaryotic cell. The origin of the basic unit from which all complex life forms are built came about by independently living, single-celled microbes becoming incorporated into an endosymbiotic association. Two types of these formerly free-living organisms became the mitochondria and the chloroplasts and as organelles continue to perform functions that are essential for maintaining the single unit of life in animals and plants respectively. This paradigm was formulated into the endosymbiotic theory of evolution, which is now accepted by most scientists. It challenges the gene-centric neo-Darwinian theory of evolution, which is entirely based on the mechanism of natural selection acting on random genetic variation as the sole paradigm. It challenges the gene-centric version of the neo-Darwinian theory of evolution, which is for the most part based on the mechanism of natural selection acting on random genetic variation.

After this primordial event and higher up on the scale of complexity, in which biological organisms have been classified into domains and kingdoms, symbiosis continued to shape evolution. Organisms belonging to different biological kingdoms such as eukaryote protists and eukaryote fungi joined into associations with unlike partners. Initially, they provided the basis for the evolution of all terrestrial plant life of the plant kingdom and continued to be instrumental in sustaining them. Plants, in turn, continued life in symbiosis and coexist today in endo- and ectosymbiotic relationships that function as ecological units. They are hosts to chloroplasts that synthesise sunlight for sustenance; to fungi that aid in the uptake of minerals and other essential nutrients, which would otherwise be unavailable; and provide protection against harmful microbes. These mutually beneficial symbiotic partnerships have not only influenced the evolution of individual organisms but, by extension, they have shaped entire ecologies on this planet.

But protists did not only forge mutually beneficial symbiotic alliances with species in the plant kingdom, they also joined up with species in the animal kingdom as was seen in the case of the sea slug, for instance.

Special mention must be made here of the protists and fungi that joined in yet another endosymbiotic partnership that resulted in a third, entirely new organism with its own distinct morphological and behavioural traits, the lichen. The merger of two, and in some cases three, heterogeneous organisms that belong to different biological kingdoms resulted in a biological entity that doesn’t fit the commonly agreed upon classification of living things. The creative process that shaped this ‘dual’ or ‘triple’ organism is termed ‘symbiogenesis’. Natural selection does not play any part in the dual organism’s emergence.

Contained In the Kingdom Monera are the countless, single-celled prokaryote bacteria, which are organisms that occupy the lower rungs on the scale of complexity**.** Some of them liaise with plant roots for nitrogen fixation and others reside in other plant tissues in partnerships that are equally beneficial for both members of the distinct kingdoms. Countless other bacteria liaise with organisms in the animal kingdom, where they take up residence inside and outside all animals, including humans, whose symbiotic relationship with their own microbiota will be discussed in more detail in the last chapter**.** As was demonstrated, several insect taxa depend entirely on their bacterial symbionts for successful growth, reproduction or protection, and some of them live with two or even three symbionts. On the other hand, some intracellular symbionts are shown to have lost several of their genes that their free-living cousins retained and have thus become totally dependent on their coexistence with their hosts. These symbiotic associations resulted in new, life-enhancing solutions that were not accessible to organisms lacking these partnerships. Importantly, new traits were produced more rapidly than random genetic variation would have allowed. In the case of the aphid, the endosymbiotic, mutually beneficial nutritional partnership with a bacterium exists since hundreds of millions of years and is passed through the host egg to successive generations. During that time, some hosts and several matrilineally transmitted symbionts went through *co-cladogenesis.* These were events when parent species of hosts and symbionts branched off into distinct species and diversified in parallel.

Finally, there are the endosymbiotic parasitic bacteria like the *Wolbachia* strains that infect their insect hosts' reproductive systems and thus not only alter their biology but also distort the sex ratio in the host population in favour of females that spread the infection to the next generation. Approximately one-third of sequenced invertebrate genomes show incorporated *Wolbachia* bacteria genes. These genetic insertions may cause chromosomal rearrangements, which has the potential for playing a part in reproductive isolation and speciation. Speciation is commonly understood to mostly come about through geographical or external barriers. Nevertheless, evidence has accumulated that support the hypothesis for symbiont-induced speciation. Microbial symbionts have induced changes in the behaviour of their host that has led to intrinsic barriers resulting in the microbial association serving as an extension of the heritable genetic variation present in the host species.

Chapter Four

Hybridisation and Other Evidence of Crossed Lines

In his earlier years, Linnaeus believed that plants and animals had been created by God and to be of permanent form. In later life, however, he recognised that new species could emerge through hybridisation, but he considered them to be derived from the original forms provided by God in accordance with the teachings of the Bible. At the beginning of the 19th century, these views of hybridisation were largely contested by the clergy and naturalists alike. God had ordered the mechanics of life in a rational manner to safeguard against chaos and disorder. There were no sudden jumps in nature, and hybrids were monstrosities that were endowed with sterility by the good Lord. Joseph Gottlieb Kölreuter (1733 – 1806) and Karl Friedrich von Gaertner (1772 – 1850) were two influential German botanists who both sought to disprove Linnaeus and his claim that crosses could lead to new species. Hybrids, in their eyes, were nearly all sterile and those that were not would eventually return to type. By this they meant that varieties could be produced but eventually, they would die out or through back-crossing return to the original forms of plants created by God. Accordingly, the two botanists set out to prove by experimentation the truth of their sentiments. The problem, however, was that fertile hybrid plants could be produced in cultivation and were in evidence in nature. The 'solution' put forward by the experimenters came from producing several generations of hybrid plants that seemed to demonstrate that eventually, sterility returned and the new hybrid lineage ceased. The veracity of these experiments was to be challenged, however, by the Swiss botanist Carl von Nägeli (1817 - 1891) and Charles Darwin.

Two obstacles to evolutionary theory were widespread at this time; one was the concept of 'reversion to type'; the other was "natura non facit saltum" or nature does not make jumps. Charles Darwin was particularly worried about the problem of reversion to type, but he accepted the latter concept and incorporated it into a gradualist theory of evolution, where natural selection applied only to small changes in variation. (Reversion to type should not be confused with the modern concept of atavism in genetics, where traits are expressed in a phenotype after they were unexpressed for several generations.) Darwin had employed the analogy that Jean Baptiste Lamarck had devised of artificial selection. To convince the evolutionary sceptics, an analogy was presented by which, for instance, through husbandry cattle could be selected and bred for certain desirable traits. These small, gradual changes could also occur in nature. A gradual accumulation of almost unperceivable changes over many generations could eventually produce a new species. The problem for Darwin was that his vast communications with farmers and various breeders of animals reflected the view that traits could be artificially selected, but if the animal in question were to be returned to the wild, these traits would disappear over a few generations. In other words, the animal would revert to its ancestral or aboriginal type.

Darwin believed that he could solve the ‘problem’ of reversion with natural selection eliminating any small traits that disadvantaged an organism in the struggle for existence. Eventually, after many generations, a new form would emerge and the original or ancestral form would be eliminated. Thus, natural selection could preserve and also provide an answer to the extinction of many organisms. There was no more need to invoke biblical floods; natural selection would gradually and almost without perception to the contemporary eye explain extinction. Darwin had tailored natural selection as a gradualist agency to answer the problem of reversion. In reality, however, there is no necessary reversion to type and there are no aboriginal forms as specified in divine Providence.

“Natura non facit saltum” was, nonetheless, embraced by Darwin, securing it to a natural selection that operated by small steps and disregarding hybridisation as a potent force for evolutionary change. In fact, he even went as far as saying later in his life the following –

> It throws light on the origin of the two sexes and on their separation or union in the same individual and lastly on the whole subject of hybridism, which is one of the greatest obstacles to general acceptance and progress of the great principle of evolution. (Darwin 1876)

Notwithstanding, Darwin was aware that in the plant kingdom new species or varieties could arise from natural hybridisation, but he considered these to be very rare events and unlikely to provide a clear mechanism of evolution. It is important to note that Darwin saw hybridisation as an unviable alternative to natural selection and not something subsumed under it. Darwin’s view was to have a profound effect on evolutionary theory to this day.

Plant Hybridisation

If there ever was a myth in evolutionary biology it was that hybridisation was not a significant factor in the evolution of new forms and species. Although biologists have recognised the occurrence of hybridisation in plants since the 18th century, its importance has been greatly underestimated until fairly recently. It may surprise many that familiar fruits and vegetables such as strawberries, grapefruits, sweet oranges, plums, tomatoes, wheat, maize, rice and potatoes are all hybrids. In fact, researchers now believe that up to 80% of all extant land plants underwent hybridisation sometime in their ancestry.[134] Moreover, much of the agricultural produce one finds on the supermarket shelves or the flowers one sees in the garden centres are the products of human manipulation using hybridisation methods. They allow farmers and researchers in horticulture to duplicate and fast-track naturally occurring processes in their plant breeding efforts. What exactly are those processes? To begin with, it is important to know the difference between two different forms of polyploidy, *autopolyploidy* and *allopolyploidy.*

Polyploidy

Autopolyploidy

The term autopolyploidy comes from *auto* meaning 'self' and *polyploid* meaning 'many-fold'*.* 'Manyfold' refers to the number of chromosomes. The number *n* refers to the number of copies of each chromosome in the *gamete* (reproductive cell) of the plants listed (see box 1). For example, cultivated bananas are triploid (3n), which means they have three copies of each of their chromosome. Many of the examples in the box below are artificially induced, usually in order to increase the size of the fruit or the plant. Generally, the more chromosomes there are in a cell, the larger the cell size, and often it follows that the overall structure of the plant increases too.

In the most common form of sexual reproduction, the chromosomes of the individual parents are divided and each parent provides half of the chromosomes to the offspring, whose chromosome count remains the same as the parents'. In autopolyploidy, the whole genome can be found multiplied in the offspring of sexually reproducing parents of the same species. Autopolyploidy takes place when the chromosomes are not divided and instead are passed on whole to the offspring, thus doubling the offspring's genome count. Sometimes the number of chromosomes can be multiplied more than twice; hence, plants that have a tripled chromosome number

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[134] Dan Graur. 2016, Evolution by Polyploidy.
nsmn1.uh.edu/dgraur/molevol/fall2010/slides/6a4_polyploidy.ppt

are triploid. If the multiplication is four times, then the plants are tetraploid and so forth.

Not all plants are *angiosperms* (flowering plants), of course, and in evolutionary history they were preceded by *gymnosperms* (non-flowering plants) by 200 million years. It has been estimated that gymnosperms such as the ferns and conifers arrived 360 million years ago. Nonetheless, even in the asexual reproduction of gymnosperms, the genome count can be multiplied. Some ferns have up to an amazing 100 copies of their chromosomes.

Examples of Autopolyploidy

1. Haploid (one copy, n). Haploids are found in seedless plants such as mosses but are also found in some algae, male bees, wasps and ants.
2. Diploid (two copies, 2n). Most common form of sexually reproducing organisms.
3. Triploid (three copies, 3n). Examples are mostly cultivated bananas, apples, ginger, citrus and seedless watermelons. Triploids generally do not have seeds and cannot reproduce.
4. Tetraploid (four copies, 4n). Examples are wheat, maize cotton, potato, durum, cabbage, leek, tobacco and peanut.
5. Pentaploid (five copies, 5n). Pentaploid organisms will generally be sterile.
6. Hexaploid (six copies, 6n). Examples are kiwifruit, oat and bread wheat (*Triticum aestivum*)
7. Heptaploid (seven copies, 7n). An example is the boysenberry.
8. Octaploid (eight copies, 8n). Examples are the most common variety of cultivated strawberry (*F. × ananassa*), sugar cane and pansies.
9. Nonaploid (nine copies, 9n). An example is the Japanese persimmon (*Diospyros kaki*)
10. Decaploid (ten copies, 10n). An example is the wild strawberry of the genus *Fragaria*.
11. Ferns can have a chromosome number of up to 100n.

Box 1.

Allopolyploidy

Although autopolyploidy is an important factor in speciation and diversity, it does not result from hybridisation; hybridisation comes with allopolyploidy. Allopolyploidy is the chromosomal condition in which the genome is doubled when undivided chromosomes are received in an offspring of parents from two distinct species. *Allo* means 'other' or 'different', the rest of the term follows the above for polyploidy. How then do plants undergo this form of chromosome duplication? Well, this condition happens frequently in nature but also results from human endeavours in horticulture, where the natural processes are given a helping hand to speed things up. In nature, plants can cross-fertilise each other even when the male pollen from one species or variety is transferred to a plant *ovule*, the female egg or germ cell of a different species or variety. The transfer of pollen can occur in several ways; by insects moving from plant to plant, by the wind, by water and by birds and bats. Normally, successful fertilisation happens between neighbouring plants of the same species, but the vectors that carry the pollen are not bound by this and pollen is sometimes transferred to unrelated species. The resulting cross between different species is an *interspecific* hybrid. Gardeners are usually aware of how 'promiscuous' plants can be when they find new varieties amongst their existing garden flora. Crosses are most likely where the contributing plants are closely related genetically and are in close proximity to each other. When two plants hybridise and the hybrid's chromosome number is doubled, the offspring is fully fertile and the process is referred to as *amphiploidy*. One very important hybrid that has benefited human nutrition is bread wheat (*Triticum aestivum*), which is a composition from three different diploid species of goatgrass (genus *Aegilops*) through a tetraploid intermediary durum wheat (*Triticum durum* or *Triticum turgidum* subsp. *durum*). Another important example is oilseed rape (*Brassica napus*) the amphiploid species that resulted from a cross between *Brassica oleracea* and *Brassica rapa* and from which Canola oil is produced. From an evolutionary perspective, such hybrids prove to be highly sustainable and display 'hybrid vigour'. This has attracted the attention of horticulturists, who mimic the process in domestic plant breeding in order to produce commercially more valuable plants.

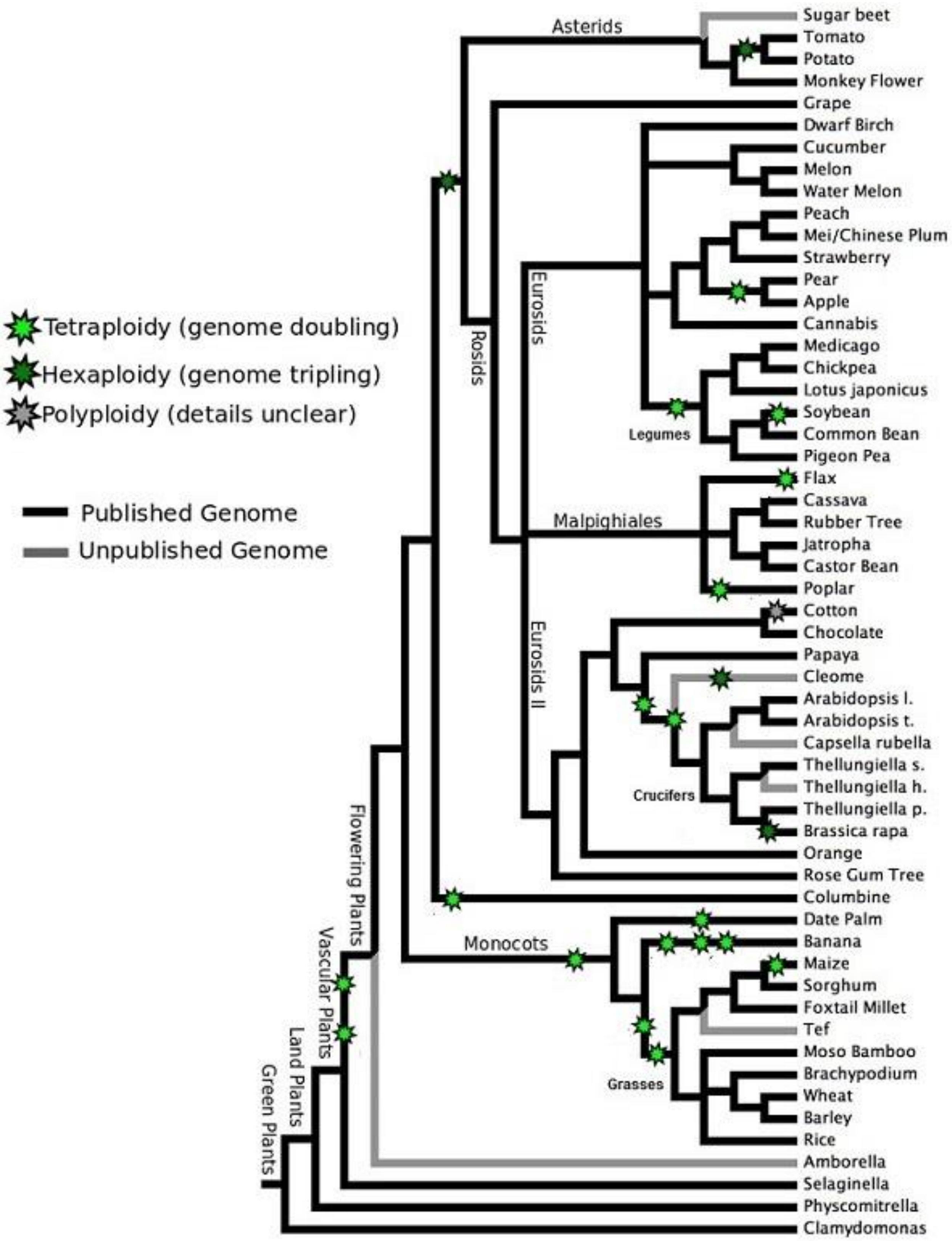

Figure 27. Phylogenetic Tree of Plants. The stars indicate where the whole genome is doubled or tripled.
(Adapted from https://genomevolution.org/wiki/index.php/Plant_paleopolyploidy)

The diagram above (figure 21) shows just how common polyploidy has been in evolution. With the recent developments in genome analysis, it is much easier to see the relationships between organisms and the gene count in historical terms. Allopolyploidy is more common in nature than autopolyploidy, where approximately 80% of all land plants may be allopolyploids.[135] This demonstrates the importance of the two types of polyploidy to evolutionary theory.

Hybrid vigor has been perceived as something of a mystery. Why do some hybrids outgrow their parents and produce larger seeds? Recent research on the arabidopsis plant (*Arabidopsis thaliana*) has revealed that *transcriptional repressors* are more repressed during the day in both hybrids and polyploids. These repressors are responsible for the regulation of circadian clocks which allow the plants to photosynthesise more effectively.

> The key, Chen and his colleagues studying Arabidopsis plants found, is the increased expression of genes involved in photosynthesis and starch metabolism in hybrids and polyploids. These genes were expressed at high levels during the day, several-fold increases over their parents. The hybrids and polyploids exhibited increased photosynthesis, higher amounts of chlorophyll and greater starch accumulation than their parents, all of which led to their growing larger.[136]

Fungi and Polyploidy

The importance of fungi in symbiosis with either plants, bacteria, algae or animals was demonstrated earlier. Fungi have also undergone both autopolyploidy and allopolyploidy, as can be seen on the diagram (figure 22).

> Although fungal polyploidisation has been long illustrated solely through yeast WGD, there is other evidence indicating that polyploidy has played a preeminent role in the evolutionary history of the fungi kingdom, as it has in plants and animals. It is highly probable that the non-exhaustive list of past and recent polyploidisation events presented here will increase greatly in the future because until now fungi are less studied than plants and animals.[137]

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[135] http://www.slideshare.net/zohaibhassan581187/allopolyploid
See also Gregory.T& Mable. B. 2005, *The Evolution of the Genome,* The Academic Press, Elsevier: Netherlands.

[136] Chen et al. http://www.sciencedaily.com/releases/2008/11/081123150255.htm

[137] http://rspb.royalsocietypublishing.org/content/early/2012/03/29/rspb.2012.0434Warren Albertin, Philippe Marullo. Polyploidy in fungi: evolution after whole-genome duplication. Proc. R. Soc. B 2012 -; DOI: 10.1098/rspb.2012.0434. Published 4 April 2012

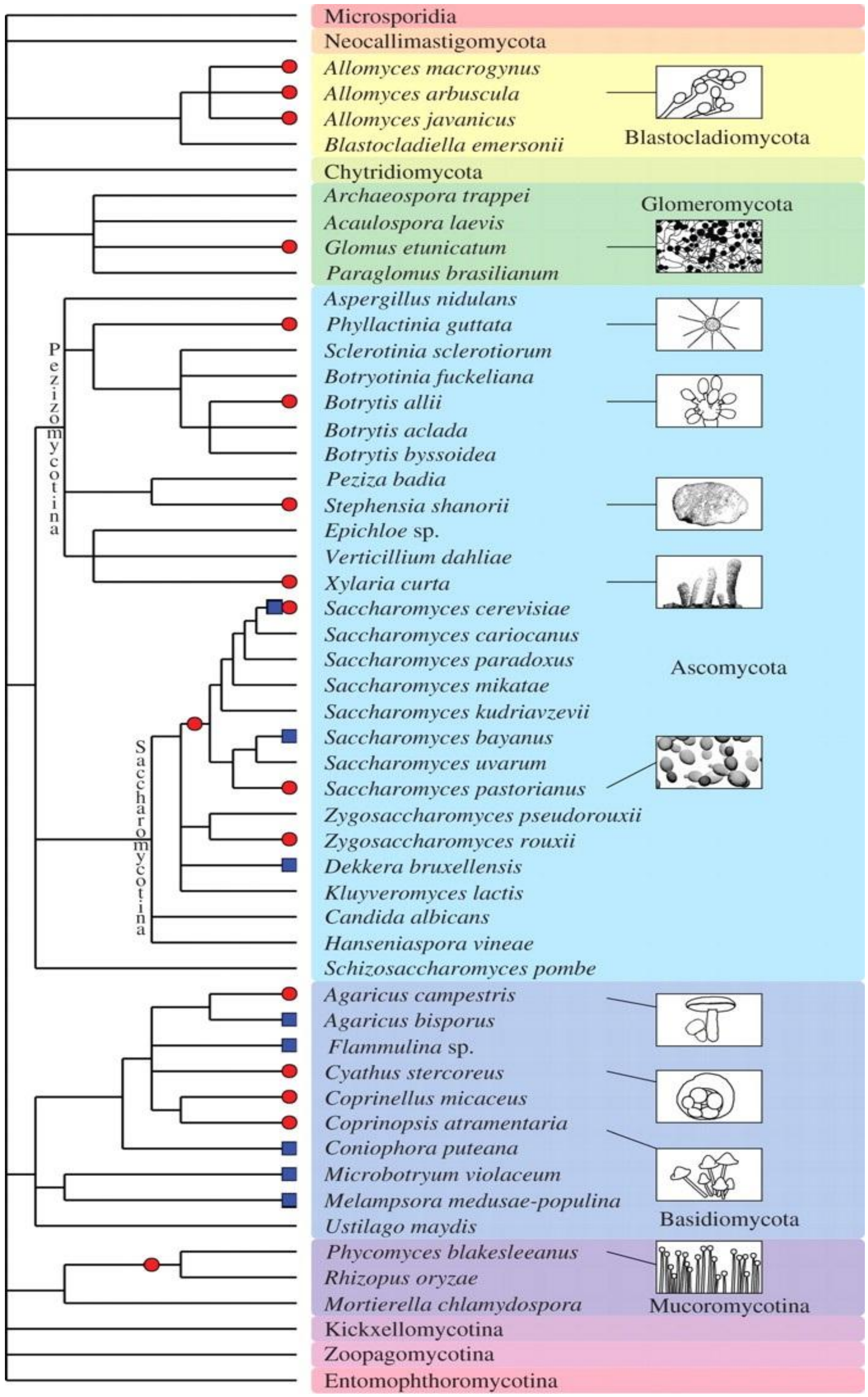

Figure 22. illustrates where there are suspected polyploidy events (red circles) and where there are hybridisation events (blue squares). Source: rspb.royalsocietypublishing.org/content/early/2012/03/29/rspb.2012.0434

Attitudes to hybridisation have altered greatly over the centuries, take, for instance, the potato. This hybrid is today one of the most popular vegetables, especially in the western world, with millions of tonnes consumed each year. The desire for French fries and crisps seems indefatigable, however, it was not always like this. When in the 16th century the potato was brought from the South American Andes and introduced to the Europeans, it was considered by many with suspicion. In fact, it was commonly thought that the potato was the creation of the devil, as it was not a vegetable that was ever mentioned in the bible. Its irregular shape and the susceptibility to blight were seen as evidence of a degenerate produce that should not be eaten. Travellers returning from the New World often carried diseases and the potato was believed to be the culprit. Peasants were reluctant to grow them and where they did, they were held to be a food fit for pig and cattle only. During a famine in Naples in 1770s, those afflicted even refused to eat a relief load of tubers that had been sent as a gift. Over time, the potato gradually gained acceptance but only by the working classes at first. Denis Diderot (1713-84), the French philosopher and writer thought that the potato might produce flatulence and should be avoided by the upper classes with the finer palettes. However, when it came to the peasantry he had no concern and said, "What matters windiness for the vigorous organisms of peasants and labourers?"

Many plants are subject to photoperiodism and the length of daylight with some responding to long days and others to short days. The problem with the potato plants imported from the Andes was that they did not like the shortening of days and the coming of autumn in the European countries of the higher latitudes. There was a desire, therefore, to discover other varieties that were better adapted to northern hemispheres. This desire was given greater motivation after the infamous Irish Potato Famine (1845-1851), which killed over a million people and caused another million to emigrate. The crop failure was caused by a fungus (*Phytophthora infestans*), originally introduced to Ireland via the holds of ships travelling from North America to England. At the time, some religious believers thought the blight to be an act of divine punishment for the sins that the affected people had committed. Some, unbelievably, saw the affliction as a blessing-

> In England, religious-minded social reformers viewed the blight as a heaven-sent 'blessing' that would finally provide an opportunity to transform Ireland, ending the cycle of poverty resulting from the people's mistaken dependence on the potato.[138]

The main problem was that the poor Irish peasants were dependent on one variety of potato, the Lumper, which matured late in the year in September or October. This particular variety was well suited to the damp conditions in the south of Ireland but

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[138] http://www.historyplace.com/worldhistory/famine/begins.htm

unfortunately, its susceptibility to blight was its failing. Inopportunely, at that time, there were no viable alternative varieties available.

Attitudes to Hybridisation in Horticulture

Hybridisation was not a European invention, in fact, it was recognised and utilised long ago in South America for crossing corn varieties. Of course, hybridisation in horticulture is not an invention at all but just an act of copying what happens in nature. The naturally occurring hybrid plants were, nevertheless, a problem for the majority of people steeped in religious belief and the teachings of the bible. It was commonly held that all living things were created by the good Lord, for the benefit of man. These creations were fixed in time and immutable, and the belief was often called the ‘constancy of species’. In other words, after the Lord’s creation there could be no new species arriving. Linnaeus certainly began his career in taxonomy with this belief in ‘constancy’. However, in later life, this was to change. As he went about his meticulous categorisation of flora, fauna and minerals, he observed that new plants had come into existence and he was certain that they had not previously existed. In the early editions of *Systema Naturae*, Linnaeus had frequently written of nature that it brings forth "n*ullae species novae*", which means ‘no new species’. In the twelfth edition, however, he removed the phrase and instead said that God had provided the original species but since then, new species had arisen through hybridisation. This is an important revision to his beliefs and something still not widely appreciated today.

Needless to say, this view was not popular at the time and Linnaeus was admonished by the clergy. Nevertheless, others would come to realise that Linnaeus was correct and new species of fertile plants did come into existence, sometimes with hybrid vigour.[139] Farmers and gardeners began to notice that within a field of plants of mixed varieties new forms could emerge that might bear desirable characteristics. Hybrids can often be larger in form, more striking in colour and sometimes enjoy good reproduction rates, hence the application of the term ‘vigour’.

Attributes that can overcome religious dogma are the desire to feed oneself and the desire to make a profit. Discovering a hybrid that produced a higher yield or was less susceptible to disease or unfavourable climatic conditions was of benefit to all and in particular to those who could produce it and sell its seeds. As a consequence, processes of human selection followed by taking potential hybrids and breeding them

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[139] Today, scientists use the term ‘heterosis’, which indicates that the hybrid offspring does not share many of the characteristics of either parent. If the growth of the offspring exceeds that of the parents it is a case of ‘positive heterosis’ but if the growth is diminished in the offspring then it is referred to as ‘negative heterosis’.

through several generations for viability. These were painstaking operations and were executed largely by trial and error. There was also a clash between what the horticulturists 'ought' and what the consumer sought. Consumers, when it comes to fruit and vegetable, desire a symmetrical shape without irregularities or 'degenerative' form. Moreover, consistency and depth of colour are also desired. Hybrids, however, did not necessarily fit the consumers' criteria though to the producer, hybrids were attractive options as they could be higher yielding, better adapted to the prevailing climatic conditions and more resistant to disease. The ideal hybrid was the one that satisfied all these desired characteristics, but this was unlikely and compromises had to be made. Instead of leaving it up to nature to produce new hybrid forms, horticulturists began to select varieties and intervene by helping along the cross-pollination processes. What was normally done by insects, the wind and other natural ways of transmission could be supplemented and controlled by humans. Below is a sample list of well-known plants, all of them hybrids-

The strawberry (*Fragaria × ananassa*) is a widely grown hybrid species of the genus *Fragaria*

The clementine (*Citrus × clementina*) is a hybrid between a mandarin orange (*Citrus reticulata* and a sweet orange (*Citrus × sinensis*)

The plum (*Prunus × orthosepala*) is a hybrid of *P. americana* × *P. angustifolia.* The genus *Prunus* contains *a*pproximately 40 species.

The orange is a hybrid between a pomelo (*Citrus maxima*) and a mandarin (*Citrus reticulata*). It has genes that are one-quarter pomelo and three-quarter mandarin.

The orange (specifically, the sweet orange) is the fruit of the citrus species *Citrus x sinensis* in the family Rutaceae

The grapefruit (*Citrus x paradisi*) is a hybrid between a sweet orange (*C. sinesis*) and a pomelo or shaddock (*C. maxima*)

The loganberry (*Rubus* × *loganobaccus*) is a hexaploid hybrid produced from pollination of a plant of the octaploid blackberry cultivar 'Aughinbaugh' (*Rubus ursinus*) by a diploid red raspberry (*Rubus idaeus*)

Peppermint is a hybrid between spearmint (*Mentha spicata*) and water mint (*M × piperita*), also known as *M. balsameawilld.*

There was a financial reward for anyone who discovered or cultivated a hybrid and then sold on the seed, but not everyone accepted this form of research, development and profit. To some religious people, these actions were against the teachings of the bible and were turning God's garden into a brothel. Nevertheless, some important crosses were made to improve staple vegetables such as corn and potato. The Lumper potato was succeeded by hybrid potatoes that were selected for maturation earlier in the year. In the USA, William James Beal cultivated hybrid corn in 1876 and Robert Reed and son James continued this work, producing a golden colour maize, which had more than double the number of rows of kernels per ear compared to the original corn grown by the indigenous Indians. The hybridisation of rice, another staple food crop, was not achieved, however, until 1966 in Maoist China but then resulted in a 30% increase in yield. Not surprisingly, by the late 1970s, this hybrid was cultivated worldwide. Attempts to hybridise wheat commercially have been less successful, probably because it is a cereal that self-pollinates very effectively. It may require the labour intensive breeding of several generations of progeny before a suitable seed is produced and this adds, of course, to the final costs.

Nowadays, due to the decline of religious influence in the West and in China, there is very little concern about the consumption and use of hybrid plants. Indeed, it would be difficult to know what to eat if one were to look for ‘pure’ or ‘aboriginal’ species. In fact, one can never be sure that a plant has not undergone a hybridisation event sometime in its distant history, although it is probable that very few people realise that nearly all the plants we consume are the result of ancient natural hybridisation or more recent commercial crossings. Humans have evolved with the consumption of plant foods, but of greater concern these days is the processing of foods and the added sugars, fats and chemicals. There is also disquiet over genetically engineered foods, but that is not hybridisation and therefore not a topic within the bounds of this work.

Hybridisation in the Animal Kingdom

Polyploidy in Animals

The occurrences of autopolyploidy and allopolyploidy that were earlier discussed are not exclusive to the kingdom Plantae and the kingdom Fungi, they also feature within the kingdom Animalia. Although polyploidy is not an uncommon manifestation within the kingdom Animalia, it is thought to be rare in birds and mammals. The true extent of it is not yet fully realised and until the 1990s was not considered to be of evolutionary significance. Now that the sequencing of genomes is possible and not prohibitively expensive, one can more clearly see the importance of polyploidy in speciation and the increase in the diversity of life forms. When an organism undergoes polyploidy, the cells of the polyploid organism contain multiple copies of genetic material and this has huge consequences for the organisation of the cell, the eventual production of proteins and the viability of gametes. Many questions have arisen concerning epigenetic influence over gene expression, genomic rearrangement and the loss of gene fragments over several generations of the polyploid. Furthermore, there is the fundamental question that asks, what are the factors that trigger polyploidy in the first instance? This is a new area of research and the casual chains and interactions are not yet understood. The relatively limited research into the subject of polyploidy has revealed that it is present in -

Invertebrates such as
Beetles (Coleoptera)
weevils (order Coleoptera, family Curculionidae)
flies (Diptera)
webspinners (Embioptera)
planthoppers (Homoptera)
bees and sawflies (Hymenoptera)
moths (Lepidoptera)
cockroaches (Orthoptera)
bush crickets (Tettigoniidae) [140]
common house spiders (Arachnida)
corals (see also below)

Vertebrates such as
ray-finned fishes (Actinopterygii)
paddlefish and sturgeons (Acipenseriformes)
silversides (Atheriniformes)
suckers, loach, carp and minnows (Cypriniformes)
livebearers and mollies (Cyprinodontiformes)
lungfish (Lepidosirenformes)
gar (Lepisosteiformes)
walleyes (Perciformes)
salmon, trout and char (Salmoniformes)
catfish (Siluriformes)*Fish*

Amphibians
frogs and toads (Anura)
salamanders, newts and sirens (Caudata)

Reptiles
geckos, lizards and snakes (Squamata)
twist-necked turtles (Chelonia)

Birds
chickens and quails (Galliformes)
blue and yellow macaw (Psittaciformes)

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[140] www.zoology.ubc.ca/~otto/Reprints/OttoWhittonWebTable.pdf. Accessed2016

Mammals
red viscacha rat (Rodentia)

The Strange world of the Coral

It was witnessed earlier that many reef-building corals have a shared existence with photosynthetic algae called zooxanthellae that live within the corals' tissues. Moreover, a sign of their flexibility is their ability to reproduce sexually or asexually. With sexual reproduction, it is thought to be possible that sex cells can receive, through transfer, mutations that exist in the *somatic* or body cells. These cancerous growths are called *neoplasms*, which mostly consist of a large number of polyps, and it is believed that these growths may lead to the formation of new species. This is not all. Corals can also form a new colony by means of hybridisation, in which eggs from two or more different origins fuse together after fertilisation. Like bacteria, corals are very difficult to categorise into distinct species because, in addition to their symbiotic relationship with algae, there are fuzzy boundaries and much overlapping.

> In most major coral regions, not only do different colonies of the same species synchronise their spawning, but colonies of different species have the same synchrony. The outcome, in many regions, is 'mass' spawning. When mass spawning occurs, the ocean surface becomes a soup of genetic material creating endless possibilities for cross fertilisation. The extent to which hybridisation occurs – that eggs of one species are fertilised by the sperm of another – is not known. What is known is that different species within the same genus (and rarely between species of different genera) can readily hybridise and that the progeny can be normal-looking corals.[141]

Hybrids and Fertility

> Mating between the rare Californian tiger salamander and the introduced barred tiger salamander has created a monster –at least for animals that dwell in the ponds of California's Salina River Valley.[142]

Monster Hybrids? This contradicts common wisdom. Aren't all hybrids inviable or sterile like the mule?

The mule, the iconic hybrid all are familiar with, is a cross between two equine species, a male donkey (*Equus asinus*) and a female horse (*Equus caballus*). The offspring of the reciprocal cross between a female donkey and a male horse is a hinny. Mules are

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[141] Vernon, J.E.N. 2016 http://coral.aims.gov.au/info/reproduction-sexual.jsp
[142] http://news.nationalgeographic.com/news/2009/06/090629-salamanders-hybrid.html

larger and stronger than donkeys and have more endurance than horses of equal size. They have been bred for this particular combination of traits for thousands of years. In the oldest western literature, Homer's epic poems the *Iliad* and *Odyssey,* thought to have been written in the 8th century BCE, mules are part of everyday life. They are also mentioned in the Hebrew Scriptures but had to be bought from non-Jews because Mosaic Law forbids the production of hybrids. The mule is an exceptionally sterile hybrid, a fact of nature that is reflected in an ancient Roman proverb "cum mula peperit". It means 'when a mule foals' and was used whenever something was not expected to happen. People today base their ideas of hybrids on what they know about the mule and therefore, they commonly but erroneously believe that all hybrids are sterile.[143] Evidently, this is not even true in the case of the mule. There have been reports of scientifically undocumented cases of mules giving birth. However, in China, where they are bred extensively for their versatility and hardiness, a female mule and a female hinny, whose hybrid status could be verified by chromosomal investigation, produced each a filly foal after mating with a donkey.[144] No cases are recorded about fertile male mules, however.

A major underlying cause for hybrid non-viability or sterility in animals is a mismatch in parental karyotype; the mule is a good example. Each animal species possesses its characteristic number of chromosomes that differ in size and shape from other species and, at certain times in the cell cycle, are arranged in matched up pairs. For instance, humans have 23 pairs of diploid chromosomes for a total of $n = 46$, of which 23 came from the mother and 23 from the father. During the process of meiosis when gametes are formed, the diploid chromosomes recombine and then divide, and each egg cell and each sperm cell is left with a single copy or haploid version of the chromosomes. When male and female haploid copies of chromosomes meet at the moment of fertilisation, they match up and the developing embryo winds up with the complete diploid set of chromosomes, except, this is not what happens when a mule is conceived. Horse and donkey chromosomes differ in number. The horse has $n = 64$ chromosomes, so the egg cell carries 32 while the donkey has 62 chromosomes, of which 31 are contained in each sperm cell. In addition, being of different species, the chromosomes not only differ in number, they also differ in the way the genetic instructions are arranged along the chromosomes. As a consequence, horse and donkey chromosomes don't match up properly. Because the extra chromosome that came from the mother cannot make a *homologous* pair (corresponding in relative position or structure) at meiosis, the process is disrupted and the hybrid will be infertile. In the case of the mule, the extra chromosome may not be of special concern

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[143] http://www.macroevolution.net/mules-donkey-horse-hybrids.html access date 30/3/16

[144] http://www.ncbi.nlm.nih.gov/pubmed/3378453 abstract

in respect to its overall well being, but in humans, for example, an extra chromosome like in Down's syndrome and Edward's syndrome can cause serious health problems.[145]

The sex bias of absent or diminished fertility in male mammalian and female bird hybrids was observed by the British evolutionary biologist John Burdon Sanderson Haldane (1892 – 1964) and formulated as Haldane's rule for hybrid sterility, which says: "[w]hen in the F1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex" (the sex that has two different sex chromosomes like XY in the mammalian male). The same bias is also seen in backcrosses.[146]

Human-Bred Hybrids

Since the earliest beginnings of farming, man has domesticated not only wild plants but also animals and in the process, he has crossed different species to produce hybrids with new traits, a practise that is ongoing. Several of today's farm animal species are themselves the unsuspected descendants of hybrids. Among them is the domesticated chicken, whose Asian origin is a cross between the red jungle fowl with the gray jungle fowl; the latter contributed the genes for the yellow colour of the skin.[147] The bovine family is another example. There is strong archaeological and genetic evidence attesting to several independent domestication events of *Bos promigenius*, the wild auroch of Asia, Africa and Europe, that resulted in *Bos Taurus* in the near East and Africa and in *Bos Indicus* or Zebu in the Indus Valley. Investigation of genetic variation in modern cattle has revealed that they all descend from either the Asian Zebu or the African or European taurines or are hybrids of both. On the African and European continents, cattle show either *introgression* (gene flow) from the respective native auroch or they are the descendants of a cross between the taurine and the now extinct auroch.[148] Today's cattle is further crossbred with its own kind and with other species for traits such as increased meat or milk production, endurance, disease resistance or heat and cold tolerance. For example, in the United States, breeders have crossed domestic cattle with the American bison, also known as buffalo, to produce the 'beefalo'. First filial generation (F_1) hybrid males generally don't produce sperm. However, it was also noted that sperm from one cross could be virtually normal, while another cross's *spermatozoa* (sperm cells) were few and abnormal in structure. F_1 females, on the other hand, are partially fertile and when

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[145] http://genetics.thetech.org/ask/ask225

[146] E.M. McCarthy, 2008, On the Origins of New Forms of Life, A New Theory, http://www.macroevolution.net/support-files/forms_of_life.pdf footnote a), p.38

[147] https://www.sciencedaily.com/releases/2008/02/080229102059.htm

[148] http://onlinelibrary.wiley.com/doi/10.1002/%28SICI%291520-6505%281998%296:3%3C79::AID-EVAN2%3E3.0.CO;2-R/abstract

backcrossed with taurine cattle, males of the resulting backcross (BC_1) produce sperm, but spermatozoa may be abnormal in size and shape. With subsequent backcrossing, though, male fertility will increase.[149] Beefalo meat is said to be leaner and has a lower cholesterol content than regular beef meat. Furthermore, in the northern U.S.A., beefalo herds will graze in open winter range, which is not suitable for common cattle breeds.

The Bizarre Story of an Auroch

In 2009, a farmer in Devon, England, imported from Belgium 13 cattle for breeding/to breed as a conservation project. Things did not, however, turn out quite as the conservationist Derek Gow had planned. Although he did manage to breed a further seven calves, he eventually had to send six of his stock to slaughter because of their aggressive behaviour. This is what he said to the Guardian newspaper-

> The ones we had to get rid of would just attack you any chance they could. They would try to kill anyone. Dealing with that was not a lot of fun at all. I have worked with a range of different animals from bison to deer and I have never come across anything like these. They are by far and away the most aggressive animals I have ever worked with. Some were perfectly calm and quiet and they are the ones we have kept. The others you could not go near. We made sure no one went near them so there were never any incidents. To get them into the trailer to get them off the farm we used a young and very athletic young man to stand on the ramp and they charged at him before he quickly jumped out the way.[150]

What is the story behind the cattle that became known as the 'Nazi Cows' and where is its significance to hybridisation? The story begins with an extinct species known as the auroch. Earliest records suggest that these mighty, wild bovines lived at least 275,000 years ago and spread across the northern hemisphere from China to Britain and from St Petersburg to Northern Africa. These hardy creatures used to occupy the forests, fens, bogs and the areas along rivers and lakes, avoiding where they could, predation from wolves (figure 23). The paintings that were discovered in the ancient caves of Lascaux and Chauvet in France depict bovines that are considered to be representations of the auroch. Human expansion and hunting are believed to have led to their eventual extinction with last survivors remaining until 1627 in central Poland.

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[149] McCarthy E.M., On the Origins of New Forms of Life, A New Theory, Copyright © 2008 by Eugene M. McCarthy, Macroevolution.net, p.40

[150] (Gow,D. 2015, http://www.theguardian.com/world/2015/jan/05/devon-farmer-forced-offload-nazi-bred-super-cows)

Figure 23. Heinrich Harder's painting of an auroch bull being attacked by wolves. Source unknown.

During the 1920s, there was much interest in the philosophy of eugenics and it generated great optimism for what could be achieved with this then-fledgling science. The brothers Heinz and Lutz Heck were German conservationists and were influenced by Teutonic mythology. They decided to attempt a back-breeding project to see if they could bring back into existence creatures that figured within Teutonic mythology such as the auroch (*Bos primigenius*) and the horse (*Equus ferus ferus*). The programme attracted the interest of leading members of the Nazi Party, who had recently come to power. The aurochs were, indeed, a symbol of strength, independence and aggression, which fitted well with fascist ideology. If the scientists could recreate such a creature, it would be a combined triumph of German mythology, politics and modern science. Selecting the features that most resembled their understanding of what an auroch looked like, the brothers imported cattle from around the world and boasted that within twelve years, they would succeed in recreating the auroch. The progeny is now called 'Heck' cattle and can be found in a few protected conservation areas across northern Europe (figure 24) and, of course, at the farm of Derek Gow.

Figure 84. Heck cattle in a conservation park.

The leading question is, however, did the Heck brothers actually achieve recreating the animal through the process of selective hybridisation? Modern consensus seems to deny this for the most part. From what is known or can be inferred from fossil remains, old accounts, drawings and paintings, there could be several important differences.[151] For instance, the original aurochs were larger in size, their horns were of a different shape, and their skulls were longer and thinner. Moreover, it is believed that the modern Heck cow had a more pronounced udder than the auroch cow, and there are thought to be differences in the coat colour as well, with Heck cattle displaying much more diversity. In fact, it has been suggested that the Spanish Fighting Bull is more characteristic of the extinct auroch than the Heck bull, in spite of being an animal originally chosen by the brothers for their back-breeding programme. Nonetheless, one trait displayed in the Heck cattle that is possibly similar to the one in the aurochs is their aggressive nature.

Although the Heck brothers did not achieve what they set out to do, their endeavours do illustrate several issues concerning hybridisation. First, it is important to note that new and fertile species can be achieved in a very short period. Closely related higher order organisms can easily reproduce and add to the marvellous diversity in nature. Second, the lesson learnt is the unpredictability concerning the form and behaviour of

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[151] See T. van Vuure, History, Morphology and Ecology of the Aurochs (BOS PRIMIGENIUS) Nude 45, NL-6702 DK Wageningen, The Netherlands. (Undated)

the offspring produced by hybridisation. Why did the Heck brothers fail to achieve creating an auroch by back-breeding? Part of the problem lies in an overly simplistic view of the gene and how genes are expressed. The idea that there are genes *for* particular traits distorts the reality of a very complex series of interactions. Darwin was probably one of the first to recognise that, with artificial selection, one might breed successfully for one desired trait like a longer beak in a bird, for instance, only to discover that there may be unexpected correlated changes elsewhere in the phenotype of the progeny. The phenomenon is called *pleiotropy* and is only one of several factors in gene expression. It is important to know that genes or the products of gene expression can interact with each other. Take the textbook example of the horse. A particular horse may have dominant genes that express the proteins for a chestnut-coloured coat but they may not be expressed because of the presence of other genes like the 'cream dilution gene', which can alter the colour of the horse's hair to a more yellow or gold colour. When two alleles of this gene are present, the horse will be blue-eyed and have a lighter coat. There are other dilution genes such as the champagne, dappled, pearl and silver genes. All of them can impact on the phenotype of the horse and to cause even more complication, a mixture of different dilution genes can also be expressed, providing unpredicted outcomes in the phenotype.

Although an organism may have within its DNA a sequence of base pairs that normally codes for proteins, it does not follow that transcription will take place. It is now known that genes can be switched on or off by the presence of regulatory genes or by epigenetic markers that make genes context sensitive. For example, one individual gene can code for up to 38,000 different proteins[152]. These are just a few examples of what must be taken into consideration but they are by no means the least. The journey from the cell to the phenotype is a complex one and hybridisation introduces many variables that give results difficult to predict.

More Human-Bred Crosses

In Nepal, Tibet and Mongolia, domestic cattle have been crossbred with yak (*Bos grunniens*), another bovine species, since earliest times. In general, the hybrids grow faster and larger than yak and some of the local cattle, but when backcrossed with yak or cattle, they are smaller than the F_1 offspring. As they don't endure harsh conditions in high altitude as well as yak, they are kept at lower elevations. F_1 hybrids yield more milk than yak and most of the local cattle, though the milk has less fat content than pure yak milk. When it comes to backcrosses, they yield less milk than F_1 offspring,

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[152] Alberts B, Johnson A, Lewis J, et al. *Molecular Biology of the Cell*. 4th edition. New York: Garland Science; 2002.

however. As to hybrid fertility, F_1 males' semen does not contain sperm and it will take three to four backcrosses for the production of sperm to resume. Anyway, consecutive backcrossing is not practised much since sterile males have no value in livestock economy. On the other hand, F_1 female fertility is comparable to yak fertility and because they sexually mature earlier than yak, the overall reproductive rate is higher than in yak.[153]

In an effort to combine desirable traits of different species, sometimes even different genera in one animal, man has bred hybrid populations nearly everywhere in the world. In Southeast Asia and Central Africa, one finds crosses between the domestic pig (*Sus scrofa domesticus*) and the feral pig as well as serendipitous crosses between the domestic pig and the wild boar (*Sus scrofa*). In western Asia, the Bactrian camel (*Camelus bactrianus*) is crossed with the dromedary (*Camelus dromedarius*) and on the South American continent, one finds an intergeneric hybrid, the 'cama', a cross between the Asian dromedary and the South American llama (*Lama glama*). The llama is a domesticated guanaco (*Lama guanicoe*), which is the native camelid.

Goats (*Capra aegagrus hircus*) and sheep (*Ovis aries*) belong to different genera and have different chromosome counts; the goat has 60, the sheep 54. In spite of widespread sharing of the same pastures, they are believed not to hybridise naturally. When researchers attempted to cross these animals, the hybrid embryos either didn't develop or the foetuses were stillborn. Among the unsuccessful experimental pairs were a North African Barbary sheep (*Ammotragus lervia*) and a domestic goat. Eventually, though, using the same species pairing, renewed hybridisation attempts resulted in a healthy and partially fertile male offspring.[154] Since then, several cases of unintentional crosses between domestic goats and sheep have been reported in different countries. In one case, the 'geep' is the offspring of a sheep and a pigmy ram and is described as having a goat face and goat hoofs, while its body is covered in wool. In three other cases where female goats mated with male sheep, F_1 male hybrids were infertile, though hybrids of female sex were fertile and when backcrossed with rams, one hybrid produced two offspring, one dead and one live $B\ C_1$ male. Elsewhere, sexes were reversed and mating occurred between rams and female sheep. In a case in New Zealand, the male hybrid offspring had 57 chromosomes and when backcrossed with a female sheep, he sired two healthy BC_1 offspring.[155] In an experimental study, female goats received skin grafts from sheep and injections of male sheep *leukocytes* (white blood cells) and when subsequently mated with sheep, an increase in hybrid

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[153] http://www.fao.org/docrep/006/AD347E/ad347e0i.htm

[154] McCarthy E.M., On the Origins of New Forms of Life, A New Theory, Copyright © 2008 by Eugene M. McCarthy, Macroevolution.net, p.39

[155] https://en.wikipedia.org/wiki/Sheep%E2%80%93goat_hybrid#cite_note-4

embryo deaths was registered, which was assumed to be due to antibody development.[156]

Accidental and Natural Hybrids

There is a site on the internet that shows dozens of images of animal hybrids. Almost all are phantasmic creations of photo-shopping such as the half-gorilla half-elephant hybrid that reminds one of the half-man-half-horse centaur of Greek mythology; or the kitten in a squirrel coat. But some of them are genuine hybrids and are either human-bred like the ‘zorse’, a cross between a zebra (*Equus zebra*) and a horse (*Equus* caballus) or ‘accidents’ of close encounters in captivity like the’ liger’ the hybrid offspring of a lion (*Panthera leo*) and a tiger (*Panthera tigris*). Marine mammals are no exception and the ‘wholphin’ is an accidental intergeneric hybrid offspring of a bottlenose dolphin (*Tursiops sp.*) and a false killer whale (*Pseudorca crassidens*) who were kept in the same pool. The hybrid is intermediate between its parents in size, colour and shape and even in the number of its teeth; it has 66 teeth, while dolphins have 88 and false killer whales have 44. Wholphins are reported to also exist in the wild[157] where, so the saying goes, interspecific or intergeneric hybridisation events are extremely rare. In the case of the blue whale (*Balaenoptera musculus*) and the fin whale (*Balaenoptera physalus*), the existence of some hybridisation between them is, however, not in doubt. Several hybrids were caught and their hybrid status was confirmed by molecular analyses, as was also the case with the offspring of an Antarctic minke whale (*Balaenoptera bonaerensis*) and a common minke whale (*Balaenoptera acutorostrata*) from the Arctic waters. There is even a surprising but well-documented report of an intergeneric hybrid between a blue whale (*Balaenoptera musculus*) and a humpback whale (*Megaptera novaeangliae*), surprising when one considers the considerable difference in size and morphology between the parent species.[158]

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[156] McCarthy E.M., On the Origins of New Forms of Life, A New Theory, Copyright © 2008 by Eugene M. McCarthy, Macroevolution.net, p.39

[157] http://www.nytimes.com/2010/09/14/science/14creatures.html?ref=science&_r=1

[158] McCarthy, E.M. 2013, Mammalian Hybrids, www.macroevolution.net/mammalian hybrids.htlm, access date 6/4/2016

Is the platypus a hybrid?

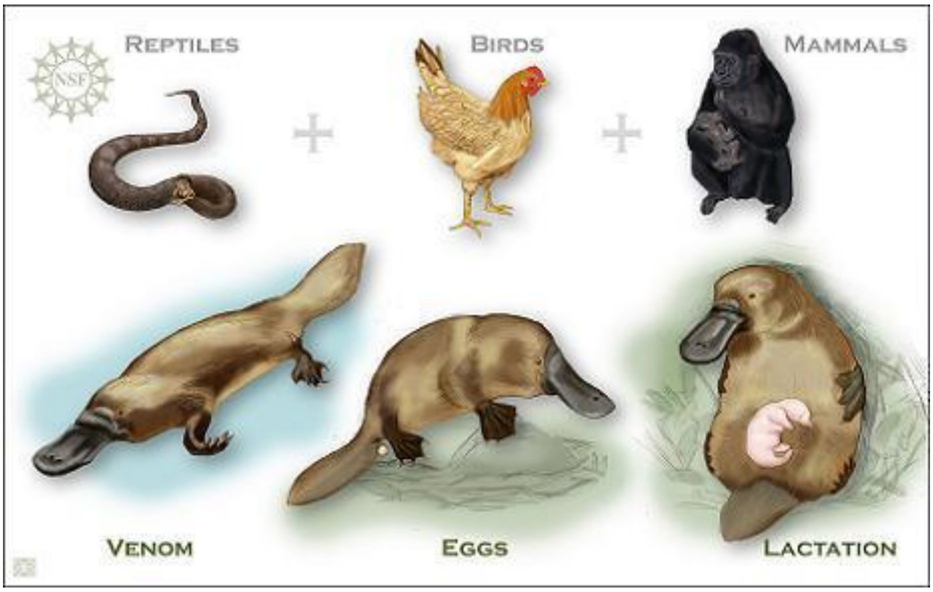

Figure 25. The Platypus and its unusual characteristics
Illustration by Zina Deretsky, National Science Foundation

Of the many aquatic and semi-aquatic mammals that exist, several breed across the species or even genus lines and produce hybrids but none produce offspring like the platypus, which looks as if it was assembled from the parts of several distinct animals. This furry creature has a tail that reminds one of a beaver; a pliable, leathery snout that resembles a duck bill; webbed feet with claws; and it lays eggs in the manner of a reptile but rears its young on milk (figure 25). Could this animal be a hybrid between a beaver and a duck?

The platypus is a semi-aquatic predator endemic to eastern Australia and Tasmania, where it is found in freshwater streams, rivers and lakes. Diving for food a few minutes at a time, it seals off eyes, ears and nostrils and finds its prey with the help of its electrosensory bill, which detects faint electric fields that are emitted by the animals it pursues. Underwater, it forages for molluscs, crustaceans, tadpoles and other little critters, scoops them up from the bottom, mud, gravel and all, brings them to the surface and then, using the sand and grit, grinds them up for consumption. The grit replaces the function of teeth, which the animal loses when it becomes an adult. Platypus dig their burrows at the water's edge, one for camping out and another where the female lays her eggs that she keeps warm until they hatch after about ten days. The blind and hairless infants are the size of lima beans and are nursed for three to four months until they can swim and hunt on their own. The female lacks nipples, so the young suckle milk from the specialised fur that surrounds the mammary glands from where the milk oozes through the skin. The platypus is also one of few venomous mammals. Males have a tiny spur on their hind limbs, which they use to deliver a poisonous, snake-like venom that can fend off competitors during the mating season. The venom will cause pain to humans but it is not deadly. In 2008, the genome of a female platypus was sequenced and subsequently, a consortium of scientists from eight different countries participated in studying this unlikely creature's genetic architecture by comparing it with that of humans, mice, dogs, opossums, chickens and one species of lizard.[159]

The scientific name for the platypus is *Ornythorhynchus anatinus*, which is a combination of Greek and Latin for 'bird snout' and 'duck-like' respectively. This unusual creature is the last living species of its family in the mammalian subclass of *monotremes.* Beside the platypus, monotremes include also the echidnas or spiny anteaters (Tachyglossidae) and represent one of the three major lineages of mammals that lay eggs as opposed to the other two lineages, the marsupials and the placental mammals that bear live young. Mammalian phylogeny is to a great extent decided on tooth morphology but adult platypus lack teeth and, therefore, much dispute used to exist about how the three lineages are positioned with respect to each other in evolutionary terms. Analysis of the platypus genome has settled the issue; monotremes split from the rest of the mammalian lineages about 200 million years ago (figure 26).

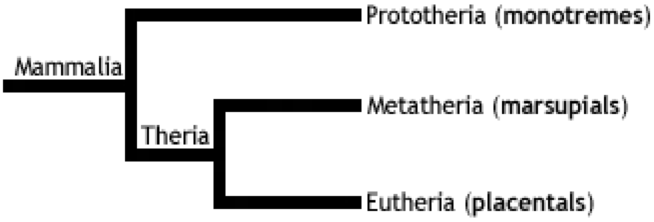

Figure 26. Mammalian phylogeny

Mammals evolved from a group of reptiles, the Synapsids that evolved into the Therapsids, which were mammal-like reptiles. Because of the early divergence of the monotremes from the reptiles, the platypus genome possesses a range of ancestral reptilian characters and is invaluable for tracing the evolutionary process from mammal-like reptilian, or should one say reptile-like mammalian, beginnings to having

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[159] http://www.nbcnews.com/id/24504461/ns/technology_and_science-science/t/scientists-decode-mixed-up-platypus-genome/#.VO_bq-mCzIUbbc.com/news/world

fur, producing milk and bearing live young.[160] Much insight is expected to be gained by knowing which of the genes have been conserved or accrued and which have been lost in the course of evolution from a reptilian to a mammalian existence. Of particular interest was finding features within the genome that illustrate reptilian and bird-like characteristics and those that depict mammalian ones. For example, researchers found genes for the production of egg yolk proteins that are shared only with reptiles and fish alongside genes that code for proteins that make up milk for lactation. The latter is one of two iconic traits of mammals and is thus proven to have evolved before the event of bearing live young[161].

Another unusual platypus characteristic is its complex *karyotype* (the number and appearance of chromosomes) consisting of 52 chromosomes, of which 10 are sex chromosomes. Like most mammals, the platypus has an X and a Y chromosome; however, they don't determine sex. In *therian mammals* (marsupials and placentals), sex is determined by an XX chromosome system in females and an XY chromosome system in males, with the SRY gene on the Y chromosome affecting male sex determination. By the way, the naming of the chromosomes is only coincidental with the fact that right before cell division, the otherwise linear chromosomes condense for a brief moment into what roughly appears to be XX and XY shapes. Sex determination in *amniotes*[162] such as the monotremes, birds and some reptiles is different from that in mammals. In birds, for example, the sex chromosomes are reversed. The female sex is determined by the XY chromosomes while the male sex is determined by the XX chromosomes. In order to avoid confusion, the chromosomes are, therefore, arbitrarily named W and Z with the female sex being the ZW and the male being the ZZ system.

As monotremes are phylogenetically the most basal group of mammals, they are well suited for determining how the therian XY system evolved. Comparative mapping shows that there is no homology between the platypus and the therian X chromosomes but that platypus sex chromosomes have instead strong homology with bird sex chromosomes. This implies that the therian X and Y sex chromosome system, and with it the SRY gene, evolved after the divergence of monotremes from the mammalian tree.[163]

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[160] http://www.ucl.ac.uk/museums-static/obl4he/vertebratediversity/monotremes.html

[161] Norris S., 2008, Platypus Genome Reveals Secrets of Mammal Evolution, National Geographic News, http://news.nationalgeographic.com/news/2008/05/080507-platypus.html

[162] From Greek denoting the membrane that surrounds the developing foetus seen in reptiles and birds that lay their eggs on land and mammals that retain their fertilised eggs within the mother.

[163] Veyrunes F. et al, 2008, Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes, Abstract, Genome Research, 18(6) at https://www.researchgate.net/publication/5388939_Bird-like_sex_chromosomes_of_platypus_imply_recent_origin_of_mammal_sex_chromosomes

Climate change is thought to be behind another confirmed natural hybrid, the 'prizzly', a rather rare cross between a polar bear (*Ursus maritimus*) and a North American brown bear or grizzly (*Ursus arctos* ssp.), whose habitats overlap when polar bears spend more time on land waiting for the ice to form. The animal that was shot by a hunter in the Canadian Arctic had brown patches in an otherwise white coat, the hump of a grizzly bear and long claws.[164] Some experts believe that one may see more hybrids in future as polar bears are being driven from their habitat because of Arctic warming.

In North America, well-documented natural hybridisation is ongoing between the mule deer (*Odocoileus hemionus*) and the white-tailed deer (*Odocoileus virginianus*), which has resulted in a hybrid population that extends from Canada all the way down to the American state of Texas in the south. Investigation in hybrid sperm production found that F_1 male hybrids that had been sired by a buck produced mature spermatozoa, albeit in a lesser amount than their parent and showing many abnormalities, while when born from a reciprocal cross they produced no spermatozoa at all.[165]

Caribou (*Rangifer tarandus*), in Europe known as reindeer, are the only members of the deer family (Cervidae) in which both sexes grow antlers. Earliest fossil evidence for their presence in North America dates from 1.3 - 1.8 million years ago. Being well suited to harsh winter conditions, their ranges cover the northern regions of North America, Europe, Asia and Greenland, where they are sustained by ground and tree lichens during the long winter months. Like most herd animals, many caribou populations migrate with the seasons. In spring, pregnant cows head for their calving grounds and the first flush of nutritious forage. Followed by the rest of the herd, they steadily move northward in search of new grazing areas and to escape the harassment of biting mosquitoes and flies. The onset of cold temperatures in fall dictates the beginning of the fall migration southward again.

The separation of caribou herds by a massive glacial ice sheet during the late Pleistocene resulted in two highly diverged lineages, the Beringian-Eurasian and the North American lineage and the emergence of two of the three subspecies that are recognised today. The migratory barren-ground caribou (*Rangifer tarandus groenlandicus*) inhabited the tundra in the north, while the *R. t. Caribou* followed a sedentary lifestyle in the boreal forest and mountains of western North America to the south. A study by researchers at the University of Calgary in Canada has shed light on the Rockies' present-day woodland caribous' ancient ancestry and discovered that

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[164] http://www.nytimes.com/2010/09/14/science/14creatures.html?ref=science&_r=1

[165] McCarthy E.M., On the Origins of New Forms of Life, A New Theory, Copyright © 2008 by Eugene M. McCarthy, Macroevolution.net, p.34

they are the relics of interbreeding between the northern tundra and the southern woodland subspecies. When after the last glacial maximum the arctic ice cap began to recede and about 14000 BP an ice-free corridor opened up, it apparently allowed the two lineages to come into contact and hybridise. When trekking herds in the study area, researchers were able to witness a wide range of migratory behaviour among individuals with some moving up to 100 km every year between the foothills and the mountains and others not migrating at all. Due to deforestation, changes in predator-prey dynamics and a change in climate pattern, most woodland populations in the Canadian Southern Rockies are now in decline and are listed as endangered or threatened. Hybridised and varied woodland populations consisting of a mixture of migrating and non-migrating individuals are believed to be equipped with the necessary flexibility to withstand challenges under changing ecological conditions that the future may bring.[166]

Three-way Hybridisation

Biologists believe that human-induced changes to the habitat of the North American grey wolf (*Canis lupus*) and the decimation of its numbers drove the species eventually to crossbreed with other species of the canine family. When farmers expanded into the northern Midwest, clearing forests and thus destroying the wolf's habitat, they unknowingly invited the coyote (*C. latrans*) to spread from the prairies into new territory. Further campaigns to destroy wolves led to dwindling populations and to their crossbreeding with the native coyote. The resulting hybrids, currently named eastern coyotes, are viable and fertile and are thriving since many decades.

Their increasing numbers, reckoned to be now in the millions, have colonised eastern North America from Labrador in the north to Florida in the south, filling niches that were once occupied by the wolf. When their genetic make-up was studied, it was discovered that they are three-way hybrids, the third in the mix being the domestic dog (*Canis familiaris*). Even the time of these hybridisation events was estimated, suggesting that coyotes mated with wolves about 100 years ago, and fifty years later they bred with dogs. Depending on the geographical location, percentages of the three species' contribution of DNA vary: in the northeast, hybrids are 60 – 80% coyote with 8 – 12% wolf and 8 – 11% dog admixture; moving south or east the ratio of genetic contribution changes, averaging 85% : 2% : 13% respectively. In the Deep South, the admixture of wolf and dog DNA is minimal, resulting in 91% coyote, 4% wolf and 5% dog. Nowhere were hybrids found that have no dog DNA at all. Unlike wolves, pure-bred coyotes from the prairie would not hunt in forests but as in many other hybrids, different parental traits are combined here as well. Eastern coyotes hunt in forests but

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[166] http://www.ucalgary.ca/news/january2009/caribou

are also found in metropolitan areas, where they tend to be nocturnal ; their fur varies in colour, displaying red, dark and light *morphs*[167] or colour variants; they are smaller than wolves but bigger than coyote with a stronger build and larger jaws, which enables them to take down small deer that is over-abundant in eastern forests**.**[168] Their howl too is a blend of a wolf's deep pitch, followed by a coyote's higher-pitched yipping.[169] The eastern coyote is more likely to breed with coyotes, to kill dogs but to be killed by wolves, whose numbers are low but in some areas slowly increasing.

The media tend to call this new predator 'coywolf'. Roland Kays, Research Associate Professor at North Carolina State University in Raleigh, USA, prefers 'eastern coyote' and calls the appearance of this new type 'evolution that is happening under one's eyes'. He concedes the hybrid to be a subspecies or an *ecomorph*, a local variety of a species, whose appearance is determined by its ecological environment, but he does not consider it to be a new species. However, research scientists Jonathan G. Way and William S. Lynn, both at Clark University Massachusetts, USA, disclaimed earlier published data and disagree *with* the hybrid's taxonomical status quo. They argue that their findings warrant the rating as the new species *Canis orient an*d accept 'coywolf' as the more accurate name for this animal[170]. Throughout this chapter, the reader will be repeatedly reminded of an existing problem concerning the biological species concept, which will be discussed in detail in the next chapter.

In the southwest of the USA, extensive, natural hybridisation occurs between Harris' ground squirrel (*Ammospermophilus harrisii*) and the white-tailed antelope squirrel (*Ammospermophilus leucurus*). On the lower Colorado River where the white-tailed antelope squirrel from northern Baja California comes into contact with Harris' ground squirrel, the former is morphologically more similar to the latter. Southeast Asia has its own hybrid squirrel population.

In Europe, there is confirmed evidence of hybridisation between the western hedgehog (*Erinaceus europaeus*) and the northern white-breasted hedgehog (*Erinaceus roumanicus*) who meet in two contact zones; one exists from the Baltic Sea eastward and the other lies in Central Europe. On the basis of its nuclear DNA (nDNA) and its mitochondrial (mtDNA), the hybrid that was found near Moscow was evidently the result of repeated backcrossing. In the USA there is hybridisation among some shrew species (Soricidae); and among the snowshoe hare (*Lepus americanus*) and the European rabbit (*Oryctolagus cuniculus*), both belonging to different genera. In eastern Africa, it's the elephant shrew species (Macroscelididae) and in China and Europe the hare species (Leporidae) that hybridise. Hybridising species are found in the large

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[167] any of the different forms found in a polymorphic species

[168] http://theconversation.com/yes-eastern-coyotes-are-hybrids-but-the-coywolf-is-not-a-thing-50368

[169] Greater than the sum of its parts, The Economist, October 31st, 2015

[170] More about this can be read at: https://theconversation.com/why-the-eastern-coyote-should-be-a-separate-species-the-coywolf-59214

family of rodents (order Rodentia), of felines (Felidae), among genets (*Genetta sp.*), mongooses (Herpestidae) and otters (Lutrinae), moles (Talpidae), prairie dogs (*Cynomys sp.*), chipmunks (Sciuridae), marmots (*Marmota sp.*), civets(*Civettictis sp.*), horseshoe bats (Rhinolophidae),.... The list goes on and on.

Extensive hybridisation is ongoing in eastern Afghanistan between the long-eared hedgehog (*Hemiechinus auritus*) and the Afghan hedgehog (*Hemiechinus auritus megalotis*), which is classified as a subspecies of the long-eared hedgehog. The hybrid was treated as a separate species until its hybrid status was genetically proven. In western central India exists a population that is geographically and morphologically intermediate between the Indian hedgehog (*Paraechinus micropus*) and the bare-bellied hedgehog (*Paraechinus nudiventris*). In this case, the hybrid has been treated as a new species. Is this confusing? One begins to wonder, are hybrids species in their own right or are they not?

Mammals Down Under are no Exception

In the Eocene, placental mammals and *marsupials* or pouched mammals lived alongside in Australia, but only the marsupials have survived to the present and are no exception to hybridisation. A number of zoos in different countries have reported the births of live hybrids from matings between various species of kangaroos. In addition, several researchers have recorded and described hybridisation between kangaroo species in the Australian wilderness. In northeastern Australia, extensive, natural crossbreeding is reported between Godman's rock wallabies (*Petrogale godmani*) and male Mareeba rock wallabies (*Petrogale mareeba*), although the parents are found to have different karyotypes. The male offspring have very small testes and are unable to ejaculate semen, while females are partially fertile. In coastal northeastern Australia, the allied rock wallabies (*Petrogale assimilis*) cross with unadorned rock wallabies (*Petrogale inornata*). Again, the species differ in karyotype and male hybrids are either sterile or of very low fertility. A male hybrid offspring from a cross between an allied rock-wallaby and a Sharman's rock-wallaby (*Petrogale sharmani*) was found to have many abnormal spermatozoa. Herbert's rock wallabies (*Petrogale herberti*) cross extensively with brush-tailed rock wallabies (*Petrogale penicillata*) in coastal eastern Australia and a stable hybrid zone[171] exists in south-eastern Queensland. The parental species differ in karyotype and partial fertility is found in both hybrid sexes.[172]

Fishes in the Oceans Hybridise too

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[171] A hybrid zone is a geographic area where distinct species converge and hybridise

[172] McCarthy, E.M. 2013, Mammalian Hybrids, www.macroevolution.net/mammalian hybrids.htlm access date 11/5/16

Hybridisation among species of placental marine mammals was already discussed earlier but the majority of ocean dwellers are fishes, which are the largest and most diverse group of vertebrate animals in existence. The question is do they hybridise as well? The answer to this question is decidedly affirmative. In 2011, researchers discovered in Australian waters 57 shark hybrids that were spanning several generations. The parent species are the Australian blacktip shark (*Carcharhinus tilstoni*) that lives in the tropical waters of northern and eastern Australia and the global, slightly larger common blacktip shark (*Carcharhinus limbatus*) from cooler waters. Unlike the other types of shark and most fish that release sperm and eggs into the water, these sharks mate and bear live pups.[173] The hybrids are slightly larger than the purebred Australian blacktips and were detected because of their physical dissimilarity with other individuals in the temperate waters some 2,000 km further down the coast in a region of overlapping distribution of the two species. They exhibit their own morphological features such as length at birth, length at sexual maturity and number of vertebrae combined with their own distinct mtDNA sequence. Their hybrid status was later confirmed by sequencing a certain nDNA marker that is inherited from both parents.[174] Clearly, the introduction of DNA from the common blacktip has made it possible for the Australian blacktip to expand its range along the coast from the warmer down into the temperate waters. Several questions present themselves here: is shark hybridisation a recent event or is it an ongoing process that was only recently discovered? Why is it happening? What may have triggered it? Is it fishery practices or climate change?

Hybridisation of Sweetwater Fishes is Big Business

The occurrence of hybridisation among fishes is most common in freshwater fish as is evidenced in public aquariums. It is also practised in the community of hobby aquarists, where hybridisation between cichlids and between other species oftentimes also occurs unintentionally. The cichlids are a group of some 2,000 or more described and undescribed marine and freshwater species, representing a substantial part of the 25,000 species of fishes that are believed to exist in total. In terms of sheer numbers of species, fishes are the most successful of all families of vertebrate animals on this planet. Lake Malawi alone contains as many as 850 different species of cichlids in an endless variety of shapes, sizes, colours and behaviours, all derived from just one lineage, which has made them a classic example organism in evolutionary biology. George W. Barlow (1929 – 2007), the late American ichthyologist, ethologist and evolutionary biologist specialising in fish, celebrated their diversity in his book *The Cichlid Fishes: Nature's Grand Experiment in Evolution*[175]*.* In the Old World, the

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[173] http://evolution.berkeley.edu/evolibrary/news/120101_sharkhybrid

[174] http://www.uq.edu.au/news/article/2011/12/world-first-discovery-of-hybrid-sharks-australias-east-coast

[175] published by Perseus Publishing, Cambridge, Massachusetts

freshwater species are found in Africa, on Madagascar and in parts of the Middle East; in the New World, they are found in Middle America, South America and in the Caribbean. In these geographical areas, they live in any body of fresh water that has a minimum temperature of 20°C and while empirical data is lacking, it is believed that cichlids rarely hybridise in the wild. However, an evolutionary ecologist at the Swiss Federal Institute for Aquatic Science and Technology in Kastanienbaum claims to have "unpublished evidence that the many species of cichlid fish in Lake Victoria in Africa arose from a three or even four-way hybridisation in the distant past. "[176] In the captivity of an aquarium, nevertheless, many cichlid species hybridise readily. For example, almost any Central American cichlid species will hybridise with any other, and many of the rock-dwelling cichlid species from Lake Malawi will freely hybridise with each other. Commercial breeders exploit this fact to produce ever more striking colours and patterns and more elaborate finnage. Many cichlid species in the wild are today under threat of extinction and captive breeding can be a positive approach to conservation while interspecific hybridisation can be a problem and raises many philosophical and ethical questions.[177]

Another commercial use of hybridisation is the creation of hybrid game fishes. In hatcheries in the north-eastern USA, biologists create novel fish species for stocking lakes and rivers with a catch for sport fishing. They also create hybrids for the purpose of preying on overabundant foraging fish. For the most part, fish hybrids display *heterosis* or hybrid vigour and grow faster than either of their parent species. They are also often more aggressive in chasing a lure or bait, two traits that are welcome among sport fishers. The intergeneric tiger trout (*Salmo trutta* × *Salvelinus fontinalis*), for example, is created by fertilising brown trout (*Salmo trutta*) eggs with brook trout (*Salvelinus fontinalis*) milt. Then the fertilised eggs are exposed to a heat shock treatment that induces the production of an additional chromosome set, turning the hybrid into a triploid, which is a condition that confers two conveniences: (1) increased survival chances and (2) sterility, which is supposed to prevent hybridisation with native fish stock. The hybrid tiger trout displays a spotted pattern that is different from the pattern of either parent species and with ample food supply, the hybrid grows more quickly and to a larger size.

Yet another hybrid created for fisheries is the sterile tiger muskellunge (*Esox masquinongy x Esox lucius*), also called tiger muskie. It is the cross between a male muskellunge (*E. masquinongy*) and a female northern pike (*E. lucius*) and is used as a game fish but also to control the population of panfish (a wide range of pan-size edible fish) in a freshwater body. The hybrids are voracious predators but since they are sterile, their numbers can easily be controlled. Yet another hybrid species that is created for its sporting qualities and high growth rate is the splake (*Salvelinus*

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[176] https://www.newscientist.com/article/dn14373-alien-fish-creates-three-way-hybrid/

[177] http://cichlidresearch.com/hybrids.html

namaycush x Salvelinus fontinalis). This is a cross between the lake trout (*S. namaycush*) and the brook trout (*S. fontinalis*). Again, the splake has a better survival rate and grows faster than either of its parents. It is a highly predatory fish and survives in waters where brook trout does not because the hybrid feeds on species that out-compete stocked brook trout. It resembles the brook trout to the extent that differentiation is only possible when looking at their tail fin. The brook trout has a squared-off tail, while the splake has a forked tail like a lake trout. In hatcheries, splakes are capable of reproducing, though, this has not been observed in the natural environment.[178]

On the other hand, natural hybridisation between a number of different sunfish species of the genus *Centrarchus* in ponds and small lakes is, however, relatively common when there is limited spawning ground. Dominant traits vary and depend on the sex and species of each parent and crosses resulting from a male bluegill (*Lepomis macrochirus*) with a female green sunfish (*Lepomis cyanellus*) are most desired. In addition to a blending of beautiful colour patterns, hybrid vigour is expressed in a larger size and an aggressive nature, traits that are both found in the parent species but to a lesser extent. They eat nearly anything that they can gobble up with their over-sized mouths, grow quickly and out-compete other game fish. But, although F_1 hybrids are fertile, they are between 85 and 95% male and have therefore few opportunities to reproduce. This is beneficial when one considers that many sunfish species are prone to overpopulating small bodies of water and becoming stunted.[179]

Climate change is at the root of a natural hybridisation event that is endangering one of the American West's most highly prized fish, the cutthroat trout (*Oncorhynchus clarkii*). Cutthroat trout spawn in cold mountain streams with just the right amount of water from spring snowmelt. Down in the valleys, rainbow trout (*Oncorhynchus mykiss*) were introduced in lakes and streams for anglers some decades ago. At higher elevations, Climate change has in recent years reduced streamflow and increased water temperature. These altered conditions are now more favourable for the rainbow trout and as a result, they have moved upstream where they meet the native cutthroat trout and produce hybrid offspring. It has become apparent that the hybrids' reproductive success is sharply reduced from that of the parent species and investigators are concerned that not only hybrids will die out but that changed environmental conditions might drive the cutthroat trout to genetic extinction.[180]

There is a report of remarkable and solid evidence of a three-way hybridisation event of vertebrates in a muddy creek in some remote part of Wyoming in the western USA, which is inhabited by two native fish species, the flannelmouth sucker (*Catostomus*

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[178] http://www.onthewater.com/game-fish-northeast/

[179] http://www.hbpondmanagement.com/article_bluegills.pdf

[180] http://www.npr.org/2014/05/27/316316952/hybrid-trout-threaten-montanas-native-cutthroats

latipinnis) and the bluehead sucker (*Catostomus discobolus*). Over the span of seven years, researchers at the University of Wyoming in Laramie have witnessed a third, introduced, non-native species, the white sucker (*Catostomus commersonii*) hybridising with the two native species. Extensive hybridisation between the white and the flannelmouth sucker has created several genetic intermediates. In addition, DNA samples have attested for yet another sort of fish that contains genetic material from all three species, the intruder and the two natives. This one was named 'muttsucker'. Of special interest is the fact that previously, flannelmouth and bluehead suckers had never been found to hybridise, but evidently, the white sucker has acted as a 'genetic bridge' between the two native species. Researchers hypothesise that eventually, all once distinct species could merge into a single, non-distinct *hybrid swarm*, which is a population of parent species, hybrid offspring and several intermediate types all intermingling and backcrossing with each other.[181]

Most are familiar with the controversy about genetically modified (GM) foods. After numerous crops and vegetables, the first GM animal for human consumption, a genetically engineered salmon, was approved in late 2015 by the US Food and Drug Administration and predictably, it was called 'frankenfish' by its opponents. Most Atlantic salmon (*Salmo salar*) one buys nowadays comes from commercial aquaculture production. By inserting two genes from other fish species, one modifying the expression of a growth hormone and the other allowing the fish to continue its development in near freezing waters, the Canadian company AquaBounty genetically engineered a new type of salmon. It reaches market size in 18 months as opposed to 24 to 30 months for the non-transgenic salmon. In addition, the transgenic fish requires 25% less feed and can be reared nearer to the markets, they say.[182] The company claims that it has several safety measures in place to avoid accidental breeding with the wild salmon. For instance, all transgenics will be female and, having a third set of chromosomes (being triploid), they will be reproductively sterile and also will be kept in tanks on land. But at the same time, they also said that a small percentage of females will remain fertile. Furthermore, the company pointed out that the hybrid crosses between the transgenic salmon and the wild brown trout would be sterile just like their naturally hybridising cousins in the wild.[183] However, that inadvertent hybridisation of fertile GM fish with wild populations is possible and represents a potential risk with unknown consequences was demonstrated by a research team from the Memorial University of Newfoundland, Canada. Intentionally bypassing safety measures, experimental crossing in hatchery-like conditions of GM Atlantic salmon with a related species, the wild brown trout (*Salmo trutta*) resulted in hybrids carrying the growth hormone transgene. Transgenic hybrids were viable and

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[181] https://www.newscientist.com/article/dn14373-alien-fish-creates-three-way-hybrid/

[182] http://en.wikipedia.org/wiki/Genetically_modified_fish

[183] http://www.geneticliteracyproject.org/2013/05/31/transgenic-salmon-can-breed-with-brown-trout-if-you-make-them/

grew more rapidly than wild salmon, wild trout and other non-transgenic crosses; they even outgrew the GM salmon. The experiment was taken one step further and under conditions that mimic natural streams, transgenic hybrids appeared to outcompete and suppress the growth of transgenic and non-transgenic salmon by 82 and 54%, respectively. The authors concluded -

> If this advantage is maintained in the wild, transgenic hybrids could detrimentally affect wild salmon populations. Ultimately, we suggest that hybridization of transgenic fishes with closely related species represents potential ecological risks for wild populations and a possible route for introgression of a transgene, however low the likelihood, into a new species in nature.[184]

And What About Amphibians?

The word amphibian derives from Greek and refers to the animals' 'double life' on land and in the water. About 370 million years ago, a group of primitive fishes, known as the Crossopterygians, gave rise to the amphibians, who became the first four-limbed vertebrates to colonise terrestrial habitats. About 6,000 species are known today and are divided into three groups: 1) the largest and most diverse is the group of frogs and toads, comprising several thousand species; 2) a group of an estimated 470 species of newts and salamanders; 3) and the smaller and least-known group, the Caecilians, which are limbless creatures that have a superficial similarity with snakes or worms. Most amphibians' life cycle proceeds from the egg stage to the larva and on to the adult form via a striking metamorphosis, of which the frog's change from an all aquatic tadpole with a tail to an air-breathing amphibian with four limbs is perhaps most impressive. Amphibians can be compared with canaries in the coal mine; their demise serves as an indicator for declining environmental conditions and it is estimated that about 122 species have become extinct since 1980.[185]

Frogs are the best known and most numerous of the amphibians. Many thousand species exist worldwide and scientists continue to discover new ones. Among the European water frogs (*Pelophylax sp.*), hybridisation is very common, though, the offspring have reproductive dysfunctions or are sterile. There are, however, three hybrid species that reproduce *hemiclonally* by backcrossing with one of their parental species, a reproduction mode that is known as *hybridogenesis*. The European edible frog (*Pelophylax esculentus*), also known as green or common frog, is such a fertile

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[184] Krista B. Oke et al, 2013, Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions, Proceedings of The Royal Society, Volume: 280 Issue: 1763 http://rspb.royalsocietypublishing.org/content/280/1763/20131047

[185] http://animals.about.com/od/amphibians/a/amphibian-facts.htm

hybrid. It is a cross between the *marsh frog* (*Pelophylax ridibundus*) and the pool frog (*P. lessonae*) and in its somatic cells, it carries the sexually recombined chromosomes of both parents. When the hybrid produces gametes, however, only one of the parental genomes is used un-recombined, while the other is excluded in the process. Reproduction occurs when backcrossing with the parental species, which provides the second genome that makes up for the discarded one during gametogenesis. Thus, half of the parental genome is passed to the next generation clonally (hence hemiclonally) and the other half sexually. Because the resulting hybrid *P. kl esculentus* needs the genome of one of its parent species to complete its reproduction cycle, it is called a 'klepton' (greek for 'stealing'), which explains the 'kl' in its species name. All-hybrid populations of *P. kl. esculentus* are also known to exist and the way they reproduce is rather intriguing. These populations consist of first-time hybrid individuals with a diploid genotype LR (derived from *P. lessonae x P. ridibundus*) and individuals with triploid genotypes (LLR and/or LRR). In the absence of the parental species *P. Lessonae* (genotype LL) *and P. ridibundus* (genotype RR), the triploid hybrids are providing the missing L or R genome.[186]

> Water frogs in mixed-ploidy populations without any parental species (i.e. all-hybrid populations) can be viewed as evolutionary units that may be on their way towards hybrid speciation. Maintenance of such all-hybrid populations requires a continuous exchange of genomes between diploids and triploids.[187]

At the beginning of this chapter, the reader made the acquaintance with a 'monster' hybrid, an interspecific cross between the endangered Californian tiger salamander (*Ambystoma tigrinum californiense*), and the invasive barred tiger salamander (*A.tigrinum mavortium*). The latter was introduced as larval bait for sport fishing in the 1940s and 1950s. As is at times the case, the hybrid shows an unexpected high level of heterosis, growing larger than either of its parent species and being more aggressive and more voracious. In outdoor experimental ponds and still in the larval stage, these hybrids devoured other amphibians and preyed on the native species' larvae. Aquatic salamanders are suction feeders and the hybrids, who have much bigger mouths, are able to suck up a large variety of prey such as the Pacific chorus frog (*Pseudacris regilla*) and the California newt (*Taricha torosa*), whose numbers were found to be seriously reduced. Researchers believe that the same could be happening in natural ponds, where hybrids already occupy a certain percentage of the native species' range. Clearly, due to the invader, the native salamander's survival is in danger. The hybrid's impact on the native Californian tiger salamander and on the ecology as such poses philosophical questions. Should the hybrid, whose genes are changing the native's

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[186] https://en.wikipedia.org/wiki/Hybridogenesis_in_water_frogs

[187] Hoffmann A. et al, 2015, Genetic diversity and distribution patterns of diploid and polyploid hybrid water frog populations (Pelophylax esculentus complex) across Europe, Molecular Ecology (2015) 24, Abstract

genome, be eradicated or should it be protected because it is partially native? And what about the argument that the introduction of new genetic material from another species confers the native species a new-found fitness?[188]

Two North American salamanders, the silvery salamander (*Ambystoma platineum*) and Tremblay's salamander (*A. tremblayi*) were shown to have been produced by a two-step hybridisation process. First, the blue-spotted salamander (*A. laterale*) crossed with a female Jefferson salamander (*A. jeffersonianum*) *and* produced a female hybrid. Then this female hybrid produced diploid eggs that were fertilised by (*A. Jeffersonianum*) males and the result was another hybrid, (*A. Platineum*). When the same eggs were fertilised by *A. laterale,* the resulting hybrid was *A. tremblayi.* Both of the second step hybrids are parthenogenic, meaning their eggs develop without fertilisation.[189]

Lizards, Hybridization, Parthenogenesis and Polyploidy

When in the early 1960s scientists investigated several species of whiptail lizards (Teiidae) from the south-western part of the United States, they noticed that some species' genomes were rather unusual. As was expected, the chromosomes were diploid sets, however, on closer examination, it was discovered that they were not homologous copies and paired up with each other in the usual way but that each copy appeared to have come from a different species. In addition, they found that many species didn't produce any males and that females reproduced asexually by parthenogenesis. In the process, their chromosomes were restored to diploidy by duplication and the resulting offspring were all female clones. Based on their findings that mating between two different whiptail species had produced hybrids with two different copies of chromosomes, the scientists hypothesised that this, in turn, had triggered females to reproduce by parthenogenesis. In addition, they reasoned that because they differed in their karyotype from their parental species, the cloned offspring represented an instantaneous new species.

Scientists were even more surprised when they subsequently found whiptail lizards with triploid sets of chromosomes. They hypothesised that in this case, male lizards that were born from sexual reproduction had crossed with parthenogenetic females. Their sperm had fertilised eggs that already contained two copies of chromosomes, leaving the offspring with three copies, two from the female and one from the male parent. Was this yet another new species?

The story becomes still more perplexing. In 1967, Harvard graduate student William B.

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[188] http://news.nationalgeographic.com/news/2009/06/090629-salamanders-hybrid.html

[189] McCarthy E.M., On the Origins of New Forms of Life, A New Theory, Copyright © 2008 by Eugene M. McCarthy, Macroevolution.net, p. 95

Neaves found in New Mexico a whiptail lizard that had not two, not three but four copies of chromosomes. He established that three of them had been contributed by the species *Aspidoscelis exsanguis* and the fourth by *A. inornata*, both natives of the same area, but he didn't investigate any further at that time. Nearly four decades later, when he was president of the Stowers Institute for Medical Research in Kansas City, Missouri, USA, he mentioned his discovery to molecular biologist Peter Baumann, also at Stowers. Both decided to use the latest research tools and study this unusual kind of whiptail lizard. Baumann and his team, including now retired Dr. Neaves, set out to recreate the tetraploid hybrid, which Neaves had come upon as a graduate student. In the lab, they crossed a parthenogenetic triploid *A. exsanguis* female with a diploid *A. inornata* male. The experiment succeeded; the species mated and all resulting hybrid offspring, four of them females, had four copies of chromosomes. Moreover, the females and their female offspring kept reproducing by parthenogenesis. Was this tetraploid hybrid yet another new species? To answer this question, the team consulted herpetologist Dr. Charles J. Cole at the American Museum of Natural History in New York City, USA, whose speciality is evolutionary biology and genetics of reptiles and amphibians. He agreed with the research team and argued that this hybrid deserves species status on the merits of its characteristics being distinctly different from those of its progenitors. He helped to formally describe the species, which they named *Aspidoscelis neavesi* in honour of its discoverer.

Not all evolutionary biologists agree, however; David Hillis at the University of Texas at Austin, USA, is one of those who question whether any lineage of the hybrid whiptail lizards should be considered a species. It is important to remember that the biological species concept (BSC) defines a species as a population in which individuals mate only with individuals of the same population, thus producing viable, fertile offspring and maintaining a common gene pool. According to this concept, *A. neavesi* would be denied species status and its progenitors would be downgraded to a subspecies classification. Furthermore, the concept lacks any capacity to account for animals that reproduce without male participation by parthenogenesis, like *Aspidoscelis neavesi*, for example. And there is one additional complication; by now, dozens of different hybrid lineages have been produced in the lab from different pairings of *A. exsanguis* and *A. inornata*; how should they be classified? Hillis suggests calling them 'hybrid clones', but some biologists point out that this would cause ambiguity and complicate communication among scientists. Clearly, the current BSC needs to be reconsidered and perhaps adjusted to be more inclusive.

Since lab-produced individuals of *A. neavesi* whiptail lizards are healthy and vigorous, one might wonder why these hybrids are not found near their parental species in the wild. The answer could be that they sometimes arise, but being low in numbers, have a lower chance of survival. Or could they be there and have not been detected? Their discovery should become easier now that a detailed description of the species exists.[190]

> Graduate Student Aracely Lutes, Neaves, Baumann, and colleagues published a 2011 PNAS paper in which they created a novel parthenogenic species from male and female lizards of different ploidy, suggesting a mechanism for evolution of unisexual species. And in a 2010 Nature paper the group solved a fundamental molecular question of how lizards reproduce asexually while retaining genetic variation inherent in their hybrid origin.[191]

The account presented here is only a selection from a plethora of well-documented hybridisation events between animals that belong to different species but are able to mate and produce viable hybrid offspring, albeit of varying fertility.

Insect Hybrids

The "Killer" Bee

The Africanised bee, by many known as the 'killer' bee, is a hybrid of the African bee (*Apis mellifera scutellata*) and one of several European honey bee subspecies (*Apis mellifera subsp*.) and has its origin in a well-intended experiment gone wrong. When European settlers arrived in the Americas, they brought with them their livestock and honeybees, however, the bees did not produce well in the tropical climate of South America. In the late 1950s, the Brazilian government presented one of their entomologists specialising in genetics with the task to create a species that would combine the traits of the African bee for withstanding tropical heat and predation with the gentleness and productivity of European bee species. Unfortunately, some of the experimental subjects escaped from their confinement in the laboratory and have since successfully hybridised in the wild. Spreading northward at the rate of more than 300 km per year, they arrived in California in 1995 and could possibly spread even farther north due to climate warming. The Africanised bee's success lies in its high fecundity, shorter development times and higher production of new colonies, outcompeting the European subspecies. Furthermore, they are well adjusted to the unpredictability of resources, surviving when pollen and nectar are inadequate for the European species. In addition, their behaviour is unlike that of their European counterparts, defending their hives much more aggressively and pursuing and

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[190] Zimmer C., 2014, The Strange Tale of a New Species of Lizard, The New York Times, available at: http://www.nytimes.com/2014/12/18/science/strange-tale-of-new-species-of-lizard.html?_r=0

[191] http://www.stowers.org/faculty/president-emeritus

attacking their perceived threats in larger numbers and for longer distances. "Since their introduction into Brazil, they have killed some 1,000 humans, with victims receiving ten times as many stings than from the European strain."[192]

The Lonicera Fly

The 'Lonicera fly' has the reputation of providing the first scientific evidence for fertile animal hybridisation in the wild. It is a parasitic fruit fly, whose name derives from the shrubby honeysuckle (*Lonicera sp.*), around which its life cycle evolves. At the time of the fly's discovery in the late 1990s, botanists believed that more than 50% of all plants have originated by hybridisation. Conversely, zoologists considered hybridisation to be a theoretically possible but rare phenomenon in their field and as a result largely ignored it. When Dietmar Schwarz, then a graduate student at the department of entomology at Pennsylvania State University, USA, noticed that an invasive honeysuckle was infested with a particular fruit fly that resembled two other, closely related fruit fly species, he believed he was on to something. As his Ph.D. thesis he proposed that the infestation was the result of a change of host by host-specific fruit flies and an ensuing hybridisation event and, together with his advisor and several colleagues, he began his research.

In subsequent studies, he found that the brushy honeysuckle, an invasive weed and the host plant of the parasite he was investigating, was a recent introduction to the north-eastern flora of the United States. It had been brought to the colonies in 1750 and become established when the Department of Agriculture introduced it as an ornamental plant in 1880. Fruit flies are classified into two families, the Drosophilidae, to which *Drossophila melanogaster*, the model organism in the study of genetics, belongs and the Tephritidae, which includes the genus *Rhagoletis.* Every species in this genus is specialised for only one particular host plant, on which it feeds, mates and lays its eggs in the berries.[193]

By carrying out DNA analyses, Schwarz and his group established that the newly discovered Lonicera fly was the result of hybridisation between two closely related fruit fly species, the blueberry fly (*Rhagoletis mendax*) and the snowberry fly (*R. zephyria*). As their common names indicate, blueberry bushes are the preferred hosts of the former and snowberry bushes are visited exclusively by the latter. It follows that this host specificity acts as a reproductive barrier between species lines. Schwarz

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[192] http://www.columbia.edu/itc/cerc/danoff-burg/invasion_bio/inv_spp_summ/Apis_mellifera_scutellata.htm

[193] http://news.psu.edu/story/209155/2005/07/27/invasive-honeysuckle-opens-door-new-hybrid-insect-species

proposed that the barrier was breached, possibly as far back as 250 years ago, when the two aforementioned fruit fly species strayed from their specific hosts and met and mated on the newly introduced brushy honeysuckle. In ensuing behavioural experiments in the laboratory, the group investigated and demonstrated hybrid and parent species' host preferences. It was shown that blueberry and snowberry flies discriminated against each other's host plants but accepted to meet on the invasive honeysuckle, while the Lonicera fly preferred the honeysuckle over the hosts of its parent species. This demonstrated that the Lonicera fly's preferential behaviour served as its own isolating barrier from the parental species. In a paper published in 2007, Schwarz and the team suggested that the results of their experiments demonstrated that the shift by two ecologically isolated species to a novel host and the resulting hybrid's preference of a new and, so far, unoccupied ecological niche was a mechanism for hybrid speciation for parasitic animals in general.[194]

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[194] Schwarz D. et al, 2007, A novel preference for an invasive plant as a mechanism for animal hybrid speciation, National Center for Biotechnology Information PubMed, Feb; 61(2) Abstract at: http://www.ncbi.nlm.nih.gov/pubmed/17348936

Figure 27. Brown Argus butterfly
Credit: Peter Eeles, Butterfly Conservation

The Brown Argus Butterfly

> Ecozones are a method of dividing up the Earth's surface. Each ecozone is a large area that contains a number of habitats, which are linked by the evolutionary history of the animals and plants within them. For instance one ecozone is Australasia[195], because its marsupials evolved in isolation to mammals in the rest of the world.[196]

One other ecozone and the largest of a total of eight is the Palaearctic. Its western region encompasses Europe and extends north to southern Sweden. In North Africa it reaches south to the Tropic of Cancer; it covers the northern and central parts of the Arabian Peninsula and extends roughly to the Aral Mountains. Temperate Asia belongs to the eastern Palaearctic. The brown Argus butterfly (*Polyommatus subgenus Aricia*), named after the many-eyed giant of Greek mythology and easily recognisable by its white-fringed wings and orange spots along its wingtips (figure 27), is found throughout much of the western Palaearctic.

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[195] Animals had migrated there from North America via Antarctica in the late Cretaceous or early Tertiary period before Australasia broke off, moved northward and became isolated from other landmasses

[196] http://www.bbc.co.uk/nature/ecozones

Two closely related though morphologically and genetically different lineages within the subgenus *Aricia* that also vary in their usage of host plants are found in different parts of Europe. *P. (A.) agestisis*, for example, which has two broods a year and is *bivoltine*, which means it has two adult flight periods per year, is found in southern and central Europe, southern Britain and southern Sweden. The univoltine, northern brown Argus (*P. (A.) artaxerxes*) is restricted to northern England and Scotland and most of Scandinavia. Populations in Scotland have a distinct white spot on their forewing but genetically, they group with the rest of the European *P. (A.) artaxerxes* populations, which mostly lack this spot. Since around 1980, *P. (A.) agestisis* has during recent regional warming expanded northward by approximately 200 km while *P. (A.) artaxerxes*, the northern brown Argus, is retreating further northward from its established habitats.[197] In a geographical band that is 150 to 200 km wide in a north/south direction and is crossing northern England and north-east Wales, *Aricia* populations display intermediate colour patterns between the southern *P. (A.) agestisis* and the northern *P. (A.) artaxerxes.* In 2005, DNA sequencing of the nuclear Tpi gene revealed that not only their morphology but also their Tpi gene is a blend of Tpi haplotypes of the two species. In addition, almost any combination of mtDNA and nuclear haplotypes, as well as voltinism, could be found in these populations. A study in 2002 had hypothesised that nuclear and mitochondrial mismatch could be explained by hybridisation and concluded “whether or not the UK Peak District [the Yorkshire Wolds and North Yorkshire Moors] populations are of hybrid origin awaits a more detailed study[198].” Hybridisation was considered to be unlikely at the time; however, the data from 2005 not only confirmed the hypothesis, but populations from North Wales were also included in the same results.[199] The initial occurrence of this hybridisation event is unknown but could have earliest been possible after the arctic icecap had receded about 11,500 years ago. In any case, *P. artaxerxes/agestis* populations have been recorded in northern England and Wales for the last 100 years, and since they were geographically separated from their putative parental species populations for most of the 20th century, it can be assumed that little or no gene flow occurred during that time. Consequently, hybrid populations could have originated hundreds, if not thousands of years ago.[200]

As of 2005, populations of bivoltine *P. (A.) agestisis* were expanding northward beyond the southernmost univoltine hybrid populations and further introgression of alleles from warmer-adapted *P. (A.) agestisis* into the gene pool of *P. (A.) artaxerxes* was expected. Based on the rate by which in eastern England southern bivoltine

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[197] Mallet J. Et al,2010, Hybridisation and climate change: brown argus butter-flies in Britain (PolyommatussubgenusAricia), Insect Conservation and Diversity, pp. 1-2, http://abacus.gene.ucl.ac.uk/jim/pap/mallet%20aricia%2010.pdf

[198] Idem, p. 4

[199] Idem, p.4

[200] Idem, pp. 4-5

populations had moved north during the years 1970 to 1982 and again between 1995 and 1999, it was predicted that few populations of the northern brown Argus butterfly *P (A.) artaxerxes* will remain in Britain by 2100.[201]

By 2012, *P. (A.) agestisis* had expanded its range in Britain by 79 km northward over the previous 20 years. This was a substantial increase in habitat and it is believed that it was made possible by climate warming during that time, allowing the butterflies to use an additional host plant on which to lay their eggs. In the past, the species was restricted to using the rockrose, which grows only in particularly warm microclimates. Since summer temperatures have increased, they also use several common geranium species that were formerly growing in cooler areas, which have now become warm enough for the brown Argus butterfly to thrive.[202]

Heliconius Butterflies

It is the year 2006 and in the June 15 issue of the journal *Nature*, scientists report the laboratory creation of a hybrid butterfly of the genus *Heliconius*.

The Heliconian butterflies are native to Central and South America, where more than 60 species display wings in a stunning diversity of colours and contrasting patterns, which evolutionary biologists cannot explain by genetic mutation alone. Butterflies and caterpillars, preyed upon by birds, spiders, lizards and other animals, have evolved with features such as camouflage, disguise and mimicry that enable them to hide from predators. The first person to offer a scientific explanation of protective mimicry in animals was the English naturalist and explorer Henry Walter Bates (1825 – 1892), whose theory about mimicry of an unpalatable or noxious species by a harmless species is known since then as Batesian mimicry. In 1848, Bates departed with his friend, the British naturalist and explorer Alfred Russel Wallace (1823 – 1913) on a joint expedition to the Amazonian rainforest where the young men amassed an outstanding collection of insects. Upon his return home 11 years later, he compiled the findings of his travels into his best known and widely regarded work *The Naturalist on the River Amazons.* He also published important papers on entomology. *Contributions to an insect fauna of the Amazon Valley: Heliconiidae* (figure 28) was one of them, which he read before the Linnean Society in 1861. His friend Wallace called it a “remarkable and epoch-making paper”[203] and said in his obituary of Bates:

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[201] Idem, p. 6

[202] http://blogs.scientificamerican.com/observations/once-rare-butterfly-species-now-thrives-thanks-to-climate-change/

[203] From Bates’s obituary written by Alfred Russel Wallace, http://people.wku.edu/charles.smith/wallace/S446.htm

> In this paper, besides making important corrections in the received classification of this group and its allies, he discussed and illustrated in the most careful manner the wonderful facts of "mimicry," and for the first time gave a clear and intelligible explanation of the phenomena, their origin and use, founded on the accepted principles of variation and natural selection.[204]

Figure 28. Heliconiidae
Plate from Bates' 1862 paper Contributions to an insect fauna of the Amazon Valley

Two Heliconian species, *Heliconius cydno* and *H. melpomene*, are found in the wilds of Mexico and northern South America and where their habitats overlap, there is a third species, *H. heurippa*, which displays wing patterns and colourings that are intermediate between the two. Biologists had for some time suspected that *H. heurippa* may be a hybrid from a cross between *H. cydno* and *H. melpomene* and in order to falsify their hypothesis, an international research team crossbred the purported parental species under lab conditions. While *H. cydno's* wings are black with white and yellow markings, *H. melpomene's* wings are distinguished by red, yellow and orange markings on black. The wing patterns and colours displayed by the lab-created hybrid were intermediate, showing red and yellow markings on black. Furthermore, they were nearly identical to the markings of *H. heurippa* found in the wild. In their experiment, F1 female hybrids were sterile while males were fertile. This finding was consistent with Haldane's rule, which asserts that hybrid sterility or nonviability affects particularly the heterogametic sex, which in butterflies and moths is, like in birds, the female sex. Researchers believed that under natural conditions, *H. heurippa* males backcrossed with females of either parent species, resulting eventually in fertile females. They also discovered that *H. heurippa* shows a strong preference for mating with members of their own rather than their parent species, a behaviour they were

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[204] Idem

able to duplicate and observe in the lab. When they covered up the red or the yellow markings of a bi-coloured hybrid female, hybrid males lost all interest in the female. The researchers argued that this sexual preference, which was based on phenotype, had the same effect as any physical reproductive isolation would have had and was the mechanism that led to hybrid speciation.[205]

Fertile Avian Hybrids in Astonishing Numbers

In the avian world, hybridisation is even more common than among ground-dwelling, air-bound and aquatic animals, presumably because it is easier for birds to cross species lines. In the preface to his book *On the Origins of New Forms of Life A New Theory*, the American geneticist Eugene M. McCarthy writes -

> Eventually, in completing a book on bird hybrids (Handbook of Avian Hybrids of the World, 2006), I found that the majority of avian crosses, at least the majority of those for which data on fertility is available, actually do produce hybrids that are themselves capable of having offspring. Moreover, about half (i.e., about 1,800) of the crosses listed in my book occur in a natural setting. Many of these crosses occur on an ongoing basis and have produced permanent hybrid populations.(McCarthy 2008)[206]

Among this astonishing number of interbreeding avian species in the wild, one finds wildfowl such as the Canada goose (*Branta canadensis*) that mates with just about every other species of geese; and the mallard (*Anas platyrhynchos*), which is notorious for hybridising, particularly with ducks of the genus *Anas*. The mallard also breeds freely with domestic ducks, which are mostly descendants of the mallard or the Muscovy duck (*Cairina moschata*), and produces fertile offspring. As a result, mallards and feral ducks and their hybrid offspring populate many a pond. Among game birds, in the family Phasianidae, pheasant species interbreed, and grouse species interbreed; so do pheasants and grouse, which actually belong to different genera. Rare interfamilial hybridisation has been described between guinea hens (Numididae) and three different types of domestic fowl cocks (Phasianidae), in which the hens produced different amounts of fertile eggs. In reciprocal crosses, fertility was much reduced, though.

Furthermore, countless passerine species are reported to hybridise. To mention just a few, there are the sparrows (*Passer domesticus* x *P. hispaniolensis*) in North Africa; the warblers (*Vermivora cyanoptera* x *Vermivora chrysoptera*) in North America; the flycatchers in Europe (*Ficedula hypoleuca* x *Ficedula albicollis*), Asia and Africa (*Ficedula*

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[205] http://www.livescience.com/800-scientists-create-butterfly-hybrid.html

[206] E.M. McCarthy, 2008, On the Origins of New Forms of Life, A New Theory, http://www.macroevolution.net/support-files/forms_of_life.pdf

sp.); oriental magpie robins (*Copsychus saularis*) on Borneo and Java; robin-chats (*Cossypha dichroa* x *Cossypha natalensis*) in South Africa; tanagers (Thraupidae) in the Andes and, Darwin's iconic 'finches' (Thraupidae) that are, as a matter of fact, tanagers as well.

Darwin's Galapagos Finches

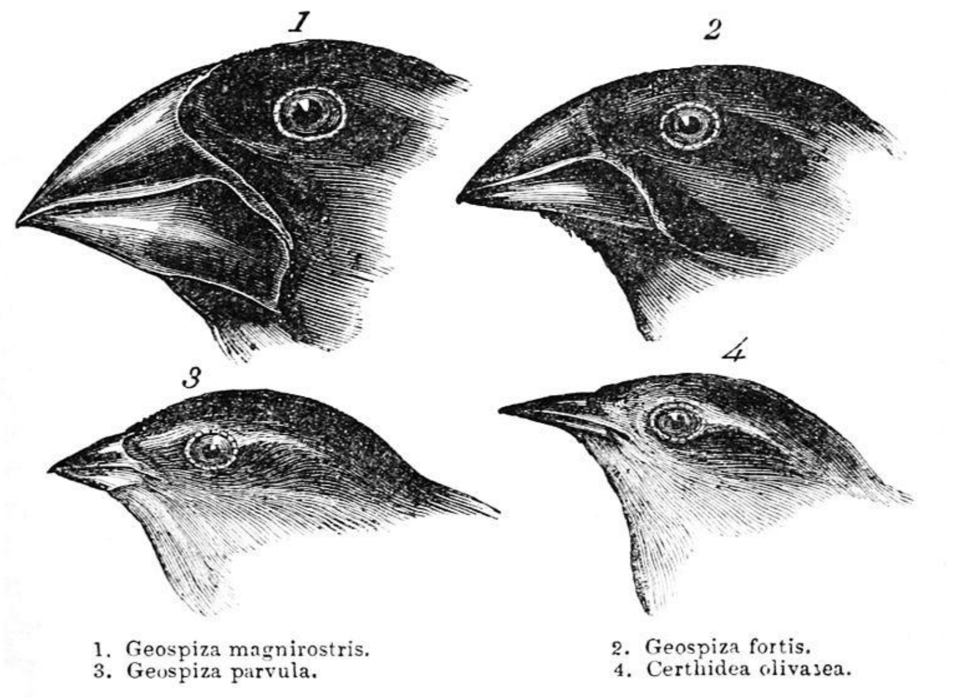

Figure 29. Darwin's finches or Galapagos finches. Darwin, 1845.
By John Gould (14.Sep.1804 - 3.Feb.1881) - From "Voyage of the Beagle"

When in 1835 Charles Darwin visited the islands in the Galapagos Archipelago, he noticed among the finches a considerable diversity in body size and beak morphology that reflected the type of food each individual group was eating. The ones with the shorter and stronger beaks ate larger and hard seeds, while the ones with the elongated beaks probed the tree bark for small insects, for example (figure 29). At the time, Darwin did not realise that they were different species. It was the English ornithologist and bird artist who later identified them as such and provided Darwin with a valuable contribution to his concept of evolution by natural selection. Following accepted rules of taxonomy, the different types were classified according to their physical characteristics and over time, key DNA sequences were added.

Recently, using whole genome *re-sequencing*[207], the complete genomes of 120 birds that inhabit the islands and represent all 15 Galapagos finch species, including two close relatives, were sequenced and the result was a surprise for some. Comparing birds with different beak shapes, researchers focussed on the genes that were responsible for beak morphology. One of these genes, ALX1, has two variants that match up accurately with big and blunted beaks as in the large ground finch (*Geospiza magnirostris*) and thin and pointed beaks as in the large cactus finch (*G. conirostris*), the two species that are found on the small island Daphne Major. What is remarkable, though, is the fact that the two variants of this gene are also found among individuals from one and the same species, the medium ground finch (*G. fortis*), which includes individuals with blunt as well as pointed beaks. Here was at long last the genetic proof to back up field observations that ecologists and evolutionary biologists Peter and Rosemary Grant had made when studying the finches over the span of forty years. Darwin's finches were hybridising, had done so for most of their evolutionary history and had a messy family tree to prove it.[208]

In 1977, 1985 and 2004, the scientist couple had witnessed and documented the species' rapid evolution following severe droughts. Rainfall on the Galapagos Islands is linked to the *El Niño* Southern Oscillation (ENSO) weather phenomenon*,* which occurs roughly twice in a decade and brings large quantities of rainfall interspersed with years of little or no rainfall at all. The *El Niño* event of 1982 to 1983 was especially severe and altered the ecology of the islands completely. Before the event, plants producing large and hard seeds were predominant but with intense rainfall, a wide variety of grasses and herbs appeared that produced small and soft seeds from which the small, pointed-beaked medium ground finch *G. Fortis* profited disproportionally. Over the ensuing 20 years, Peter and Rosemary Grant watched beak sizes and population dynamics change and oscillate in step with the type of seed that was predominantly produced according to environmental conditions. They called what they were witnessing 'fission and fusion'. Over the course of their studies, which had begun in 1973, beaks of the medium ground finch had become more pointed and beaks of the common cactus finch (*Geospiza scandens*), another inhabitant of the island, had become smaller and blunter. Through hybridisation, the morphological traits that had served as a distinction between the ground finch species had converged in one species, allowing a glimpse of evolution in a time frame that a person can observe in a life time. It should be noted that, while most female chicks of interspecific crosses are mostly

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[207] The sequencing of part of an individual's genome and comparing it with the standard genome of the species in order to find sequence differences.

[208] https://www.princeton.edu/main/news/archive/S42/36/79O60/index.xml?section=topstories

sterile, both sexes of F_1 finch hybrids are viable and fertile. They rarely breed with each other and instead backcross with individuals of their parental species.[209]

Floreana, another island in the Galapagos Archipelago, is the home of three species of tree finches, the large *Camarhynchus psittacula*, the small *C. parvulus* and the medium *C. paupe.* In the absence of distinct characteristics, they are largely differentiated by their body and beak sizes only. As their name implies, they are arboreal and with their sharp, grasping bill, well suited for poking in tree bark crevasses, they feed mainly on insects. The medium tree finch evolved on the island from a morph of the large tree finch but is now critically endangered due to a parasitic fly, which was accidentally introduced on the island in the 1960s. The adult fly (*Philornis downsi*), does not harm the finches, but its larvae infest their nests and suck blood from their nestlings' nasal passages, causing up to 98% of nestling mortality. It had been observed previously that parasite infestation is increased when precipitation is high. Moreover, tree finch body size appeared to be correlated with parasite density.

In order to investigate how these factors played together, researchers chose 2005, a year of low rainfall and low parasite incident and 2010, when precipitation and parasite density were high. Using museum tree finch species from 1852 to 1906 that had not been exposed to the parasite as their benchmark, they sorted birds from each time period according to their body size. The museum sample could be grouped into three distinct populations, small, medium and large. In the 2005 sample, the three groupings were still discernible but showed increased morphological overlap between them. In the 2010 sample, a year with high precipitation and highest density of parasites, tree finches could only be sorted into two groups, a small and a larger size and the question was, which species had disappeared from the record? Closer observation clarified that the larger finches were much smaller than the large ones in the museum sample of the 1800s. It appeared that the large tree finch had become extinct. Genetic investigations then revealed that this species had already been extinct by 2005 and that the third, morphologically intermediate group was the result of hybridisation between the small and medium species. The researchers had discovered that, even in years of high precipitation and high parasite density, the nests of hybrids and also of the smaller-bodied populations were less infested by parasites, which had resulted in an increased number of hybrid young. In 2005, 19% of the small and medium finches were hybrids; in 2010, this proportion had increased to 41% [210].

It was also observed that females of the medium tree finches choose to mate with the smaller males of the other species that have a comparatively lesser parasite load, again, favouring hybridisation. Why this is the case is not yet understood. A possible

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[209] http://rstb.royalsocietypublishing.org/content/363/1505/2821#sec-5

[210] http://www.sciencenews.org/article/charms-small-males-may-collapse-darwin%E2%80%99s-finch-species

reason could be that males of the smaller species escaped serious, parasite-induced injuries and deformation to their airways and sing more irresistibly than their larger competitors.[211] On one island, the environmental impact of the recurring and disruptive weather pattern of *El Niño* led to interspecific hybridisation and appearance of novel traits, on another island, an introduced parasitic fly caused high nestling mortality and the ultimate demise of one species while the other two are fusing into one by hybridisation. The example of the Galapagos finches serves to aptly demonstrate three evolutionary phenomena, hybridisation, the formation of new traits and extinction, all occurring alongside each other and observed in the short temporal space of a human lifetime.

Hybridisation among Non-human Primate Species

The word Primate derives from Latin for “primas” (of first rank) and is the name given to what is considered to be the ‘highest’ mammalian order that includes monkeys, apes and humans. In his first edition of *Systema naturae*, published in 1735, Linnaeus introduced a new classification system, in which he grouped animals, plants and minerals into their respective kingdoms. He divided the animals into six different classes that he named *Quadrupedia* (four-legged), and he placed man, whom he called *Homo sapiens* (wise man), together with animals that had a morphological affinity with man in the order *Anthropomorpha* (from Greek for human form or human-like). After this classification had earned him much criticism, Linnaeus changed the terms in the 1758 edition of *Systema naturae* from Quadrupedia to Mammalia and from Anthropomorpha to Primata, which are still in use today. [212]

Primates are subdivided into two groups, the *prosimians* (from Greek for pre-monkey) that include lemurs, lorises and tarsiers and the *anthropoids* (Greek for resembling human). The latter group comprises the monkeys; the apes, which are man’s closest relatives; and man himself. The anthropoids consist of six families that are divided into two groups: (1) South American marmosets and tamarins (Callitrichidae) and (2) South American monkeys other than marmosets (Cebidae); both families are called the New World monkeys or Platyrrhini, (from Greek for flat-nosed). The second group are the Catarrhini (from Greek for nostrils that face down). They comprise the African and Asian old world monkeys (Cercopithecidae); the Southeast Asian siamangs and the

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[211] http://evol-eco.blogspot.pt/2014/03/the-lifetime-of-species-how-parasitism.html The lifetime of a species: how parasitism is changing Darwin's finches

[212] The Linnaean Correspondence http://linnaeus.c18.net/Letters/display_notes.php?id_letter=L0783&id_person_from=1112&id_person_to=981&code_from=LinnaeusC&code_to=GmelinJG

gibbons (Hylobatidae); and the African great apes such as the gorillas, the orangutans and the chimpanzees (Pongidae); and living and extinct man (Hominidae)[213]

Hybridisation among non-human primate species in captivity is widespread, but even under natural circumstances -

> "Seven to 10% of all primate species hybridise, which is common considering a lot don't ever come into contact with each other," says physical anthropologist Rebecca Ackermann of the University of Cape Town in South Africa.[214]

During recent decades, an extensive and comprehensive literature has accumulated that records observations of primate hybridisation in captivity as well as in the wild.[215] Not wanting to tire the reader with too much detail, here is just a small sample demonstrating the possible consequences of interspecific and intergeneric crossbreeding among non-human primate species. The reader may be reminded of the species conundrum and notice that hybrids are not always recognised as such.

Prosimians, the most primitive of the extant monkeys, comprise of lemurs (superfamily Lemuroidea), which are endemic to Madagascar and include the aye-aye (genus *Daubentonia*), the sifakas (genus *Propithecus*) and the indri (genus *Indri*); the lorises that are found in Asia and the pottos, native to Africa and both belonging to the family Lorisidae; and the galagos (family Galagidae). Most of these animals are tree-dwelling and nocturnal, lack the dexterity of monkeys and apes and, unlike other primates that reproduce according to their individual biological cycles, reproduce during distinct breeding seasons.[216]

At Anjamena in north-western Madagascar, brown lemurs (*Eulemur fulvus*) and mongoose lemurs (*E. mongoz*) share the same habitat. During a field study, researchers observed animals that were in their pelage intermediate between the two species and subsequently, when a brown lemur was found having the mtDNA genotype of a mongoose lemur, the existence of a hybrid zone was confirmed. To clarify the extent of hybridisation in this zone, a survey using mtDNA and nDNA markers was carried out on a total of 162 animals. Among the mongoose lemurs, two F_1 hybrids were identified; one of them carried the mtDNA of a brown lemur. Six more individuals were found to be hybrids on account of brown lemur genetic introgression. Statistical assessments and genetic analyses determined that the two confirmed

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[213] formerly named Hominids included the genus Homo and modern and extinct great apes and their ancestors. A more recent classification of Hominins includes beside the genus Homo all immediate ancestors such as Australopithecus, Paranthropus and Ardipithecus.

[214] http://www.bbc.com/earth/story/20151013-how-interbreeding-shaped-us

[215] McCarthy E.M., Mammalian Hybrids Cross Listings, http://www.macroevolution.net/mammalian-hybrids.html access date 9/8/16

[216] http://animals.about.com/od/primates/p/prosimians.htm

hybrids were the products of interbreeding between *E. fulvus* and *E. mongoz*. Furthermore, one male hybrid was found to be fertile. All eight brown lemurs that were screened showed either genetic admixture from *E. mongoz* and/or were offspring of *E. mongoz* females. Overall results showed that in this hybrid zone interbreeding among the two species is bidirectional, in other words, introgression occurs into both distinct populations, with a bias toward brown lemurs.[217]

In the Andringitra region of eastern Madagascar, a hybrid zone exists that is inhabited by red-fronted lemurs (*Eulemur rufifrons*) and grey-headed lemurs (*E. albocollaris*). While the red-fronted lemurs' habitat extends over a large area, two remaining gray-headed lemur populations are living in a rather fragmented territory and are threatened by hunting practices. Currently, they are on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species. There were concerns that crossbreeding with *E. rufifrons* might hasten their genetic extinction, but a study carried out in the hybrid zone suggests that hybridisation could present a conservation opportunity. Around 180 animals were captured at ten sites along a transect in the hybrid zone and data from morphological measurements as well as blood samples for genetic analyses yielded following results: crossbreeding in the study area is bi-directional; hybrids appear to be as fertile as their parents are; and they display characteristics that are unique to them, such as longer tails, for instance. This suggests that hybrid populations are living and breeding in their own distinct range, separated from their parental species. Gene flow from *E. rufifrons* into the gene pool of *E. albocollaris* appears to make evolutionary innovation possible and it can, therefore, be assumed that this may aid in the survival of the species.[218] According to the fossil record, Old World monkeys were once present in Europe. Today, they are native to Africa, the Middle East and South and East Asia where they inhabit tropical rainforests, arid grasslands and mountainous areas with heavy winter snows. Most Old World monkeys are partially omnivorous but their preferred diet is plant matter, and most have tails, though they are never prehensile like the tails of their cousins, the New World monkeys.[219]

The territory of Gombe National Park in Tanzania, Africa, is confined by Lake Tanganyika in the west and by human settlements in the north, east and south and is,

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[217] Pastorini J. et al, Genetic analysis of hybridization and introgression between wild mongoose and brown lemurs, abstract, results, conclusion, http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-9-32

[218] Delmore K., June 01, 2011, Conservation Implications of Hybridization Between Two Species of Brown Lemur, http://www.alltheworldsprimates.org/all_the_worlds_primates_blog/11-06-01/conservation_implications_of_hybridization_between_two_species_of_brown_lemur.aspx

[219] https://animalcorner.co.uk/animals/old-world-monkeys/

therefore, labeled a ‘terrestrial island’. In this enclave, a high incidence of interbreeding occurs between red-tailed monkeys (*Cercopithecus ascanius*) and blue monkeys (*C. mitis*) with hybrids of different ages and sexes living in mixed as well as pure species populations, which testifies to bidirectional interbreeding. Female hybrids are commonly observed nursing their young and leaving no doubt as to their fertility.[220]

Gelada baboons (*Theropithecus gelada*) are native to the Ethiopian Highlands, while hamadryas baboons (*Papio hamadryas*) occupy a much larger range throughout north-east Africa. Notwithstanding their belonging to different genera, they occasionally interbreed. At Bihere Tsige Park near Addis Ababa, Ethiopia, a female hamadryas baboon produced several hybrid offspring with a gelada; the offspring were large but otherwise normal in their development. In morphological characteristics such as skull and tooth form, the hybrids were intermediate. However, they did not exhibit any of the parents' traits that are normally employed for attracting the opposite sex during courtship. In another case, a female infant from a backcross of a young hybrid female with a hamadryas baboon appeared to be thriving when she was 2.5 years of age.[221]

On closer look, the New World monkeys, also known as neotropical primates, are easily distinguishable from the Old World monkeys by their broad, flat noses. Their ancestors diverged about 35 million years ago[222] from African Old World monkeys by possibly either crossing the Atlantic to South America on a vegetation raft or by migrating via a land bridge that has since submerged. Today, they are inhabiting the tropical and sub-tropical forests of Central and South America.[223] Some researchers have observed that -

> “hybrids of all of the south-east Brazilian marmosets are genetically fertile, [and this hybridization] “along with founder effects and genetic drift may cause rapid ‘speciation’, or at least the production of localized uniformly distinct, true-breeding phenotypes.”[224]

The neotropical owl or night monkeys (genus *Aotus*) are unique among monkeys for their nocturnal activity. Another distinguishing feature is their wide range of diploid counts from 46 to 56 chromosomes. Because of their natural resistance to the

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[220] Detwiler K.M., Hybridization between Red-tailed Monkeys (Cercopithecus ascanius) and Blue Monkeys (C. mitis) in East African Forests, summary, http://link.springer.com/chapter/10.1007%2F0-306-48417-X_7

[221] Jolly, C.J., Woolley-Barker, T., Beyene, S. et al. International Journal of Primatology, Abstract (1997) 18: 597. doi:10.1023/A:1026367307470

[222] https://mbe.oxfordjournals.org/content/20/10/1620.full.pdf

[223] http://animals.about.com/od/primates/p/new-world-monkeys.htm

[224] McCarthy, E. M. 2013, Mammalian Hybrids, http://www.macroevolution.net/old-world-monkey-hybrids.html access date 7/9/2016

parasites that cause malaria, colonies of owl monkeys are kept in biomedical and other facilities for research purposes. Until 1983, the genus *Aotus* was represented by only one species (*Aotus trivirgatus*) and ten subspecies. Subsequently, all subspecies were moved up to species level and after genetic profiling, their taxonomy was further adjusted. However, these changes are not accepted by all researchers and some still treat all owl monkey lineages as *A. trivirgatus.*[225] This is another glaring example of the species conundrum, which will be discussed in the next chapter.

A research team at the University of California at Davis, USA, recorded hybrid production in a group of sexually mature adult owl monkeys when they were still taxonomically considered to belong to one species, *A. Trivirgatus,* only. For breeding purposes, the majority of pairs had been selected according to chromosome morphology and/or diploid count. Because of limited space, three pairs were joined together in spite of the incompatibility of their karyotypes and, although they were not expected to breed, two of the pairs produced a total of four hybrid offspring. Overall, pairs that were compatible in their karyotype had more pregnancies and more viable offspring over the same period of time. Nevertheless, the three pairs with different karyotypes produced viable offspring as well. In fact, one of the pairs produced as many offspring during the same amount of time as the carefully matched pairs. One pair, a male with a diploid count of 53 chromosomes and a female with 56, had three offspring and another pair, the male with 54 and the female with 55 chromosomes had an offspring every year after they were housed together. The third pair did not produce at all. One of the four hybrid offspring was karyotyped to be $2n = 55$ from a female parent with $2n = 56$ and a male parent with $2n = 53$. The results demonstrated that different chromosome counts are not necessarily a barrier to reproducing healthy offspring.[226]

In another study, this time at the Primate Research Institute at Kyoto University in Japan, a small group of adult owl monkeys were used in a study to record and evaluate the sleeping behaviour of captive-born hybrids. The hybrids had been produced inadvertently by pairing animals of different species before multiple taxonomic determinations had come into existence. The subjects of the study were four first generation female hybrid siblings, whose female parent was, according to the chromosomal count of $2n = 50$, an *A. azarae*. Their male parent was an unknown species with a chromosomal count of $2n = 53$. Also part of the study were three pure-bred owl monkeys consisting of a mother and her two female offspring. Karyotype analysis revealed that one of the four hybrid siblings had the chromosomal anomaly of three instead of two X chromosomes. In spite of this condition, the animal was healthy

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[225] Cawthon Lang KA. 2005 July 18. Primate Factsheets: Owl monkey (Aotus) Taxonomy, Morphology, & Ecology . <http://pin.primate.wisc.edu/factsheets/entry/owl_monkey>. Accessed 2016 September 21

[226] SIMPSONI J.S., JONES A.C., 1982, Hybrid production in owl monkeys (Aotus trivirgatus), Laboratory Animals (1982) 16, pp 71-72

but showed certain behavioural differences compared to the other members of the group. As far as the objective of the study was concerned, the researchers reported significant differences in the quantification of total sleep time and sleep episode length between the pure-bred and the non-trisomic owl monkey hybrids.[227] In the wild, of course, these differences could have an important impact on the bybrids' nocturnal behaviour.

Some of the largest neotropical or New World primates are the howler monkeys. They inhabit Central and South American forests and are also found in southeastern Mexico, where two species were the subject of an extensive study that was conducted over a period of ten years. The aim of the study was directed toward investigating hybridisation in the context of human evolution and served to demonstrate the difficulties of determining hybrids by their appearances. Mantled howler monkeys (*Alouatta palliata*) and black howler monkeys (*Alouatta pigra*) are both mostly folivorous but otherwise differ in their behaviour, their physical appearance and their karyotype and populate their own distinct regions. However, in the State of Tabasco in Mexico, a natural hybrid zone exists where the species overlap and interbreed. In the study, blood and hair samples were taken from 135 live adult howler monkeys. Using various mtDNA and nDNA markers, analyses identified 128 hybrid offspring, presumably resulting from several generations of hybridisation or backcrossing. Furthermore, an assessment of the relative genetic contribution from each parent species was made. Detailed morphological measurements that had been taken from individuals inside the hybrid zone and from an additional 76 individuals living in several other adjacent states were then compared with the genetic data. The results brought some remarkable insights to light. Overall, hybrids possessed a large amount of morphological variation, but when these variations were sorted according to the amount of DNA each parent species had contributed, it emerged that they were physically identical to the parent species that had contributed the most to its genome. Even in cases where hybrids were considered to be genetically 'intermediate', their physical appearance was not intermediate. The result of this study strongly suggests that hybrids cannot be reliably determined on account of their appearance only and as a consequence, occurrences of natural hybridisation may be underestimated in general. As far as the focus of the study was concerned, the researchers suggested that these findings should be considered when inferring from the fossil record hybridisation or lack thereof among human ancestral species.[228]

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[227] Sachi Sri Kantha et al, 2007, Sleep Parameters in captive Female owl Monkey (Aotus) Hybrids, Neotropical Primates, 14(3), December 2007 pp 141-144,

[228] Michigan News, 2012, http://www.ns.umich.edu/new/multimedia/slideshows/21025-monkey-business-what-howler-monkeys-can-tell-us-about-the-role-of-interbreeding-in-human-evolution

Unlike most monkeys, all apes are tailless. Compared to the great apes, gibbons are very small in size, weighing between 4 and 13 kg only and are therefore called the 'lesser' apes. These mostly frugivorous animals inhabit the dense tropical forests of southern Asia, where they rarely descend to the ground. Instead, they move through the tree tops by leaping and *brachiating* with their especially long arms. This way of locomotion allows them to cover a distance of up to 56 km in only one hour.[229] According to their different diploid chromosome numbers, gibbons are classified into four genera. In the wild, it is difficult to distinguish the various species by the colour of their fur, therefore, identification has to be by genetics or by their songs, which can be long and complex as in the case of mated pairs. Gibbons are monogamous, which is rare among primates.[230]

Because they are difficult to identify on account of their appearances, this has led in the past to misidentification and the establishment of mixed species breeding pairs in zoos. In many cases, the problem was unknowingly passed on to other, unassuming institutions that thought to be receiving pure-bred animals, when in fact, they were receiving hybrid offspring of crosses from different species.[231]

> "...misidentification and consequent hybridisation are serious problems in zoos. (Surveys of European and North American zoo gibbons in the early 1980s found that over 4% were definitely hybrids, and the real total would undoubtedly be considerably higher.)" (Gould N. 1995)[232]

Within the group of the common gibbon (*Hylobates lar*) and its subspecies, three geographic areas are known to be shared by different species producing hybrid offspring: (1) At the headwaters of the Takhon River in Khao Yai National Park, Thailand, a ca. 100 km^2 hybrid zone of *H. larand* [sic] and *H. pileatus* exists, where hybrids make up 5% of the breeding population. (2) In the northwest of the Malaysian peninsula near the tributaries of the Muda River, a group of *H. agilis* and *H. larat* [sic] were seen with their hybrid offspring on the shores of an artificial lake. (3) On the island of Borneo in Kalimantan, Indonesia, a stable hybrid population of *H. agilis* x *H. muelleri* exists in a hybrid zone that extends over 3.500 km^2. No pure-bred individuals were sighted in the area.[233]

As mentioned above, gibbon vocalisation is elaborate and not only sex- but also species-specific and can, therefore, be used for species identification. In the 1980s,

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[229] http://animals.nationalgeographic.com/animals/mammals/gibbon/

[230] https://en.wikipedia.org/wiki/Gibbon

[231] Geissmann T., 1995, Gibbon Systematics and Species Identification, International Zoo News Vol. 42/8 (No. 265) December 1995, p 468

[232] Gould N. 1995, Editorial, International Zoo News Vol. 42/8 (No. 265) December 1995, p 466

[233] Geissmann T., 1995, Gibbon Systematics and Species Identification, International Zoo News Vol. 42/8 (No. 265) December 1995, p 478

vocalisations of three female and two male F_1 hybrids were recorded and analysed. They were offspring from crosses between the pileated gibbon (*Hylobates pileatus*) and the white-handed gibbon (*H. lar*) that were living in several West German zoos at the time. The hybrids were found to have their own song repertoire, which was distinct from each parent's song and which, in the case of one female hybrid, did not change over the span of six years. One other female hybrid acted like a male and performed male-like songs for several years, as long as she was part of her natal group. After one male hybrid mated with a white-handed gibbon, he sang, though infrequently, duets with his mate. His song was a typical male song; however, one phrase that he sang in synchrony with the female resembled closely that of the great calls[234] of female gibbons. "Although this male has never heard other great calls than those of *H. lar* females, his great call-like vocalizations were virtually identical to those of female F1-hybrids." [235]

Great apes, humankind's not so distant cousins in the animal world, comprise of the gorilla (genus *Gorilla*); the bonobo, also known as pygmy chimpanzee (*Pan paniscus*); and the chimpanzee (*Pan troglodytes*). All are native to Africa, where they inhabit the rainforests, forests and savannahs. They are mostly herbivorous but will supplement their diet with insects and, in the case of the chimpanzees, also with the occasional small animal they hunt down in groups. Lowland male gorillas weigh up to 180 kg while their mountain gorilla counterparts may reach a weight of 220 kg. The African great apes live in groups, which is the extended family in the case of gorillas or, in the case of the chimpanzees, large communities comprising up to 120 individuals. The orang-utan is the Asian large ape that lives in the tropical forests on the islands of Borneo and Sumatra. Males can reach 200kg as well, but unlike the African apes, orangutans are semi-solitary and only get together with their kind for procreation.[236]

Gorilla taxonomy had been controversial for some time, but four distinct taxa are now recognised: the western gorilla (*Gorilla gorilla*), comprising the two lowland gorilla subspecies *G. g. gorilla and G. g. diehli*; and the eastern gorilla (*Gorilla beringei*), comprising the eastern lowland gorilla subspecies *G. b. graueri* and the mountain gorilla subspecies *G. b. beringei*. Based on morphological analyses in combination with mtDNA sequence data, a research team at the University of Capetown in South Africa, has convincingly argued that the eastern lowland gorilla (*G. b. graueri*) is a hybrid of the mountain gorilla (*G. b. beringei*) and the western lowland gorilla (*G. g. gorilla*). Skulls for measurements had come from museum collections and for molecular analyses published mtDNA sequence data had been used. In a previous study, one of the authors and colleagues had identified a high frequency of traits in baboon hybrids that were not shared with their parental species, which were one or two additional

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[234] see: Singing Behavior at http://www.gibbons.de/main/index.html

[235] Geissmann T., 1987: Songs of hybrid gibbons (*Hylobates lar* x *H. lar*). International Journal of Primatology 8: 540 (Abstract only).

[236] http://www.factmonster.com/spot/ape2.html#go

teeth in the lower jaw and unusual facial bone sutures. These same traits were found in the eastern lowland gorilla with a higher frequency than in the mountain gorilla or in the western lowland gorilla. In addition, cranial measurements for the eastern lowland gorilla could be described as ‘intermediate’ between the mountain gorilla and the western lowland gorilla, with some measurements falling outside the range of either of the putative parental species. Results for *G. g. diehli* were less definitive due to the paucity of specimens. The findings that *G. b. graueri* is a hybrid of the mountain gorilla (*G. b. beringei*) and the western lowland gorilla (*G. g. gorilla*) are supported by genetic analyses that show introgression from the western lowland gorilla, which had evidently migrated from the west to the east.[237]

The river Congo in west-central Africa is a natural barrier between the habitats of the common chimpanzee (*Pan troglodytes*) and the pygmy chimpanzee or bonobo (*Pan paniscus*). Consequently, there are no accounts of any hybridisation in the wild between the species. However, in captivity, they are known to have crossbred and produced viable hybrid offspring. In a brief paper, two researchers reported on such occurrences involving one bonobo male and two female chimpanzees. Of five pregnancies, three resulted in three hybrid offspring, one male and two female. When compared to infant chimpanzees, the hybrids’ ears were dark and smaller and their faces were darker and less *prognathous* (jaws projecting beyond the upper face). They had the pink lips and the prominent side whiskers of the bonobo but were lacking the webbing in the second or third toe, which is yet another distinction of the bonobo from the chimpanzee. In addition, the females’ genitalia were larger than chimpanzees’. As to their vocalisations, the researchers detected characteristics that belong to both parental species. When the male hybrid was one year old, he did not show the typical aggressiveness of a chimpanzee. He also displayed a liking for water and had the habit of walking bipedally with ease.[238]

The Asian representative of the great apes, the orangutan (genus *Pongo*), originally native to Malaysia and Indonesia, is now only found on the islands of Borneo and Sumatra, where they are listed as endangered. Beginning in 1971, orangutans had been taken to a camp in Tanjung Puting National Park on the island of Borneo and subsequently, 90 animals had been released into the park. At the time, all orangutans were thought to belong to the same species, until genetic analyses classified them as two distinct species, the Sumatran orangutan (*P. abelii*) and the Bornean orangutan (*P. pygmaeus*), the latter containing three subspecies. When 44-year-old park records

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[237] Ackermann R.R., Bishop J.M., 2009, Morphological and molecular evidence reveals recent hybridization between Gorilla taxa, Evolution 64(1):271-90

[238] Vervaecke H., Elsacker V., 1992, Hybrids between common chimpanzees (Pan troglodytes) and pygmy chimpanzees (Pan paniscus) in captivity, Mammalia 56(4):667-669

were reviewed, it was discovered that inadvertently two non-native females had been released, which had then mated with native males and born twenty-two hybrid offspring between them. While one of the females had produced 14 healthy offspring (two had died in infancy) that were still alive at the time, the other female had produced fewer surviving offspring than any other female in the park. One of them was stillborn, one died when young, two died in infancy, and of the ones that survived, one male and one female were sickly and needed frequent medical attention. These findings were found to be alarming because more than 1500 orphaned and displaced orangutans are currently scheduled to be re-introduced into the wild. Researchers are calling for genetic testing beforehand and international guidelines, in order to prevent the potential for endangering the health of future wild populations through hybridisation.[239]

Hybridisation in Animals and its Many Outcomes

The reader may agree with the authors that the picture that has emerged is astounding. The abundance of cases the American geneticist Eugene M. McCarthy has described in his book *On the Origins of New Forms of Life, A New Theory* (McCarthy 2008) and the examination of the relevant academic literature available on the internet are demonstrating two important points:

(1)Hybridisation, accepted to be common among plants, is also common in the animal world and especially so among birds, fishes, insects and non-human primates. It is, however, not always identified or recognised as such -

> Most estimates of the extent of hybridization among animal species are based on morphological identification of hybrids in hunted or museum specimens. Thus, it is possible that some hybrids are erroneously recorded. However, it is also not unlikely that the frequency of hybridization has been underestimated. Groups with high recorded rates of hybridization tend to contain brightly coloured or otherwise morphologically rather distinct species. Hybridization may be strongly underestimated when we consider that many pairs of species are only weakly diagnosable using morphology. Probably, many rare hybrids are simply lumped with one of the parents in such groups. (Mallet)[240]

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[239] Beall A., FOR MAILONLINE, 2016, Rise of the hybrid 'cocktail' apes: Orangutans interbreeding in Indonesia are threatening the already endangered species, http://www.dailymail.co.uk/sciencetech/article-3465359/Rise-cocktail-apes-Different-species-orangutans-interbreeding-Indonesia-create-hybrid-animals.html

[240] Mallet J. Hybridization as an invasion of the genome. Trends Ecol. Evol. 2005;20:229–237

In a similar vein, innumerous cases are mentioned in the literature where populations lost their species recognition when they were found to be of hybrid origin. Instead, they were given either subspecies status or were lumped together with a parent species.[241]

(2) Contrary to what is widely assumed, hybrids are frequently partially fertile and are by no means the evolutionary dead-end they are commonly believed to be. As a matter of fact, hybrid fertility is dependent on many determinants and is very much a matter of degree, of sex and the direction of the cross.

Indeed, there are hybrids that are partially fertile in both sexes like the crosses between the Bactrian camel and the dromedary. Furthermore, there are cases where both sexes are fully fertile like the hybrids from certain lemur crosses. Similar to matings within single species populations, fertility in species that cross with other species depends on the obvious requirements such as the age of the individuals, conditions under which matings occur and whether ecological needs are met. In addition, hybrid viability and fertility vary to a great extent from species to species, individual to individual and from cross to cross and may also depend on the direction of the parental cross. A cross may be partially viable or fertile in one direction, while the reciprocal cross may be nonviable or infertile, as was shown with hybrids of the North American mule deer and the white deer, for instance. Fertility varies with the sex of the hybrid, as was observed by J.B.S. Haldane and formulated in Haldane's rule. Mammalian male hybrids are generally sterile while female hybrids are mostly fertile. In avian hybrids, the roles are reversed; females are virtually almost sterile while the males are partially fertile. But, of course, there are exceptions to the rule as mentioned above. Although the mismatch of the parental karyotypes, as was seen with the example of the mule, can be an important cause of hybrid sterility, here too are exceptions to what is believed to be the rule as is demonstrated in hybrids from crosses between owl monkeys of different karyotypes. One is reminded of the gibbons; there were no chromosome mismatches suspected or detected among hybridising gibbons that were held in various zoos before the application of molecular technologies pointed to the existence of different species. In short, among F_1 hybrids or after backcrossing, nearly every condition can be found on a spectrum of fitness from complete sterility to complete fertility. It would indeed be correct to say that hybrids are partially fertile or sterile and that in countless cases partial fertility increases to full fertility after backcrossing with one of the parent species. Immunological factors can also play a role in embryo viability as was described with turkey hens (*Meleagris gallopavo*) that were fertilised with sperm from chicken cocks (*Gallus gallus domesticus*) and the hens developing antibodies against the

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[241] For more detailed information see: McCarthy E.M., 2008, http://www.macroevolution.net/hybrid-species.html

spermatozoa[242]. A similar immune reaction was suspected in the earlier mentioned experiment with a mother goat that had received skin grafts and injections of ram leukocytes with subsequent hybrid embryo death.

Once a viable hybrid is born, its fertility, or lack thereof, depends on a number of conditions. Testes or ovaries may not be fully developed, or, if developed, they may be incapable of producing sperm or eggs. If they are present and functioning, reproductive cells may be of varying quality. In the case of spermatozoa, some or all may be of a variable size or they may be irregularly shaped as is the case in earlier described rock wallaby and beefalo hybrids. But when the partially fertile hybrid backcrosses with the parent species, fertility improves with each subsequent generation and is eventually restored.[243]

What is to be said about hybrid traits? As a general rule, the phenotype, the observable traits of any kind, may that be physical ones like body shape, colour, size etc. or behavioural ones are found to be in many cases a mixture of those possessed by the parents. Among the countless reportings of 'intermediate' morphologies, one recalls the intermediate size of the mule and the number of the wholphin's teeth. In addition to gestation and incubation periods that are for the most part also intermediate, the vocalisations of many hybrids are a mix as well. Avian hybrids often combine the song of their parents, as do the hybrids of certain gibbon crosses. Even traits like the echolocation call frequency is found to be intermediate in the presumed hybrids of the smaller or eastern horseshoe bat (*Rhinolophus megaphyllus*) and the greater large-eared horseshoe bat (*Rhinolophus philippinensis*)[244] and, astonishingly, some hybrids also produce a mixture of proteins according to the different genetic instructions that were received from each parent. For instance, the venom of crosses between the jumping pit viper (*Atropoides nummifer*) and Picado's pit viper (*Atropoides picadoi*) was analysed for its proteins and found to be intermediate between that of the parent species[245].

Furthermore, parents' traits can also be compounded and appear in the hybrid offspring in a combination, making them look more like one or the other parent. Sometimes this may cause confusion as was described in the case of the cross between a dusky grouse (*Dendragapus obscurus*) or a sooty grouse (*Dendragapus fuliginosus*), both known as the blue grouse, and a sharp-tailed grouse (*Tympanuchus phasianellus*) that could be mistaken for either of its parents, depending on from which end it was

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[242] E.M. McCarthy, 2008, On the Origins of New Forms of Life, A New Theory, http://www.macroevolution.net/support-files/forms_of_life.pdf footnote a), p.38

[243] Idem, p.40,

[244] idem, p. 43

[245] Idem, p. 44

seen.[246] Combined traits were also noticed in the vocalisation of the eastern coyote, the three-way or compound hybrid, where one finds the distinct deep pitch of one parent added to the high pitch of the other.

And last, there is heterosis or hybrid vigour in animals. In plants, it is a well-understood phenomenon that is expressed by higher yield and greater performance and robustness. When a hybrid trait like size, vigour or resistance falls well outside the range of parental variation, the trait is said to be heterotic. The 'monster' salamander comes immediately to mind, or the mule with its increased stamina. Animal breeders have exploited this phenomenon since time immemorial to produce animals that develop faster, grow larger and stronger and are easier to work with than either of their progenitors. As was seen earlier, heterosis is also found in hybrid fishes and extra vigour is often the reason behind their creation for sport fishing and pond management. One example is the earlier described hybrids from certain sunfish crosses that are more vigorous and aggressive than their progenitor species. Research showed that this was at least partially due to the combination of distinct haemoglobin subunits they had received from each parent, which was having an enhanced effect on the hybrids' blood chemistry.[247]

Summary

Except for a book on interspecific avian hybridisation by the American geneticist E. McCarthy, the subject of animal hybridisation is so far still only discussed in academic journals and not yet made accessible to a wider audience. The examples presented in this work are but a very limited selection of what has been recorded and which, quite likely, is an underestimation of their occurrences in the natural world. As has been demonstrated, hybrids cannot always be reliably determined according to their appearance only and, therefore, occurrences of natural hybridisation may be generally underestimated. Nevertheless, in the animal kingdom, the process of natural hybridisation producing partially fertile offspring is widespread and needs to be acknowledged as a matter of biological fact. In the case of the caribou, for instance, it could be traced back to the end of the Pleistocene epoch. The examples of the Lonicera fly, the Africanised bee and the brown Argus butterfly demonstrate that hybridisation is an ongoing process that is, among other factors, driven by human activity and/or climate warming. While some hybrids may be evolutionary dead-ends, the vast majority, evidently, is not. Indeed, as is shown in the case of the eastern coyote and the cross between the mule deer and the white-tailed deer, hybrids can be reproductively so successful that their populations eventually grow into huge herds and countless bands respectively and thrive over thousands of square kilometers of

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[246] Idem, p. 43

[247] McCarthy E.M., 2008, http://www.macroevolution.net/heterosis.html access date 5/16

the North American land mass. Consequently, one begins to wonder what the position of hybridisation is in the grand scheme of evolution.

Introgression and hybrid success can be, as was demonstrated in several cases, a double-edged sword. Occasionally, introgression from a not too distantly related species can introduce variation into a declining population's gene pool and with it a much-needed boost to restoring fitness. Conversely, as is sometimes feared by conservationists, it can potentially be the final blow to sending a weakened, pure parental species into extinction by being swamped with another species' genotype. On the other hand, it can be claimed that hybridisation is an important source of evolutionary innovation. It can be seen as a creative force that forges new combinations from two (or more) distinct parental genotypes, which can lead to new traits. One is reminded of the hybrid blacktip shark with its specific morphological distinctions, including the difference in the number of vertebrae, and the new traits in fishes that are being exploited for commercial use. Hybridisation can even lead to the formation of new species as is the case of the many cichlid species, the tetraploid hybrid lizard, the Heliconius hybrid and the Lonicera fly. Nevertheless, species status may sometimes be disputed as was the case with the eastern coyote, where the argument refers back to the species conundrum. Hybridisation as a creative force for innovation is well demonstrated in Darwin's finches. It is hybridisation that conferred on them the new traits that enabled them to exist on the type of seeds that were available after *El Nino's* changing impact on the environment. What is more, these transformations happen during the lifetime of only a few generations of a population and not thousands of years, as orthodox evolutionary theory stipulates.

Hybridisation and Human Evolution

> The ape is likest to man in viscera, muscles, arteries, veins, nerves and in the form of bones.
>
> Claudius Galenus[248]

These are the words of the Greek physician and philosopher, commonly known as Galen of Pergamum (130 – ca. 216 CE). The resemblance of non-human primates to man was already recognised by the Greek philosopher Aristotle (384 – 322 BCE) and the Greek physician Hippocrates (460 – ca. 375 BCE), but it was Galen who put the proposition to the test by dissecting Barbary apes (*Macaca sylvanus*), which are not apes but monkeys. Apes were not known in the west until the 15th century. It is important to note that this resemblance was taken as pure coincidence because, at the time, no knowledge existed of biological evolution. The notion of living things changing over time through the influence of the environment or even by chance was speculated on for the first time by the French mathematician and naturalist George Louis Leclerc,

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[248] Historical background of primate studies, https://www.britannica.com/animal/primate-mammal/Snouts-muzzles-and-noses

Comte de Buffon (1707 – 1788) late in the 18th century.[249] Roughly hundred years later in 1859, Charles Darwin would posit in *The Origin of Species* that all forms of life share a common ancestry and in 1871, in *The Descent of Man*, he proclaimed that humans share a common ancestry with the African great apes. -

> If the anthropomorphous apes be admitted to form a natural sub-group, then as man agrees with them, not only in all those characters which he possesses in common with the whole Catarhine group, but in other peculiar characters, such as the absence of a tail and of callosities and in general appearance, we may infer that some ancient member of the anthropomorphous sub-group gave birth to man. (Darwin 1871)[250]

With that, man's earliest antecedent became firmly established on the human evolutionary trajectory.

In 1856, fossilised remains of a seemingly primitive being that was later named 'Neanderthal man' had been found in a cave at Feldhofer in the Neander Valley in Germany. In 1893, remains of 'Java man', another primitive human now known as *Homo erectus* (upright man), were unearthed in Indonesia. When in 1912 'Piltdown man' (decades later exposed to be a hoax) was discovered in England, the European scientific community was convinced that humans had evolved in Europe or Asia. This view was, however, challenged in 1924 by the anatomist Raymond Dart's discovery of a fossilised, humanlike child's skull and brain near Taung in South Africa that would become known as the 'Taung child'. The following year, he wrote in the journal *Nature* that he had uncovered "an extinct race of apes *intermediate between living anthropoids and man"*[251] and called the specimen *Australopithecus africanus* (southern ape of Africa). During subsequent years, more fossil remains of adult 'ape-men' were found in Africa, and with ensuing systematic investigations, a skeptical scientific community became gradually convinced that australopithecines were members of the human family and that Africa was the cradle of mankind. In more recent times, biochemical evidence has allowed for an approximate calculation that places the split of man and his closest relative, the chimpanzee, from the last common ancestor between 4 and 7 million years ago.[252]

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[249] http://anthro.palomar.edu/evolve/evolve_1.htm
http://linnaeus.c18.net/Letters/display_notes.php?id_letter=L0783&id_person_from=1112&id_person_to=981&code_from=LinnaeusC&code_to=GmelinJG

[250] Darwin Online, p 197 http://darwin-online.org.uk/content/frameset?itemID=F937.1&viewtype=text&pageseq=1

[251] Wayman E., 2011, How Africa Became the Cradle of Humankind, http://www.smithsonianmag.com/science-nature/how-africa-became-the-cradle-of-humankind-108875040/?no-ist

[252] Figures vary from 4 to 10my depending on source

The scientific disciplines that investigate and seek to understand man's evolutionary relationships with pre-human antecedents on the one hand and the evolution of the genus Homo and his extinct forebears are palaeoanthropology and evolutionary anthropology. These disciplines use a diversity of approaches that are informed by the social, medical and natural sciences. What began largely as a study in comparative anatomy and racial classification has grown over the span of more than a century into a multi-disciplinary field of study that encompasses disciplines as diverse as primatology and genetics.

Since the discovery of the roughly three-million-year-old 'Taung child' 90 years ago, thousands of fossilised remains of different species of early *Homo* and their presumed predecessors and descendants have been unearthed. South Africa and the East African Great Rift Valley in Kenya, Tanzania and Ethiopia have yielded the oldest specimens so far. Many more remains of hominins[253] were found in Europe and Asia that had either migrated there from Africa or had evolved there. Exciting discoveries of fossils and associated material cultures continue to be made in Africa and in other regions of the Old World, generating headlines and praise but also controversy. Publications and hypotheses about the findings follow in due course, many of them backed in time by solid evidence and others that don't stand up to scientific scrutiny. The story of these discoveries exceeds the scope of this work, but a few important milestones need to be mentioned here.

'Ardi' (*Ardipithecus ramidus*) got his name for his presumed closeness to the root of humanity, as 'ramidus' means 'root' in the Afar language of Ethiopia, where he was found. However, this 4.4 million-year-old (myo) and 1.2 m tall hominin was less well adapted to bipedality than 3.4 myo 'Lucy' (*Australopithecus afarensis*), whose 40% complete skeleton bears witness to her ability to move equally well in trees as on her two legs on the ground. *Homo habilis* (aka handy man), the first fossil remains to be classified in the genus *Homo,* lived between 1.5 – 2.4 million years ago (mya) and because simple stone flakes were discovered in the same stratigraphic layer as the fossils, this hominin was believed to have used stone tools, hence the name 'able' or 'handy man'. It has to be said that there is little unanimity among palaeoanthropologists, the scientists who study the fossil record. 'Lumpers' often assign variant fossils to existing species, arguing that morphological differences fall into the normal range of variation, while 'splitters' prefer to assign a fossil to a new species on account of these same differences. Accordingly, some believed that there is too wide a range of variation in the *H. habilis* specimens and therefore assigned some of the fossils to another species, *Homo rudolfensis*, named after a specimen that was found near Lake Turkana, formerly Lake Rudolf. Many more discoveries of fossil remains belonging to the genus *Homo* were to follow. The first hominin found in Africa

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[253] For recent hominoid taxonomies see: http://cogweb.ucla.edu/ep/Hominoids.html

as well as in Asia and Europe is *Homo erectus* (upright man)*,* who was fully bipedal, although he may not have walked exactly like we modern humans. The African specimen is dated to be 1.5 myo and younger, while specimens in Europe and Asia are dated to be around 1.8 myo and some much younger than that. 'Turkana boy' (*Homo ergaster*) or 'working man' with an estimated age of 1.6 myo and found near Lake Turkana in Kenya was another, spectacular discovery. The nearly complete skeleton revealed a bodily physique very similar to that of modern humans that left no doubt about its bipedality. Some believe that *H. ergaster* may have been more efficient at walking than modern humans, whose pelvis changed with the increasing brain size of their infants at birth. Even though *H. ergaster'*s skull differs in shape and size from the Asian *Homo erectus*, some palaeoanthropologists classify him with the latter.[254]

Equally, there is little consensus on how the many fossil remains are related to each other and what the human family tree or rather 'family bush' should look like. What can be said, though, is that through the millennia from partial to complete bipedality the fossil record manifests a general trend: an increase in overall body size as well as an increase in brain size relative to body size; a decrease in skeletal robustness and tooth size; an increase in sophistication of tool making from rough stone flakes to expertly crafted tools for specialised purposes: and finally the appearance of art.[255]

It should be of interest to know that earlier in hominin history, several genera existed simultaneously. For example, in Africa, *Homo* lived temporally and geographically alongside at least two, if not more australopithecine species. And, in the context of this work, it is important to know that several hominins of the genus *Homo* were alive at the same time. For instance, *H. erectus, H. neanderthalensis, H. floresiensis* (believed to have evolved from *H. erectus*) and another extinct human species, the recently discovered 'Denisovan', coexisted temporally and in some cases also geographically with *H. sapiens* in Europe and Asia. There is one more, important species to be mentioned, it is *H. heidelbergensis.* He is assumed to have originated in Africa and migrated to Europe, where he is believed to have given rise to H. *neanderthalensis about 300,000 years ago,* though there is no consensus here either. There are others who believe that *H. heidelbergensis* is the direct ancestor of our lineage, *H. Sapiens* or 'wise man', who is thought to have evolved in Africa 195,000 years ago. According to the widely accepted out-of–Africa hypothesis, anatomically modern humans left Africa about 60,000 years ago and spread throughout the rest of the Old World where they met with other hominins such as *H. neanderthalensis* in Europe and western Asia and the descendants of *H. erectus* in Asia.[256]

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[254] http://www.talkorigins.org/faqs/homs/specimen.html
[255] Idem
[256] Idem

Of all the hominins that have existed, only modern humans have survived to the present day. What, for example, happened to the Neanderthals? In the European fossil record, hominins with morphological features that would become typical of Neanderthals' first appeared about 400,000 years ago, and after having evolved into their unmistakable form then disappeared from the fossil record about 30,000 years ago. What is the reason for their disappearance a few thousand years after modern humans' first presence in Europe? Much speculation exists but little agreement about the role modern humans may have played in the Neanderthals' extinction. A few scientists argue that they did not become extinct but were assimilated by modern humans, and the Portuguese palaeoanthropologist João Zilhão is a proponent of this theory. In 1999, Dr. Zilhão and his team of archaeologists uncovered from a shallow grave in the Lapedo Valley north of Lisbon the skeleton of a young boy that was dated to be 24,500 years old. Palaeontologists were joined by Dr. Erik Trinkaus, Professor of Palaeoanthropology at Washington University in St. Louis, USA, and a widely recognised authority on Neanderthal palaeontology, who examined the remains and pronounced them to belong to a Neanderthal and early modern human hybrid. He went even further and declared the skeleton to be not only the physical evidence of interbreeding between the two groups but to be a descendant of generations of Neanderthal and Cro-Magnon[257] hybrids. Referring to the morphological evidence before him he said -

> "It's a complex mosaic, which is what you get when you have a hybrid" and added "This is the first definite evidence of admixture between Neanderthals and European early modern humans." (Wilford, 1999)[258]

A detailed report in the journal *Proceedings of the National Academy of Sciences* left the scientific community divided. Some rejected the interpretation, others found the findings intriguing, asserting that Neanderthal affinities can be seen in some specimens of archaic and modern *H. sapiens*.[259]

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[257] Cro-Magnon are the earliest modern humans in Europe, named after the rock shelter in south-western France where they were found.

[258] Wilford J.N., 1999, Discovery Suggests Humans Are a Bit Neanderthal, NY Times, April 25 http://cogweb.ucla.edu/ep/Neanderthal.html

[259] Idem

Figure 30. Human skulls. Modern human left, Neanderthal or ‘archaic’ human, right https://commons.wikimedia.org/wiki/File:Sapiens_neanderthal_comparison_en_black background.png Author: hairymuseummatt (original photo) DrMikeBaxter (derivative work,) Source: http://www.flickr.com/photos/hmnh/3033749380/ (original photo)

Two years earlier, a German/American team led by Swedish geneticist Svante Pääbo, then at the Zoological Institute of the University of Munich, Germany, had succeeded in extracting, amplifying and sequencing mtDNA from the Neanderthal type specimen that had been discovered in the Feldhofer cave in Germany in 1856. It was the first time that this kind of work had been attempted with a fossil that old (ca. 40,000 years) and for that purpose, a 3.5 gramme sample had been cut out of the *humerus* (upper arm bone) of the German national treasure, which is permanently housed in the Rheinisches Landesmuseum in Bonn, Germany.

Earlier in this work, the reader learned how the mitochondrion became an essential organelle in the eukaryotic cell and that it supplies the cell with the energy it needs to work properly. The reader will also remember that, having started out as independently living organisms, mitochondria have their own, small genome that exists alongside the nDNA, which is contained in the nucleus of the cell. Working with mtDNA has its advantages. For example, cells contain an abundance of mitochondria, which allows the extraction of sufficient amounts of its DNA. In addition, because mtDNA is transmitted through the generations only by the mother, changes derive exclusively from mutations and not from recombination as is the case with the nDNA that is provided by both parents. When the first Neanderthal mitochondrial genome was sequenced in 1996, a common reference sequence of modern mtDNA existed already, which had been obtained from sequencing mtDNA from thousands of contemporary humans around the world. This reference sequence was now used as a benchmark against which the researchers compared the sequence of the iconic fossil. The evidence before them was clear. Although showing close affinity to modern humans, the Neanderthal sequence contained changes that were not existent in any of the thousands of sequences obtained from contemporary humans. Before publishing their findings, the team asked their colleague, population geneticist Mark Stoneking at Penn State University, USA, and his team, to duplicate the work they had done. The American team arrived at the same conclusion. Between the Neanderthal sequence and that of a contemporary human, an average of 28 differences was counted, whereas differences among people alive today account for only 7 differences on average. In addition, based on the changes in the mtDNA, it was estimated that Neanderthals and modern humans shared a female common ancestor ca. 500,000 years ago, three to four times deeper in the past than modern humans had shared their common female ancestor, the famous 'mitochondrial Eve'. This calculation is more or less in accordance with the fossil record. With the added caveat that mtDNA offered only a limited view because it was exclusively transmitted by the female, their findings were published in July 1997 in the journal *Cell,* proposing[260]-

> The Neandertal mtDNA sequence thus supports a scenario in which modern humans arose recently in Africa as a distinct species and replaced Neandertals with little or no interbreeding. (Pääbo 2015)[261]

By 2000, several independent laboratories had sequenced Neanderthal fossils but none of the results contradicted these findings.

2001 saw the first draft version of the present-day human genome sequence with its completion in 2003, which was followed by a draft sequence of the chromosomal genome of man's closest ape relative, the chimpanzee, in 2005. Neanderthals and modern humans had coexisted in Europe for several thousand years, and the hotly debated question was still lingering, did they have sexual encounters and if so, would it be possible to find a trace of it in modern humans' genome?

After the collaboration of several institutions and many scientists and further improvements in sequencing technologies, the first sequencing results and analysis of Neanderthal chromosomal or nDNA were reported in the journal *Science* in 2006. Additional analysis of 1 million base pairs, the complementary nucleotides that make up the rungs in the double-stranded DNA, followed in the journal *Nature*. Sequencing

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[260] Pääbo S.,2015, Neanderthal Man: in search of lost genomes, Philadelphia, p. 1-19

[261] idem p. 19

results had been used to calculate the human/Neanderthal divergence time and according to their findings, Neanderthals and present-day humans had shared a most recent common ancestor ca. 706,000 years ago. Furthermore, ancestral populations of humans and Neanderthals had separated ca. 370,000 years ago, which was before the emergence of anatomically modern humans.[262] In addition, comparison of Neanderthal DNA sequences with human genomes had shown that they diverged about 500,000 years ago.[263]

The task of mapping and analysing the complete Neanderthal genome, which is like ours made up of more than 3 billion base pairs was too much to be accomplished by one laboratory team only, so the officially named *Neandertal Genome Analysis Consortium* was established. It consisted of 25 members from several countries with expertise in population genetics, mathematics, computer science, and it even included a man who had once worked as a code breaker for the British and American intelligence agencies. The team's goal was finding the answers to many questions and one of them was, did Neanderthals have a closer relationship with Europeans than with humans in other parts of the world? Additional questions were, in which way did the two human genomes differ, and were there genes that had changed in interesting ways between then and now? In Europe, the most pressing question for some was, had the Neanderthals contributed DNA to the extant European population and if so, when had this happened?[264]

After the team had succeeded in producing an extensive library of short fragments of chromosomal DNA sequences by using several technologies, they could, at last, begin the process of mapping them. It was a process "...much like doing a giant jigsaw puzzle with many missing pieces, many damaged pieces, and lots and lots of extra pieces that would fit nowhere in the puzzle."[265] For the purpose, they matched Neanderthal DNA segments to the reference sequence of the present-day human genome and a chimp genome using computer algorithms. Positions where single nucleotides differed between one and the other in identical locations on the chromosomes were then identified. The differences are known as single nucleotide polymorphisms (SNPs)[266] and could be described as genetic signatures. The described methodology is the bread-and-butter, so to speak, to this type of genetic analysis.

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[262] Noonan J. P. et al, 2006, Sequencing and analysis of Neandertal genomic DNA, Science Vol 314, Issue 5802 abstract only, http://science.sciencemag.org/content/314/5802/1113

[263] Green R.E. et al, 2006, Analysis of one million base pairs of Neanderthal DNA, Nature 444, 330-336, abstract only http://www.nature.com/nature/journal/v444/n7117/full/nature05336.html

[264] Pääbo S.,2015, Neanderthal Man: in search of lost genomes, Philadelphia, pp. 169-171

[265] idem p. 153

[266] idem p. 173

At long last, they were ready to publish the first genome ever sequenced of man's closest relative, the extinct Neanderthal, and their detailed analysis '*A draft sequence of the Neandertal genome'* appeared in May 2010 in the journal *Science.* The project had been a colossal task, had taken four years to complete and had involved contributions from several groups and more than fifty scientists. Supplementary online material alone accounted for 174 pages. "The paper was more like a book than a scientific paper, as one palaeontologist put it".**[267]** DNA from three different Neanderthal individuals had been sequenced and the resulting more than 4 billion base pairs had been compared with the genome of five contemporary humans from Europe, West Africa, South Africa, Papua New Guinea and China. A number of genomic regions had been identified that in humans are involved in metabolism and in cognitive and skeletal development. Results showed that Neanderthals shared more genetic variation with present-day Eurasians than they did with people in sub-Saharan Africa, which suggested that Neanderthals and the ancestors of the Eurasians had mated before the latter spread out into Europe and Asia. According to the study, this exchange of genes had occurred between 50,000 and 80,000 years ago and all present-day non-Africans were carrying a Neanderthal DNA contribution of between 1 and 4%.**[268]**

As was to be expected, the scientific publication was accompanied by much fanfare in the press. The *Daily Telegraph's* headline read *'Humans share Neanderthal genes from interbreeding 50,000 years ago'.* There could be no more doubt, Neanderthals had mated with modern humans and a small portion of their DNA was living on in most people's genomes. But where and how had it happened? The region where Neanderthals lived stretches from Western Europe to the Middle East and beyond to the western parts of Asia as far as southern Siberia. The Middle East is a natural corridor from Africa into Eurasia and was probably the place where the two groups encountered each other. The fossil record demonstrates that about 100,000 years ago, anatomically modern humans lived in the Carmel Mountains in what is today Israel, where their remains were found in the Skhul and Qafzeh caves. It is worth mentioning here that some palaeontologists believe that the Skhul 9 remains show evidence of cross-breeding with Neanderthals**[269]**. Neanderthal remains and their material culture that were discovered in nearby caves at Tabun and Kebara, date to between 65,000 and 48,000 years ago**[270]**. It can be assumed that over the span of thousands of years, the two groups met there and mated with each other and that subsequently, modern humans fanned out from there into the Old World, taking Neanderthals' genetic legacy with them to Europe and Asia and eventually into the wider world.**[271]**

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[267] Pääbo S.,2015, Neanderthal Man: in search of lost genomes, Philadelphia, p. 218

[268] Green R.E. et al, 2010, A Draft Sequence of the Neandertal Genome, Science Vol 328, Issue 5979,

[269] https://www.coursehero.com/file/p3d7l45/Kebara-Qafezeh-and-Skhul-caves-Levant-Israel-and-Palestine-Qafzeh-or-Kafzeh-is/

[270] https://www.researchgate.net/publication/31942841_Thermoluminescence_Dates_for_the_Neanderthal_Burial_Site_at_Kebara_in_Israel abstract only

[271] Pääbo S.,2015, Neanderthal Man: in search of lost genomes, Philadelphia, pp. 197-198

But the story of interbreeding among human species doesn’t end here. In a cave in southern Siberia, archaeologists uncovered in 2008 some adult teeth and a fossil of a beautifully preserved finger bone. Subsequent comparison of its genome with that of modern humans and Neanderthals revealed that it belonged to a young girl that was closely related to Neanderthals and had lived 40,000 ago. The researchers, however, decided the DNA was distinct enough for the fossil to be classified as a new species and named this archaic human ‘Denisovan’ after the cave in which it had been found.

As is now known, all present-day humans carry between 1 and 4% of Neanderthal DNA, which means, different people have different amounts of the Neanderthal contribution in their genome. It suggests that the ancestors of present-day humans encountered Neanderthals on more than one occasion and in different places in Eurasia. Far away from the Siberian cave, the people of Melanesia, a region of the Pacific that includes New Guinea and surrounding islands, carry not only their share of Neanderthal genetic material. In addition, they have between 2 and 4% of Denisovan DNA in their genome, and recent studies suggest that less than that may be carried by people in the rest of the world. In order to learn more about this newly discovered archaic human and its genetic legacy, a group of researchers in the fields of medicine, anthropology, statistics and biotechnology led by Joshua A. Akey of Washington University in the US and Svante Pääbo went to work. They compared the DNA of archaic human ancestors, some of which is of uncertain origin, to genome sequences from modern people of many geographical regions including individuals from Papua New Guinea and the Bismarck Archipelago, off the north-eastern coast of New Guinea. Previous research had exposed large regions in the human genome where Neanderthal sequences were completely absent. This new study showed that the same regions were devoid of Denisovan sequences as well, which suggests that these stretches of DNA are unique to present-day humans. It is intriguing that some of the genes in these regions are associated with language and the development of the brain and will certainly be subject to intense future research. “This project, Akey said, helps realize the influence of hybridization with other species on the trajectory of human evolution”.[272]

This is still not the end of the human hybridisation story. In spite of modern humans having originated in Africa, the main focus of genomic research has so far concentrated on populations living outside the African continent. Geneticist Sarah Tishkoff of the University of Pennsylvania in the US and her colleagues changed that by sequencing the DNA of five individuals, each belonging to one of three groups of hunter-gatherers, the Hadza and Sandawe in Tanzania and the Pygmies in Cameroon. What they found were distinct genetic variants among the groups in regions that code for smell and taste; are responsible for the functioning of the immune system; determine the fat content of breast milk; are involved in the healing process after injury; and they found

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[272] Ancient Denisovan DNA excavated in modern Pacific Islanders, https://www.sciencedaily.com/releases/2016/03/160317150805.htm

new clues about the pygmies' short stature. But what was the biggest surprise to all was the discovery that all three representatives of these diverse populations shared appreciable segments of DNA with an unknown species with whom they had mated more or less at the same time when Europeans had done so with Neanderthals.[273]

And there is more. Most of the well-studied hominin fossils come from Africa, Europe and western Asia while East Asia has largely been overlooked. That changed, however, when in 2003 *H. Floresiensis*, dubbed the 'Hobbit' because of its small size and newly dated to between ca. 100,000 and 60,000 years old, was uncovered from a cave in Flores, Indonesia. Since then, many more archaeological diggings were carried out there and in parts of China. In 2012, excavations in caves in Southwest China yielded cranial bones, a jaw bone and teeth belonging to four or five individuals and representing a mysterious group of humans that was difficult to classify. Based on certain morphological characteristics, it was proposed by some at the time that they might belong to hybrids between modern humans and an archaic species. Indirect carbon dating had revealed an amazingly young age between 14,000 and 11,000 years.

In 2015, palaeoanthropologist Darren Curnoe of the University of New South Wales, Australia, and his team published a paper in *PLoS ONE* describing a *femur* (thigh bone) that had been excavated from Maludong (Red Deer Cave) in Southwest China. Dated to be 14,000 years old, the femur shows a mixture of characteristics, some of which belong to modern humans, others to *H. erectus* or perhaps *H. habilis*, the first named species in the genus *Homo*, who lived more than 1,5 mya in Africa. The researchers believe that the femur belonged, in fact, to a hybrid between a modern human and an unknown archaic species. At the same time the Curnoe team published their findings, the group that had discovered the Denisovan specimen in the Siberian cave a few years earlier reported the recovery of more remains from the same cave. This time, they had found Neanderthals, Denisovans and modern humans in the same stratigraphic layer that was dated to be ca. 50,000 years old. Furthermore, in another, slightly older part of the cave and again in one and the same layer, they found remains of Neanderthals, Denisovans and *H. erectus.*[274] These recent fossil discoveries in China and Siberia lend themselves to the hypothesis that a third, still unknown hominin was potentially interbreeding with modern humans in that part of the world.

Lo and behold, there is, indeed, genomic evidence in support of the fossil evidence! When he was passing through Asia on his way to Australia, *H. sapiens* did not only mate with Neanderthals and Denisovans but with yet a third hominin species. When Professor Jaume Bertranpetit at Pompeu Fabra University in Spain reviewed DNA

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[273] http://www.sciencemag.org/news/2012/07/genetic-code-tells-hunter-gatherer-tales

[274] Curnoe D., 2015 Bone suggests 'Red Deer Cave people' a mysterious species of human, http://phys.org/news/2015-12-bone-red-deer-cave-people.html#jCp

sequences that had been obtained from present-day Australians, Papuans and from the people of the Andaman Islands and mainland India, he found stretches in their genomes that matched neither the Neanderthals' nor the Denisovans' nor any other known hominin for that matter. The question is, who is this mysterious third species the ancestors of today's far eastern populations encountered and bred with? [275]

Other recent evidence concerns the Middle East. The reader will remember that there is fossil evidence in the Middle East that places anatomically modern human and Neanderthal remains and their material culture near the Carmel Mountains in northern Israel. He learned that many evolutionary anthropologists who accept the out-of-Africa model believe that the Middle East is the place where anatomically modern humans coming from Africa encountered Neanderthals for the first time and from where Neanderthal DNA was taken into the Old world by the ancestors of present-day humans. Meanwhile, more supporting evidence has surfaced that puts this hypothesis on much firmer ground. It is the fossil of a *cranium* (braincase) that was found in the Manot cave in western Galilee only 40 km from Amud and 48 km from Kebara, the places where Neanderthals had lived between 65,000 and 48,000 years ago. The cranium is dated to be 55,000 years old and displays a close affinity with Cro-Magnons, the robustly built early western Europeans, but it also shows some African features. This specimen is not only the earliest anatomically modern human found in the geographical corridor between Africa and Asia, it also fits spatially and temporally with Neanderthals' presence in this part of the world and further supports the idea of a first sexual encounter between Neanderthals and modern humans in the Middle East.[276]

Evidently, modern humans in Africa and outside of Africa once shared the same living space with several archaic cousins with whom they had sex and exchanged genes. Today's advanced gene technologies are continuing to uncover the traces of these encounters that present-day humans all over the world carry in their genomes in a varying amount. In addition, genomic, as well as fossil evidence questions the hitherto widely accepted view that anatomically modern humans left Africa and quickly colonised the Old World by replacing the hominin populations that were already living there.

So far, much has been said about gene flow from ancient lineages into modern humans, which left its traces in contemporary populations' DNA. Of course, introgression was not unidirectional. Recently, scientists discovered not only the first

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[275] Homo sapiens interbred with THIRD species of hominin on way to Australia: DNA study finds mystery new ancestor, http://www.dailymail.co.uk/sciencetech/article-3707766/Mystery-ancient-human-ancestor-DNA-Andamanese-tribe-Experts-say-previously-unknown-extinct-hominin-bred-human-migration.html

[276] Skull discovery suggests location where humans first had sex with Neanderthals https://www.theguardian.com/science/2015/jan/28/ancient-skull-found-israel-sheds-light-human-migration-sex-neanderthals

evidence of modern human DNA in a Neanderthal genome, they also estimated that this admixture occurred much earlier than all other admixtures that were previously documented. The Neanderthal genetic material in question was extracted from an individual found in the Altai Mountain in Siberia and when compared with genomes from modern populations across Africa, several SNPs that most modern Africans are carrying could also be identified in the Neanderthal individual. Their findings imply that 100,000 years ago a first group of anatomically modern humans left Africa and met and mated with archaic humans who already lived in Eurasia just like successive groups of modern humans would do approximately 40,000 years later.[277]

Modern Humans' Genetic Heritage Through Admixture with Ancient Species

One might wonder what are the functional implications of the admixture of genetic material from archaic humans for *Homo sapiens*. It was mentioned earlier that a modern Eurasian individual may have anywhere between 1% - 4% of Neanderthal DNA in his or her genome and that, in addition, Melanesians may have inherited up to 6% of DNA from the Denisovans. However, the variants found across contemporary Eurasian populations differ in the sense that different people carry different variants of Neanderthal DNA. When all these variants are added together, they represent more than 20% of the complete Neanderthal genome, while across the populations in East Asia, the amount may be up to 40%. A possible explanation is that East Asians acquired an additional amount at a later time.[278]

Since the publication of the first draft of the Neanderthal genome in 2010, research methodologies are steadily being improved and genome technologies, in particular palaeogenomics, continue to advance and do so at sharply reduced cost. Sequencing now yields high-quality and detailed readouts that allow looking for patterns and formulating a host of new questions. When examining contemporary peoples' genomes, some regions stand out for being completely devoid of Neanderthal variants, while other areas are especially rich in genetic code. For instance, there are many Neanderthal variants in genes relating to *Keratin.* Researchers found that nearly 80% of Eurasians carry a Neanderthal version of the gene that codes for the production of this protein.[279] Keratin is the main structural component of feathers, hoofs, claws, horns and also of human skin, hair and nails that makes them tough. Thicker hair and tougher skin help reducing heat loss and would have been beneficial for newcomers to colder climatic conditions.

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[277] Early gene flow from modern humans into Neanderthals, https://www.sciencedaily.com/releases/2016/02/160217140315.htm

[278] https://www.geneticliteracyproject.org/2015/02/23/why-do-asians-have-twenty-percent-more-neandrathal-dna-than-europeans/

[279] http://www.bbc.com/earth/story/20151116-what-did-the-neanderthals-do-for-us

Another group of genes is involved in skin pigmentation, and findings imply that to a certain extent, Eurasians may have inherited their pale skin from the Neanderthals. The spectrum of human skin pigmentation between light and dark is, among other factors, directly related to the intensity of the sun's UVB radiation or lack thereof in the various latitudinal regions around the globe. Generally speaking, people who live in a band around the equator have a dark pigmentation from melanin that protects against too much UVB radiation, while in the northern or southern latitudes, where radiation is less intense or even absent during the winter months, skins are light.[280] Humans need exposure to a sufficient amount of UVB radiation because it induces the skin to synthesise Vitamin D, also known as the 'sunshine vitamin'. This vitamin is essential for calcium absorption for bone strength and is shown to be important for overall health. There are few foods with a significant Vitamin D content and their availability is neither general nor reliable, leaving exposure to the sun as the only sure source. Neanderthals evolved and had lived in northern climes for hundreds of thousands of years and it can, therefore, be assumed that their skins were light like those of today's inhabitants of these regions. Coming from Africa, modern humans would have had dark skin. Under diminished or absent UVB radiation in the northern latitudes, however, dark skin severely slows or prevents Vitamin D synthesis.

In past decades, the change from a predominately outdoor to a predominately indoor lifestyle of populations living in the industrialised northern latitudes is accompanied with an increasing occurrence of Vitamin D deficiency in those populations. Emigration to northern countries by dark-skinned people and women who are required by their religious traditions to cover up completely has had the same effect. For some time now, it has been observed that Vitamin D may play a significant role in the human reproductive process. Recent studies have found that deficiency during pregnancy is related to obstetrical complications such as pre-eclampsia, gestational diabetes, bacterial vaginosis among others and soft bones and hypocalcaemic seizures in newborns. Furthermore, observational and associative studies suggest that insufficient levels of Vitamin D in the female, as well as the male, are associated with reduced fertility and reproduction. In men, significantly lower levels of Vitamin D are correlated with severe erectile dysfunction, low sperm count and less sperm motility. While animal studies have provided solid evidence that Vitamin D deficiency causes reduced fertility, this causal relationship has yet to be confirmed in large, randomised human trials.[281]

For the moment, valuable insights are gained from women that undergo treatment in fertility clinics, where the success of the various stages from the development of the egg to the implantation of the embryo is directly related to their Vitamin D status.[282] If

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[280] https://www.nasw.org/article/vitamin-d-levels-determined-how-human-skin-color-evolved

[281] https://academic.oup.com/humrep/article/27/10/3015/747482/

[282] http://uscfertility.org/fertility-treatments/vitamin -d-fertility/

a causal relationship between Vitamin D and fertility and the outcome of pregnancies can be confirmed, one may assume that hybridisation between Neanderthals and modern humans was benefiting modern humans' reproductive success. Neanderthal introgression would have acted as a 'quick fix' and contributed to becoming adapted to the new climatic conditions over the course of only a few generations.

It can be assumed that Neanderthals who had lived in Eurasia for hundreds of thousands of years were well suited to their environment, while the African newcomers didn't have the natural protection they needed to fight the new, diverse microbial pathogens they encountered. Two independently working research teams with differing aims and using different approaches found that modern humans' sexual encounters with their archaic cousins endowed them with variants in three genes belonging to a group of immunity genes that are still protecting modern populations today. Two of these variants originate from Neanderthals, whereas the third is most similar to the Denisovans' variant. Computational biologist Janet Kelso from the Department of Evolutionary Genetics at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, said -

> "We found that interbreeding with archaic humans--the Neanderthals and Denisovans--has influenced the genetic diversity in present-day genomes at three innate immunity genes belonging to the human Toll-like-receptor [TLR] family."[283]

The aforementioned TLR genes code for an increased production of proteins that play a fundamental role in recognising pathogens on the surface of the cell and results in a stronger reaction of the built-in immune responses. Population geneticist Lluis Quintana-Murci at the Pasteur Institute and the CNRS in Paris, France, and colleagues arrived at a similar conclusion, saying -

> "These, and other, innate immunity genes present higher levels of Neanderthal ancestry than the remainder of the coding genome," and added "This highlights how important introgression events [the movement of genes across species] may have been in the evolution of the innate immunity system in humans."[284]

Again, it appears that modern humans gained from the introgression of Neanderthal immunity genes as opposed to going through thousands of years of mutations, some of them potentially lethal or life-threatening, while adapting to new conditions. Although this immunity boost was then and still is invaluable for the protection against invading pathogens, this increased detection sensitivity might also have increased the susceptibility to allergies that many people suffer from these days.[285] By the same

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[283] Dannemann et al., 2016, Neanderthal genes gave modern humans an immunity boost, allergies http://www.eurekalert.org/pub_releases/2016-01/cp-ngg123015.php

[284] idem

[285] idem

token, one may wonder about Neanderthals' resistance or lack thereof to the tropical pathogens that modern humans coming from Africa would have carried with them. Indeed, a new study indicates that the new arrivals may have introduced the herpes simplex 2 virus that causes genital herpes, the *Helicobacter pylori* bacterium that causes stomach ulcers, and they may also have transmitted tuberculosis and the tapeworm. Could the exposure to these new diseases and parasites have contributed to the Neanderthals' eventual demise?[286]

Denisovans may have not only left their legacy in modern humans' immune system. Another possible contribution is an almost exact match of a gene that is beneficial for coping with life in high altitudes and that was detected in about 80% of Tibetan people. Organisms that are exposed to less oxygen in high altitude produce more haemoglobin, the protein molecule that carries the oxygen in the blood. Too much haemoglobin, however, causes thickening of the blood, high blood pressure and preeclampsia, a pregnancy disorder. This particular gene regulates the production of haemoglobin and thus protects against the ill effects of high altitude living.[287] Another gene version, this time appearing to originate from Neanderthals, increases blood clotting, which would have been beneficial at a time when daily life was a risky affair and severe injuries were common. It would have reduced blood loss and wounds would have closed more rapidly, thus preventing infections from entering. However, hypercoagulation in today's populations with this variant increases the risk of stroke and pulmonary embolism.[288]

As the examples above show, not all gene variants that were beneficial or life-saving for the archaic lineages are having the same positive effect in modern-day people. There are some Neanderthal variants, for example, that might contribute to a propensity to certain diseases such as Lupus, Crohn's disease, depression and type 2 diabetes. The latter, also known as adult-onset diabetes, is the most common form and used to be considered a disease of the middle or old age and being overweight or obese. Obesity brings about resistance to insulin, the hormone that is produced by the pancreas and helps to control blood sugar. Insulin resistance will lead to abnormally high blood sugar levels that are the tell-tale of diabetes, an ailment that increases the risk of heart attack, stroke, kidney failure, blindness and amputations. Type 2 diabetes is a complex disease, which is influenced by several genes besides eating habits and lack of physical activity. Even after accounting for differences in lifestyles, people of Native American ancestry such as Mexicans and other Latin American populations are

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[286] The surprising way Neanderthals got Herpes, http://www.bbc.com/earth/story/20160412-what-really-happened-when-we-met-neanderthals

[287] https://www.newscientist.com/article/mg23030700-700-the-4-genetic-traits-that-helped-humans-conquer-the-world/

[288] 'Health risk' legacy from Neanderthals, http://www.bbc.com/news/science-environment-35547175

found to be especially afflicted by the condition. When the genomes of thousands of Native Americans with type 2 diabetes were compared with those of healthy Latinos, researchers found that they were 50% more likely to carry five linked SNPs in a gene that normally helps to move certain lipids into liver cells that deal with them. The mutation increases the likelihood of its carriers getting diabetes at a younger age while being less obese than carriers with the standard version. The gene variant could be traced back to the fossil remains of a Neanderthal that had been excavated from the Denisovan cave. The mutation that contributes to type 2 diabetes may have been advantageous to Neanderthals who led an existence that likely oscillated between feast and famine and had to exert a lot of physical activity to survive. In the modern, especially the western world, people live mostly sedentary lives and have access to food, often unhealthy and processed, at all times. Under these circumstances, the Neanderthal variant can be detrimental to people's health.[289]

Earlier, it was mentioned that Neanderthals' genomic legacy is not evenly distributed throughout contemporary non-African populations' genomes. Some regions can be particularly rich like the ones that contain immune genes or have to do with the properties of the skin, while others are either completely devoid of Neanderthal variants or show reduced presence. In the non-African modern genome, some of the regions that show hardly any occurrence of Neanderthal DNA have to do with fertility. As was discussed earlier in the chapter, humans have 23 pairs of chromosomes; 22 pairs are called autosomes and the 23rd pair, the allosomes, determine the sex of the individual. Females have two X-chromosomes, while males have an X- and a Y-chromosome. Recent studies of people's genomes have disclosed that hardly any Neanderthal genes are expressed in sperm production and the female sex chromosome is also nearly devoid of Neanderthal genes. These findings combined suggest that male offspring of Neanderthal and modern human crosses were either not viable, or they were infertile or had reduced fertility. Immediately, Haldane's rule comes to mind and the pattern that is seen in mammals when male and female of different populations are too distantly related to interbreed successfully. Furthermore, it suggests that any Neanderthal DNA that is found in modern peoples' genomes was most likely passed on through the female line.[290]

The hypothesis of Neanderthal male hybrid infertility is supported by a recent study that was carried out at Stanford University, USA. When researchers compared the Y-chromosome of a 49,000-year-old Neanderthal from El Sidrón in Spain with that of chimps and ancient and modern humans, they found that the Neanderthal Y-chromosome has left no trace in modern male populations. The loss of the Neanderthal Y-chromosome is believed to be connected with mutations in several

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[289] The deep roots of diabetes, http://evolution.berkeley.edu/evolibrary/news/140204_diabetes

[290] http://news.harvard.edu/gazette/story/2014/01/neanderthals-dna-legacy-linked-to-modern-ailments/

Neanderthal genes. One of the mutations is linked to the occurrence of miscarriages that are triggered by the mother's immune response to a male foetus's genes. If that was the case, there would have been no viable male offspring and hence no Y-chromosome would have come to be passed on.[291] Conversely, the fact that all modern humans who carry Neanderthal DNA in their genomes inherited their mtDNA exclusively from modern humans leads to yet another conclusion. Viable and fertile offspring were only produced from pairings of modern human females with Neanderthal males and not from reciprocal pairings, an outcome that is also consistent with Haldane's rule.[292] But none of this can be written in stone quite yet. Archaeologists continue to discover new fossil remains and science is only beginning to unravel the secrets that are hidden in the genomes of archaic humans and the contemporary human race.

Summary

Evolved into anatomically modern humans, the ancestors of today's modern populations left other hominin species behind in Africa and began colonising new regions in the eastern hemisphere, where they met human species that had left Africa many thousands of years earlier. Fossil evidence suggests that one of the possible regions of a first encounter with their Neanderthal cousins, a species that had evolved in Europe and then spread as far as Siberia, could have been the Middle East. Although there are differences in opinion about the timing, a growing body of evidence from numerous research projects of ancient and modern genomes tells a recurring story. Whenever the ancestors of today's peoples came across close or more distantly related cousins in Eurasia or farther afield, they mated with them. So far, three candidates are accounted for. Two of them, the Neanderthals and the Denisovans, left their enduring legacy in form of varying amounts of genetic variants in the genome of modern-day populations, while modern humans, who had stayed behind on the African continent, also mixed with a now extinct and as yet unnamed species.

Some of the archaic genomic ingressions may have increased some of the propensities for certain ailments in modern populations such as type 2 diabetes. Others appear to have not only helped their ancestors to become quickly adapted to the new climatic conditions they found themselves in, but also boosted their immune defences and helped them survive better. Others still made it possible for them to broaden their geographical range and inhabit higher altitudes as well. All in all, it can be said that the relative small introgressions acquired through interbreeding with archaic humans had a significant influence on the evolutionary trajectory of *Homo sapiens*. Last, there is the issue of hybrid infertility. Modern humans and archaic humans may have had

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[291] https://www.newscientist.com/article/2083381-missing-y-chromosome-kept-us-apart-from-neanderthals/

[292] Neanderthal-human Hybrids, http://www.hypothesisjournal.com/?p=932

problems reproducing, but the fact that human beings today carry ancient peoples' DNA in their genome is proof that their offspring were not completely infertile.

Chimeras, Telegony and Anglerfish

Chimeras

When autopsies were carried out on 59 human females, the pathologists were surprised to discover that in 63% of the cadavers, the female brains had parts of the Y chromosome incorporated in the nucleus of the neurons[293]. How on earth had parts of the male chromosome entered the female brain?

Another group of researchers discovered that women could also harbour male DNA in their blood and organs and that some of these women were childless. In fact, 21% of women screened had male DNA incorporated somewhere in their body.[294] This phenomenon is known as *microchimerism* and can be present in all placental organisms, both male and female. Microchimerism is one of the mechanisms that result in phenotypes historically called *chimeras*. A chimera is by definition any organism that contains at least two different genotypes. These originate either from the fusion of two or more embryos in the womb or by induction of cells from the mother to the foetus or from the foetus to the mother. Chimerism does occur in plants but originates from different processes, and fungal chimerism is observed but little understood at present. Here, though, examination is given to chimeric animals.

One normally associates chimeras with those exotic beings of Greek mythology like the lion with the additional goat head and a snake as a tail. It is now thought that at least 10% of the human population worldwide are chimeras.[295] This may seem a surprisingly high number. Some people may also be aware of the unusual chimeric colouring of some animals. Birds can be 'half-siders' in their colouring like green on the left and blue on the right side, for instance. A peacock's train may be divided in half displaying white feathers on the one side and on the other side the splendid colours that one normally sees. Butterflies can have wings where one wing is patterned and coloured completely differently to the other. Mostly, these colourings differ on the bilateral axis

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[293] William F. N. Chan, Cécile Gurnot, Thomas J. Montine, Joshua A. Sonnen, Katherine A. Guthrie, J. Lee Nelson**.** Male Microchimerism in the Human Female Brain**.** *PLOS ONE*, 2012; 7 (9): e45592 DOI: 10.1371/journal.pone.0045592

[294] Yan Z, Lambert NC, Guthrie KA, Porter AJ, Loubiere LS, Madeleine MM, Stevens AM, Hermes HM, Nelson JL N 2005 Male microchimerism in women without sons: quantitative assessment and correlation with pregnancy history.Am J Med. 118(8):899-906.

[295] Blocklage, C.E. (2005) Embryogenesis of chimeras, twins and anterior midline asymmetries. Hum Reprod21: 579–591

of the body plan with the symmetry of size usually maintained. There are, however, examples of animals whose colouration is patchy or mottled arbitrarily across the body surface and equally, there are exceptions to symmetrical body size as well, where one wing of a butterfly is larger than the other (figure 31). The patchiness is thought to occur when the two different embryonic cells from non-identical twins merge to become one at a later stage of development. In other words, what began as twins merged to become a single individual.

Figure 31. Bilateral gynandromorph Ornithoptera goliath procus from Ceram Island. Source: http://www.scilogs.com/maniraptora/watch-cal-academy-butterfly-collection/

In humans, twin conception is common, but twin conceptions do not often result in twin births; it is estimated that it is ten times more likely that the twins will merge. It was mentioned above that 10% or more of humans are chimeras but if that is so, why are they not conspicuous?

> We do not expect to find chimeras because most of us are ignorant of their existence and the informed few just know they are too rare and bizarre to require consideration. We don't look for them because we don't expect to find them and we don't find them until we trip over evidence we cannot ignore. The human spontaneous chimeras identified as such to date comprise only the small fraction of all chimeras in the human population which we have been unable to ignore. (Blocklage 2005)[296]

Most chimerism goes unnoticed because the presence of multiple genotypes and cell lines within an individual are not often expressed in the visible body. Recent research has delved deeper into microchimerism and discovered further evidence, so the figure

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[296] Charles E. Bocklage, 2005, Embryogenesis of chimeras, twins and anterior midline asymmetries, Human Reproduction Vol.21, No.3 pp. 579–591, 2006, p.581

of 10% of human chimeras is probably an underestimation. Microchimerism can also be the result of human interventions such as organ transplantation or blood transfusion, but the most common source is natural acquisition through maternal-fetal transfer during pregnancy. Transmission of cells between the mother and the foetus occurs in both directions. (Even bacteria can be transferred from mother to foetus)[297]. The placenta, which connects mother and developing offspring and provides it with oxygen and nutrients, has a partially porous membrane which can be penetrated by cells. Many are destroyed by the respective immune systems but some survive and eventually incorporate in the new body as blood cells or within the organs and tissues. A mother could, therefore, receive by lateral transfer cells from the foetus and these could possibly also be transferred to a foetus of a later pregnancy. There could even be a generational transfer where a foetus receives cells that originated in the grandmother.

> There is growing evidence that chimerism in one form or another may not be so unusual at all. In fact, some researchers now think that most of us, if not all, are chimeras of one kind or another. Far from being pure-bred individuals composed of a single genetic cell line, our bodies are cellular mongrels, teeming with cells from our mothers, maybe even from grandparents and siblings.[298]

In a rather bizarre legal case, a woman in America was told after DNA testing that her children were not actually hers and a prosecution of fraud was taken against her for claiming benefits for children that were not her own. This was very distressing for the woman who was obviously aware of her own pregnancies and childbirths but, unfortunately, hospital records could not be found. Eventually, it was discovered that the mother was a chimera carrying two sets of distinct DNA, opportunely the DNA taken from a cervical smear test matched the DNA of her children. In another incident, an athlete was stripped of his awards because tests showed that he had received an illegal blood transfusion. Fortunately, the athlete was able to prove his innocence, for he too was a chimera, in this case, carrying two blood lines. The message is that society must be very cautious when employing DNA or blood tests. The results are not necessarily conclusive and an innocent person could be wrongly convicted or a criminal could escape detection based on faulty diagnosis. The assumptions here rest on a faulty theory of heredity from the nineteenth century, which has dominated evolutionary thinking until present times. The idea that only germ cells and the singular DNA they carry can be passed on through the generations has excluded

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[297] Bacteria has been discovered in an offspring's meconium (first plop). Jiménez, E.*et al*. 2008. Is meconium from healthy newborns actually sterile?*Res. Microbiol.*(**159)**, 187–193.

[298]'The Stranger Within'.New Scientist vol 180 issue 2421 - 15 November 2003, page 34

acknowledgment of other possibilities like lateral transference. Indeed, many cases of the phenomenon of chimerism have probably been misdiagnosed as *mosaicism*.[299]

Mosaicism is different from chimerism. At conception there is just one cell; there is no fusion of non-identical twin cells. However, from the initial development onward through any stage in life, mutations can take place that result in two distinct genotypes. This can occur in the germline cells of heredity and in the somatic cells that build the body. The process of mosaicism is an important contributor to the diversity of life but it can also be harmful to organisms. The 'is it mosaicism or is it chimerism' debate has come to the forefront in recent years. Medical practitioners must now be more careful with their diagnoses by going beyond the generally accepted theories of the 20th century and recognising the new empirical evidence. The mechanisms that cause chimerism are complex and varied and are contingent on the number of embryos that fuse and the timing of the fusion. Nevertheless, although the details are many, the underlying principle is again one of merger or combination, not only of genetic material but also of complete cells. This adds to the complex layers of diversity in organisms and presents one with problems concerning heredity and the philosophical question about individuality. It is an even more pressing question when one considers the microbiome, which will be discussed in more detail in the next chapter.

Telegony

The word *telegony* derives from the Greek *tele* meaning 'from afar' and *gony* meaning 'combining', 'production', 'origination'. Telegony was first thought to be of possible biological importance toward the end of the 19th century. On the strength of an anecdote, it was said that a farmer first crossed a female horse with a male zebra and produced a hybrid 'zorse'. The farmer then crossed the same horse with a dark stallion. To the farmer's surprise, the offspring of this second cross still contained the characteristic stripes of the zebra. Since then, attempts with different mammals to reproduce these results have all failed and to date, there is no scientific evidence of telegony within mammals. Consequently, telegony was consigned to the bin of disreputable biological theories. However, some recent experiments may open the door for a re-assessment. Researchers have conducted an experiment, this time not with mammals but with the neriid fly (*Telostylinus angusticollis*) –

> Flies fed a larval diet high in nutrients are large and produce larger offspring, than flies fed a diet low in nutrients. To test for telegony, we mated immature females to either a small or large male, so that their eggs developed after being exposed to the

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[299] Charles E. Bocklage, 2005, Embryogenesis of chimeras, twins and anterior midline asymmetries, Human Reproduction Vol.21, No.3 pp. 579–591, 2006

> semen of this first mate. Two weeks later, we re-mated each female to a second small or large male to fertilize her mature eggs, and measured the size of these offspring. Genetic tests confirmed that the second male sired the offspring. However, offspring size was determined by the diet of the first male that the females mated with. So, if a female was initially mated to a small fly, her offspring were small, even when their father was large (and vice-versa). (Crean 2014)[300]

It is thought that the female's immature eggs absorb molecules from the seminal fluid of the first mating. Of course, it does not follow that what occurs with invertebrate flies will also apply to mammals and other animals, but it does open up the debate concerning the methods and effects of transmission over the generations. One might detect a move from 'hard' theories first expounded by the German evolutionary biologist August Weisman (1834 – 1914), who concluded "that neither injuries, functional hypertrophy and atrophy, structural variations due to the effect of temperature or nutrition, nor any other influence of environment on the body, can be communicated to the germ-cells, and so become transmissible.''[301] Weisman's view of heredity became the bedrock of classical 20th^{h} century evolutionary theory, echoed here in 1982 by Ernst Mayr, one of the 20th century leading evolutionary biologists: "The proteins of the body cannot induce any changes in the DNA. An inheritance of acquired characters is thus a chemical impossibility."[302] With so much contrary evidence, these views seem now incredible and somewhat simplistic but at the time, they were quite plausible. Some researchers have recognised the shortcomings of the past -

> Our discovery complicates our entire view of how variation is transmitted across generations, but also opens up exciting new possibilities and avenues of research. Just as we think we have things figured out, nature throws us a curve ball and shows us how much we still have to learn. (Angela Crean et al 2014)[303]

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[300] Crean, A. J., Kopps, A. M., Bonduriansky, R., & Marshall, D. (2014). Revisiting telegony: offspring inherit an acquired characteristic of their mother's previous mate. *Ecology Letters*, *17*(12), 1545–1552. http://doi.org/10.1111/ele.12373

[301] Weismann, A. 1893*. The Germ-plasm: a Theory of Heredity*, CharlesScribner's Sons.

[302] Mayr, E. (1982) *The Growth of Biological Thought: Diversity, Evolution, and Inheritance*, Belkman, Harvard.

[303] Crean, A. J., Kopps, A. M., Bonduriansky, R., & Marshall, D. (2014). Revisiting telegony: offspring inherit an acquired characteristic of their mother's previous mate. *Ecology Letters*, *17*(12), 1545–1552. http://doi.org/10.1111/ele.12373

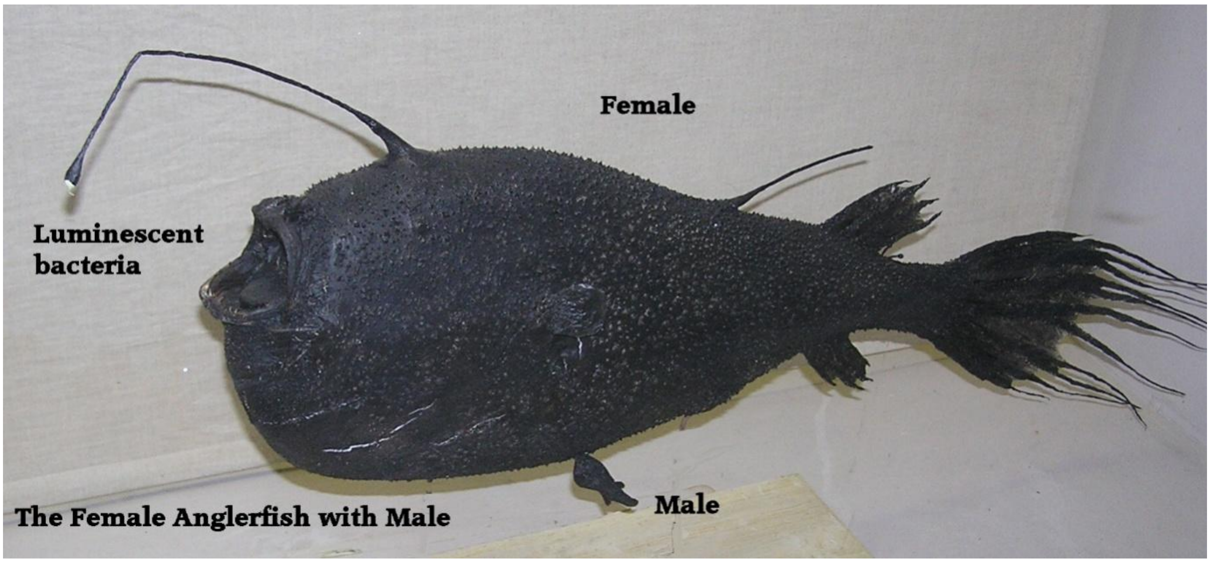

Figure 32. Derived from photograph by Andrew Butko, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=4914101

The anglerfish (genus *Ceratias*) is a deep water sea fish, which has an unusual method of reproduction that results in a form of chimerism and also shares an unusual symbiotic relationship with bacteria. As can be seen in the picture provided (figure 32), the female dwarfs the male that has attached itself to the underside of the female body. Some males begin life with well-developed olfactory organs and relatively large eyes that may help detect females in the dark, deep waters. If a male fails to find a female, the male will not develop gonads and will soon die. However, upon finding a mate, the male has a basal bone that stabs deeply into the female body. Then enzymes are released from his mouth that facilitate the fusing of the two bodies, producing shared tissues, blood vessels and a circulatory system. Eventually, the organs of the male, including the brain, *atrophy* or waste away, except for the testes, which increase in size and provide a permanent sperm bank for the female. Sometimes, several males can attach to a single female and thus increase the complexity of DNA combinations for the next generation. In this manner, some anglerfish are polyandric, hermaphroditic chimeras. But that is not all; one also has to consider the symbiotic relationship that produces bioluminescence.[304]

Most marine life is luminescent. Some luminescence is produced by chemical reactions and some is produced by symbiotic or invasive bacteria. In the case of the anglerfish

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[304] For an in-depth explanation of the mechanics of bioluminescence see Lin & Meighen http://photobiology.info/Lin.html

pictured, there is a bulbous part, the *esca*, affixed to what looks like a fishing rod, hence the name ‘anglerfish’. In this instance, the esca holds bacteria that have penetrated through the porous membrane. Once inside the esca, the bacteria find nutrients and are protected from the external environment. For bioluminescence to occur, there need to be sufficiently large numbers of bacteria so that *quorum sensing* can take place. Quorum sensing refers to the interactions from cell to cell that affect gene expression.[305] The esca ‘lantern’ can attract prey, but conversely, light in the dark ocean can also attract predators. There are many fishes that contain bioluminescent bacteria, but not all incidents are of mutual benefit; some invasive bacteria feed off and destroy their host.

Thus, besides being polyandric, hermaphroditic chimera, some anglerfish are also symbionts. What is not known at present is whether they hybridise with other fishes as well. The anglerfish is an unusual and rare composite creature but it is not uncommon in the animal world to be both, a chimera and a symbiont. Bioluminescence in its various forms is frequent amongst fishes, invertebrates and some fungi. The complex web of nature calls out for recognition of a new theory to reflect this.

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[305] https://microbewiki.kenyon.edu/index.php/Deep_sea_fish

Chapter Five

Conclusion et al

The Species Problem

Life is much easier when objects are clearly defined and classified so that one can easily identify them. Furthermore, it is imperative that everyone agrees with the labeling of these objects or groups thereof. If a customer in a pet shop asked for a puppy but was given a parrot by the sales assistant, that would be a sign of a serious breakdown in shared identification.

Dividing up nature and providing group names for organisms has always proved to be problematic for biologists and naturalists. How does one, therefore, classify organisms? Does one take account of similar form or morphology? Does one take account of similar genetic compositions? Does one take account of mating preferences? Does one take account of relationships through heredity?

After his hierarchical classification of all organisms in *Systema Naturae* in 1735, the Swedish botanist Carolus Linnaeus or Carl von Linné as he was known after his ennoblement, is generally regarded as the Father of Taxonomy. This system has, however, changed over time. For instance, Linnaeus included a Kingdom taxon that consisted of minerals, but has long been removed. There is, indeed, no universal consensus on the taxonomic classification system and the members of those taxa. Nevertheless, below is an example of a commonly used hierarchical taxonomy.

Domain

Kingdom

Phylum

Class

Order

Family

Genus

Species

This system of classification is based on the morphology of organisms and grouping them by similar characteristics. As an example, the taxon 'Domain' composes archaea, bacteria and eukarya. These three groups include all known organisms. Archaea and bacteria are single-celled organisms called prokaryotes, while eukaryotes can be single or multi-cellular and compose all animals, fungi, protists, algae and plants. Viruses, however, are not classified within this system. As one descends the list above, the organisms become more specialised. Although there are additional problems with taxonomic hierarchies, it is the difficulties at the level of the species that most concerns this work.

The most widely used criterium for distinguishing species is the Biological Species Concept (BSC), which was developed by Alfred Russel Wallace[306] and later made popular by the German evolutionary biologist Ernst Mayr (1904 – 2005). It was a subject Wallace had already discussed with Henry Walter Bates before they set off on their expedition to the Amazon. The principle of the BSC rests on the mating practices and preferences of organisms: "a biological species is a group of interbreeding or potentially interbreeding organisms that can produce viable offspring."[307] At first glance, this seems a very reasonable method for distinguishing species. After all, one sees many natural barriers like cats never mate with mice, elephants never mate with tigers and so forth. There are, however, many exceptions where the Biological Species Concept does not apply. For instance, what does one say of organisms that reproduce asexually and bacteria that reproduce by binary fission? Moreover, what does one say of extinct organisms? They too require classification but they no longer interbreed or have the potential for interbreeding. The Biological Species Concept is therefore too narrow and requires some additional criteria.

Another serious problem for the BSC concerns one of the subjects of this work, the hybridisation between different varieties of organisms that leads to fertile offspring. Brown bears and polar bears are an example. Normally they are considered to be distinct species, however, as was discussed earlier, on some occasions male brown bears have mated with female polar bears and produced fertile offspring.[308] In light of the BSC, should one, therefore, conclude that as brown bears mated with polar bears they are therefore of the same species? Or should one conclude that only the male brown bears are the same species as the polar bear, as the female brown bears did not interbreed with the polar bears? This particular problem with classification according

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[306] Wallace, A. R. ---manuscripts & archival materials: Wallace, A. R. 1860 Letter 2627 — Wallace, A. R. to Darwin,C. R., [Dec? 1860]. In Darwin Correspondence Project. Cambridge: University of Cambridge; Wallace, A. R.1868 Letter 5966 — Wallace, A. R. to Darwin, C. R., 1 Mar 1868. In Darwin Correspondence Project. Cambridge:Cambridge University.

[307] McGowan, A. 2015. http://www.askabiologist.org.uk/from_the_lab/species-concepts

[308] Cahill JA, Green RE, Fulton TL, Stiller M, Jay F, et al. 2013Genomic Evidence for Island Population Conversion Resolves Conflicting Theories of Polar Bear Evolution. *PLoS Genet*, 9(3): e1003345; DOI: 10.1371/journal.pgen.1003345

to the BSC cannot be dismissed as an exceptional example and out of kilter with normality, for this work demonstrates that hybridisation is more prevalent than previously thought. There are other methods for deciding classification, however.

Most field biologists will initially define species by observation of the organism's anatomy or overall form. An Ornithologist will commonly identify birds by their size, plumage, colour, shape of the beak and so forth. This seems a very reasonable and practical approach but once again, there are problems and shortcomings. The most obvious problem is that the morphology of the female gender is most often distinct from the male gender. A male blackbird (*Turdusmerula*) is usually black feathered with a yellow beak, but a female is usually feathered a lighter brown with speckled breast and a beak that is not distinctive yellow. Moreover, the male is usually of larger form than the female, and the young, when hatched, are very different from their parents. Yet, one recognises this diversity as part of a single species. Take another example from the world of invertebrates, where the females are usually larger in size than their male counterparts. A queen honey bee will be larger overall and with longer legs than the worker bee. A worker bee may indeed have more morphological similarities with a worker bee from what is normally considered another species than with the queen of her own species.

Both, the BSC and morphological distinctions, are insufficient to determine without equivocation what a species is. Can one, therefore, turn to genetics for a clear solution? Again, this method will not be without problems. First, should one compare the genes that organisms possess or should one compare their entire genomes? If one takes genes exclusively, then one is looking only at a fraction of the entire genome. For instance, human genes make up less than two percent of the 3.2 billion base pairs of DNA. In addition, there is variation among humans; 99.5% of genes are shared but, incidentally, if one wants to discover who is related to whom, one must examine the areas of DNA that do not code for proteins. Only identical progeny such as twins or triplets share the same DNA, everyone else is likely to have their own unique sequence of DNA. Of course, one would not wish to suggest that each unique genome represents a unique species, this would be counterproductive to useful classification. Some demarcation lines are required to sort humans into a genetically recognisable group that excludes other organisms. However, this is not so easy to do and things get more complicated still when comparisons are made between humans and mice, for instance.

> Overall, mice and humans share virtually the same set of genes. Almost every gene found in one species so far has been found in a closely related form in the other. Of the approximately 4,000 genes that have been studied, less than 10 are found in one species but not in the other.[309]

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[309] Why Mouse Matters 2010. https://www.genome.gov/10001345

It is surprising that mice and humans share so many genes, but one would be reluctant to draw the conclusion that both are of the same species or, based on this evidence, that both are extremely closely related, at the very least. Perhaps, one has, therefore, to gauge things by looking at the whole genomes of both organisms and then draw comparisons. This approach, however, is also problematic –

> Both the mouse and human genomes contain about 3.1 billion base pairs (or chemical letters). Only about 5 percent of the sequence consist of protein-coding regions (genes). More than 90 percent of the genome is non-coding DNA, sometimes called "junk" DNA, that has no known function. Because of the vast amount of non-coding DNA, it is very hard to recognize the genes simply by looking at one sequence alone; even the best of today's computational programs fail to identify many coding sequences and misidentify others. It is similarly difficult to identify regulatory regions within DNA - the "switches" that turn gene expression on or off, up or down - as they exist only as poorly defined "consensus" sequences. (ibid 2010)

Comparisons are technically difficult to draw, so conclusions concerning relationships between groups of organisms should only be considered provisional until technology and clearer identification become available. Apart from the problems of demarcation and the practical problems of identifying genes, there is another problem that concerns the fact that all organisms are endosymbiotic.

The Individuality Problem

One may think of Adam amazingly giving a name to all the animal types on the sixth day of creation before God created Eve, or one may even think of Descartes pondering the uniqueness of man. One perceives oneself as an entity in one’s own right, exclusively feeling one’s own pain and sharing self-consciousness with no other. Whether in religion or in philosophy, there is a consensual view that regards all organisms, may that be plants, fungi, animals including humans, as individuals. Early evolutionists saw nature composed of individuals struggling for existence and competing as individuals for resources or for mates. Biologically, however, one is not quite as individual as one might think; one is, in fact, composed of many different biological entities. From before birth and throughout its lifetime, every human body harbours its particular microbiome composed of bacteria, protists and fungi. As to the ratio of microbial cells to human cells a range of 'guesstimates' exists with the best current approximation being 1.3 microbial cells to each human cell. But whatever the ratio may be, the importance of the microbiome to the human health is undeniable.[310] Because these organisms are only a fraction of the size of a human cell and constitute only a small percentage of the total human body mass, one is not aware of this invasion. They occupy one's skin, hair, mouth, ear, eyes, lung and gut and without them, one cannot effectively get nutrients from one's food, repair one's skin or mount an adequate defence against microbial invaders that would otherwise do one harm. This composite existence is not peculiar to humans but pervades all nature. Kramer and Bressan believe that humans are in fact superorganisms where the microorganisms shape one's character-

> The overarching message is that we are not unitary individuals but superorganisms, built out of both human and nonhuman elements; it is their interaction that determines who we are.[311] (Kramer 2015).

How then does this consideration affect one's quest for clear taxonomy? If organisms are indeed composites of many organisms, it follows that there is also a collection of different genomes present within a single body. If one wants to classify organisms according to genomes, then there is this complication of invasive genomes that become incorporated within an 'individual'. The genes within these invasive genomes are not inert, they produce proteins that can be released into the wider body and may have some effect on the endocrine and nervous systems, for instance. One example suggests that the microbes in the gut release chemicals when they want food, which eventually reach the brain and stimulate one's appetite. Conversely, one's behaviour may be influenced by the organisms within one's body without one realising it.

Thus, the classification of organisms is a difficult pursuit. All methods employed to date are not without a problem and seemingly, the more we discover, the more difficult it becomes to establish clear lines of demarcation. One suggestion is to produce a chemical read out of all the molecules within a body.[312] This would also include molecules other than just genetic ones; it would be a sort of biological periodic table. Would this, however, help classifying organisms if they are indeed superorganisms? The idea is novel and deserves more consideration.

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[310] http://www.sciencealert.com/bacteria-cells-don-t-actually-outnumber-human-cells-in-our-bodies-study-finds

[311] Kramer, P &Bressan,P. 2015. Humans as Superorganisms. Perspectives on Psychological Sciencevol. 10 no. 4 464-481

[312] Thims, L. 2008. www.humanthermodynamics.com/evolution-table.html

As was recounted earlier, after initially believing that plants were in their form immutable, Linnaeus recognised later in life that some of them had changed during the time of his observations and the process that had caused such a change was hybridisation. The prevalent ideas of the time that were in opposition with this new insight were 'reversion to type' and the belief that nature does not make any leaps. Farmers practising animal husbandry believed that animals selected and bred for traits would eventually revert to type when they were returned to the wild. Darwin believed that the gradualism of natural selection could explain the phenomenon of reversion to type, which was believed to be true at the time but in actual fact does not exist. As to the belief that nature does not make any leaps, Darwin accepted the importance of hybridisation in the plant kingdom, but believed that events in the animal kingdom were too uncommon to have any significance for evolutionary theory. It is also important to note that Darwin did not see natural selection as the exclusive evolutionary force. From the very beginning, he included other factors such as sexual selection, Lamarckian ideas of 'use and disuse', the impact of the environment and the influence of habit[313]. Darwin was an evolutionary pluralist with natural selection as the main factor.

After Darwin's death, there was renewed interest in what were known as 'saltationist' mechanisms of evolution. Saltationism asserted that evolution was discontinuous and was not necessarily a gradual process. The English biologist St George Jackson Mivart (1827 – 1900)[314] criticised natural selection on several grounds and, what is relevant here, he argued that the gradual nature of natural selection was not evidenced in the known fossil record. If evolution was a slow and gradual process, one would expect this to be reflected in the fossil record, and that was not the case. In fact, there were long periods when no change could be determined and new species emerged without the intermediary fossils to support gradualism. Darwin's response was that the fossil record was far from complete and with time, the discovery of new fossils would vindicate his hypothesis. This, however, has not happened and in the second half of the 20th century, this debate re-emerged and resulted in the hypothesis of 'punctuated equilibrium'.[315] More on this later.

After Darwin, a period of evolutionary pluralism flourished. While there were evolutionists who supported gradualism, there were others who were sceptical of the potency of natural selection and invoked alternative processes. These included hybridisation; symbiogenesis; and the direct impact of the environment, sometimes

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[313] See: Darwin. C, 1868. *The Variation of Animals and Plants under Domestication.* London: John Murray.

[314] See: Mivart, St. G.J. 1871. *On the Genesis of Species*. London: MacMillan & Co.

[315] See: Gould, S J, &Eldredge, N. 1977. "Punctuated equilibria: the tempo and mode of evolution reconsidered." *Paleobiology***3** (2): 115-151. (p.145)

alluded to as Lamarckism. Julian Huxley called this period up to the 1930s the "eclipse of Darwinism", but this is actually a misnomer. A more accurate term would have been the 'eclipse of natural selection'. It should be said that Darwinism was actually more inclusive with respect to processes for change than natural selection alone. It was the British naturalist Alfred Russel Wallace and the German evolutionary biologist August Weismann (1834 – 1914) who took a more singular view of evolutionary change and regarded natural selection as almost the exclusive process of change.

Mendel's discoveries concerning genetic inheritance were seen even by some Darwinists as antithetical to natural selection. The Dutch botanist Hugo de Vries (1848 – 1935) considered himself a Darwinist, but his *Mutation Theory of Evolution* included hybridisation as the main source of evolutionary change and this went against the gradualist approach. Likewise, his contemporary, the English biologist William Bateson (1861 – 1926) also doubted the gradualist approach by natural selection. He argued that whatever the cause of the variation that natural selection was said to act upon, that was the force that drove evolution. In other words, if one discovers what causes variation, then one has discovered the driving forces behind evolutionary change. One could use the analogy of judges at a talent contest who select the best contender from a field of contestants, but the judges' actions do not explain why the contestants have their particular talents. Those talents are developed before any selection process. One must also not forget the contributions of the Russian symbiologists Famintsyn and Merezhkovsky who rejected the view that natural selection could provide biological novelty.

During the 1930s, scientists such as the English statistician and biologist Ronald Fisher (1890 – 1962), J.B.S. Haldane and the American geneticist Sewall Wright (1889 – 1988), developed the theoretical foundations for the Modern Synthesis, a term coined by the British evolutionary biologist Julian Huxley (1887 – 1975) in 1942. In the process, much of the pluralism previously considered was purged from evolutionary theory. Even Darwin's original formation of natural selection was altered from the 'struggle for existence' or 'survival of the fittest' to the tautological 'survival of the best reproducers'. Out went any consideration of symbiogenesis, hybridisation, Darwin's Lamarckian mechanisms and theories of the direct impact of the environment[316]. Instead, Mendel's discoveries were included but now as subject to natural selection. Population genetics was the key mechanism and was exclusively of a gradual nature with only small mutations as the source of variation. In addition, the focus of the explanation shifted from the phenotype or individual to the gene and the importance of genetic mutations. For the American geneticist Thomas Hunt Morgan (1866 – 1945), famous for his work with the fruit fly (*Drosophila melanogaster*) and influential in shaping evolutionary theory, mutations were the important cause of variation and the

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[316] The most brilliant exposition of the direct impact of the environment was by D'Arcy Thompson, called *On Growth and Form*, 1917.

driving force behind evolutionary change. If to be passed on, these mutations, however, could not be too drastic but had to be just slight alterations to the genetic sequences. Morgan endorsed Darwin's gradualist approach but he was sceptical about natural selection's creative ability -

> "Selection, then, has not produced anything new, but only more of certain kinds of individuals. Evolution, however, means producing more new things, not more of what already exists." (Morgan 1919)[317]
>
> "…………there is no evidence that selection determines the direction in which variation occurs." (*ibid* p193)

Proponents of the Modern Evolutionary Synthesis took the gradualist approach on board but mostly ignored Morgan's concerns about the causal limitations of natural selection. Genetics were to dominate evolutionary thinking in what has become known as the 'genic' or 'gene-centric' explanation of evolution. This view was fortified in the 1970s with the publication of *The Selfish Gene* by Richard Dawkins[318]. Dawkins combined gradualism with natural selection acting upon the gene, displacing Darwin's view that selection acted upon the individual and the view that selection might also target groups. Dawkin's work proved to be highly influential but also highly controversial. The debate that raged in the 19th century after the publication of *The Origin of Species* surfaced again with new evidence from palaeontology. The re-examination of the fossil record from the Burgess Shale in Canada supported the view that evolutionary change was discontinuous with long historical periods where no change was detected. Thus 'punctuated equilibrium' was hypothesised. The equilibrium became known as 'stasis', which was punctuated by episodic events of mass extinction that could be followed by rapid appearances of novel organisms. These organisms often lacked evidence in the fossil record of ancestral lineages. Supporters of 'punctuated equilibrium' contested the gradualist approach and many of those supporters also contested that natural selection acted on the gene[319].

A debate ensued, which, naturally, attracted philosophers since definitions were required. For instance, what is meant by 'gradualism' and how gradual is gradual? What is meant by 'rapid evolution', how rapid is rapid? Furthermore, questions were asked concerning the appropriate 'levels of selection'. Was it set at the level of the gene, the individual, the group or perhaps at all of the above? The latter controversy still continues unresolved today, but it will be argued later that this debate is an unnecessary one. The debate over gradualism, though, has largely petered out. Today, few evolutionary biologists believe that evolution is exclusively of a gradual nature.

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[317] Morgan,T.H. 1919. *A Critique of the Theory of Evolution,* p.154, Princeton: Princeton University Press.

[318] Dawkins, R. 1976. *The Selfish Gene*, Oxford: Oxford University Press.

[319] See: The Darwin Wars by Andrew Brown for a popular summary of the clash between "Gouldians" and "Dawkinians".

This is largely because of the recognition and acceptance of chromosomal polyploidy and a host of recent discoveries concerning genetics and the workings of the cell, together with a reappraisal of older ideas that did not seem to fit mainstream biology of the mid-20th century. Moreover, the empirical evidence of widespread symbiosis and hybridisation presented in this work endorses the view that evolution is indeed discontinuous.

If one were to browse through a textbook on evolution written in the 1970s, one would notice that there are many statements of 'fact' that are not considered to be facts anymore (see below). Moreover, important subjects are missing that are now included in contemporary textbooks. Some of the major omissions were the sciences of developmental biology, epigenetics and endosymbiosis. These are not new theories or recent discoveries, and in the past they had their advocates who were marginalised by those who supported the ruling paradigm. The classic example of exclusion is perhaps the treatment of the American cytogeneticist Barbara McClintock (1902 – 1992) by the biological establishment of her time. McClintock discovered the important genetic mechanism of *transposition*, colloquially called 'jumping genes', where whole chunks of the chromosome could be rearranged during meiosis. It explained, for example, the differently coloured spots on maize kernels and how colours could change in just one generation. By the 1950s, her studies led her to the inference that there must exist controlling elements for genes. This, however, was not understood, nor was it accepted by the establishment and she unjustly experienced hostility and disbelief. She actually discontinued her research at one point. It was not until some thirty years later that her work was recognised and she was awarded a Nobel Prize. On reflection, one may recognise that 'science marches on', but it also does have its casualties.

Lynn Margulis and her efforts to bring proper recognition to symbiogenesis have already been discussed. Once again, facing a sceptical audience of neo-Darwinists, a scientist suffered rebuff but unnecessarily so. Today, the endosymbiotic theory is widely accepted and the importance of the microbiome is becoming fully realised, not only in evolutionary theory but also in medical practice. The British developmental biologist Conrad Waddington (1905 – 1975), suffered similar ostracisation and was not invited to various conferences because of his promotion of epigenetics. At the turn of the 21st century it was very difficult to purchase a work on epigenetics; ten years later, the bookstores and libraries are brimming with them. A proponent, Nessa Carey[320], calls it a revolution in evolutionary theory. Certainly, epigenetics is an important part

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[320] See: Nessa Carey, 2011. *The Epigenetic Revolution*, London: Icon Books

of evolutionary theory, but one must remember that the science was discussed over a hundred years ago in the post-Darwinian period.[321]

As to the many 'facts' of the last century that are no longer considered to be facts, here are a few examples: It was stated that humans possessed 150,000 – 180,000 genes (in some instances as many as 400,000) and more than any other organism. After the sequencing of the human genome, it was a shock to many to discover that humans had less than 20,000 genes and far less than many plants. Much of the DNA considered to be redundant or 'junk' DNA is now known to affect how genes are expressed. The evolution of the eye was thought to be analogous, meaning that the different types of eyes in the animal kingdom had evolved through separate pathways. It is now believed that eyes share homologous genes, all derived from the same ancestor but expressed differently according to their context. When RNA was discovered in 1961, it was believed for many years to be simply the messenger (mRNA) that provided the transcription of the information in the DNA. Now it is known that there are many types of RNA that interact in many ways between the cell and the protein building blocks.

> For many years RNA was believed to have only three major roles in the cell–as a DNA photocopy (mRNA), as a coupler between the genetic code and the protein building blocks (tRNA), and as a structural component of ribosomes (rRNA). In recent years, however, we have begun to realize that the roles adopted by RNA are much broader and much more interesting. We now know that RNA can also act as enzymes (called ribozymes) to speed chemical reactions. In a number of clinically important viruses, RNA, rather than DNA, carries the viral genetic information. RNA also plays an important role in regulating cellular processes–from cell division, differentiation and growth to cell aging and death.[322]

It is not only the interactions of the RNA molecules that are found to be more diverse and complex. Take, for instance, Francis Crick's famous doctrine from the 1970s called the *Central Dogma* of molecular biology. It asserts that 'information flows' in a singular direction, starting from the DNA to RNA and then to proteins but never in the other direction. Today, this is seen as a gross over-simplification that was based on knowledge of cellular activity existing at that time. There are several flaws in this doctrine: For instance, information does not flow exclusively in one direction; there are feedback loops from both RNA and proteins that produce regulatory factors and signalling molecules. These initiate activation or de-activation of gene expression in the DNA. Furthermore, there are many other influences that affect the final quality of

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[321] See: Peter Kropotkin 1995 .*Evolution and the Environment*, Collected essays. George WoodcockEd.Black Rose Books.

[322] http://www.rnasociety.org/about/what-is-rna/

protein. The schematic drawing (figure 31) provides some idea of the complexity involved –

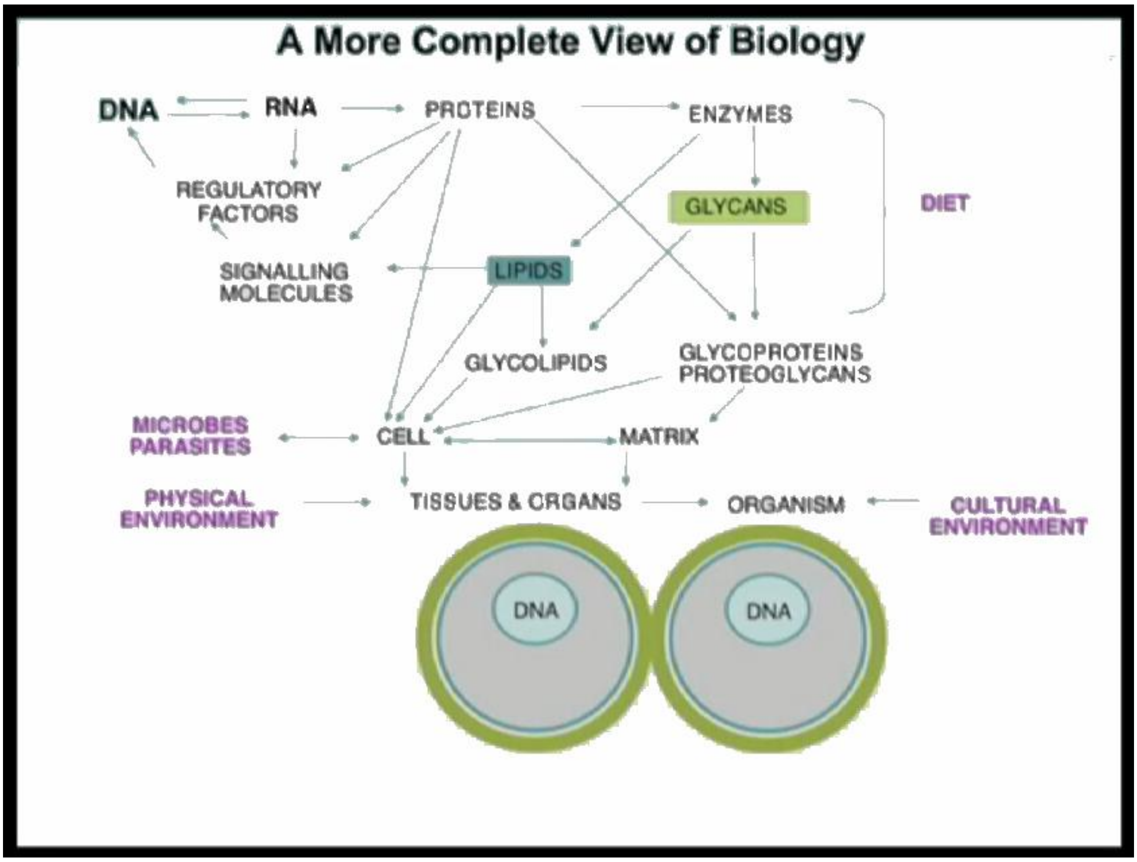

Figure 31. Source Unknown from 2014

These are just a few of the many discoveries that have taken place in recent years, and one is advised not to use any biology textbooks produced before 2006 for they will only be partially correct. The problem is that the facts determine the theory and if the facts are wrong, then it is highly likely that the theory is wrong as well. Indeed, this is very true of some theories of the late decades of the 20th century. The idea that a genotype is a blueprint for structure and behaviour of an organism is incorrect and another over- simplification. Erroneous ideas flourished at the time, believing that there were particular genes 'for' a whole range of human attributions such as mathematical genius, homosexuality, belief in religion, intelligence, to name a few. These ascriptions, however, belong to the realm of pseudoscience and not science. The question to be answered is, how are the phenomena of hybridisation, symbiosis and all the discoveries of recent years to be incorporated into evolutionary theory?

Toward a New Theory

The evidence for symbiosis and hybridisation that we have discovered and documented in this work is much greater than we imagined but has not featured sufficiently within evolutionary theory. These two processes, however, are not alone in lacking recognition of their importance, for other processes such as the different forms of polyploidy, chimerism and lateral transfer should be equally considered. It is our conclusion that the existing theory of 'descent with modification' is insufficient to explain the diversity of biological life. Moreover, the classification of biological entities and what it is to be an individual have become much more obscure. Even the 'tree of life' is a rather poor representation of the reality. A reformed or new theory is therefore requested, which must reflect the following-

- New forms are created by combination and re-combination, both in the pre-biological and the biological world.
- These combinations and liaisons lead over time to greater mass and complexity.
- Objects can also divest mass and complexity adding to the overall diversity.
- The processes which bring about change are rich and varied, there is no single explanation.

There are some biologists who have identified some of these issues and have called for reform. What has been said so far?

Stephen Gould (1941 – 2002) was an American paleontologist, evolutionary biologist and historian of science. When reading his scholarly work *The Structure of Evolutionary Theory,* published shortly before the author's untimely death, one cannot fail to be impressed by the historical depth and presentation of the theories and debates that have vexed evolutionary biologists. Impressive as this large work is, it is, nonetheless, not only missing many of the recent discoveries mentioned above, it is also missing consideration of symbiosis, allopolyploidy and hybridisation. Ironically, Gould was considered to be an evolutionary pluralist who embraced explanations that transcended adaptationist reasoning focused on natural selection. Since his death, there have been efforts to broaden evolutionary theory. The 'Extended Evolutionary Synthesis' (EES[323]), for instance, is presented by a body of biologists and theoreticians, who, as the name suggests, wish to build on the now misnamed 'Modern Synthesis' (figure 33). They are not alone. Another initiative for a new conceptual framework comes from the evolutionary developmental biologists Scott F. Gilbert and David Epel, which they call Ecological Developmental Biology (EDB[324]). Both frameworks share

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[323] Pigliucci, M & Muller, G.B ,Evolution: 2010. The Extended Synthesis. MIT Press. Some of the individuals involved in an international research project are Kevin Laland, Tobias Uller, Marcus Feldman, Tim Lewens and Jessica Flack.

[324] Gilbert, S.F &Epel, D. 2009*, Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution,*Sinauer.

considerations of epigenetic inheritance, developmental plasticity, niche construction and other fields of research.

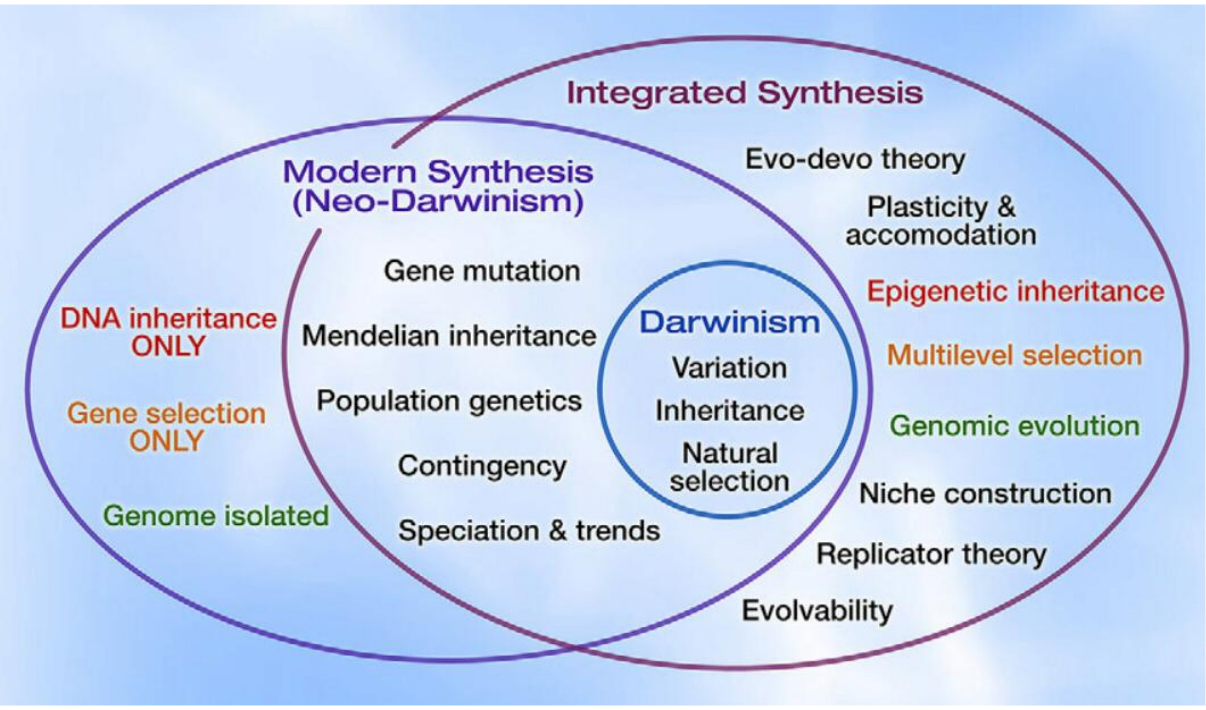

Figure 33. At the core is the original 19th century Darwinism. To the left are the principles of the Modern Synthesis and to the right are the principles of the EES. Noble argues that the processes in coloured text are contrary and those on the outer left ring should be purged from a contemporary theory.

Dennis Noble, a systems biologist, has argued for a more radical change, claiming that much of theoretical neo-Darwinism requires at least a major overhaul if not an entirely new conceptual framework. He agrees with much of the content of the EES but thinks that these new extensions are not consistent with the traditional 'Modern Synthesis'. Moreover, he argues that the language employed is divorced from actual working practice and more of a misleading set of metaphors that slide into uncontested 'facts'[325]-

> Experimental results in epigenetics and related fields of biological research show that the Modern Synthesis (neo-Darwinist) theory of evolution requires either extension or replacement. This article examines the conceptual framework of neo-Darwinism, including the concepts of 'gene', 'selfish', 'code', 'program', 'blueprint',

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[325] Noble, D. 2015, "Evolution beyond neo-Darwinism: a new conceptual framework. *J. Exp.Biol.*218, 7-13.

> ‘book of life’,‘replicator’ and ‘vehicle’. This form of representation is a barrier to extending or replacing existing theory as it confuses conceptual and empirical matters. (Noble 2015)[326]

John Reiss, a zoologist and evolutionist, has also concerns about the teleological vocabulary of neo-Darwinism. He offers alternative terminology in a quest to remove the endeavour to answering the unnecessary question of perfect design.[327] His is a more recent contribution, though many of the considerations are not new. Several biologists have previously argued about the limitations of neo-Darwinism and its fidelity to genic explanations. Jan Sapp, a professor of biology and history at York University, Canada, has written a historical account of the development of the theory of symbiosis and its alternative evolutionary explanations.[328] Lynn Margulis has already been identified as a critic with an alternative hypothesis and one can also consider Mae-Wan Ho and Peter Saunders[329], who were major critics as far back as the 1970s. Another scientist who has been largely overlooked is the Portuguese António Lima-de-Faria, geneticist and professor emeritus at Lund University, Sweden, who wrote a remarkable book explaining the many modes of evolution without any need to employ the concept of natural selection.[330] An important factor in his thesis is the recognition that the building blocks of life are a continuity of the evolution of the wider Universe, a view that is endorsed here.

Although the recent calls for revisions and a new language for evolutionary theory are welcome, they alone are insufficient and do not embrace many of the factors that explain the marvellous diversity of life. The foundation of these omissions may lie in the understanding of what an ‘individual’ is. Darwin’s explanation of evolution rested very strongly on individual organisms competing against each other in the struggle for existence. His observations of the great diversity in life brought up the problem of definition for ‘species’, as discussed earlier. However, the problem goes much deeper than this and also concerns a definition for what an ‘individual’ actually is. The biological evidence suggests that all multicellular organisms are composites of a variety of different organisms. All animals, for instance, have co-evolved symbiotically with various microorganisms and this makes it difficult to find common factors that produce clearly defined entities. Without the symbiotic microbes, one is vulnerable to invasion from microbes that would do great harm. The science writer Jon Turney has called the

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[326] Noble, D. 2015. "Evolution Beyond Neo-Darwinism: A New Conceptual Framework". The Journal of Experimental Biology 218 (Pt 1): 7–13. doi:10.1242/jeb.106310

[327] Reiss, J. O. 2009. *Not by Design: Retiring Darwin’s Watchmaker*. Berkeley: University Presses of California, Columbia and Princeton.

[328] Sapp, J.1994. *Evolution by Association : A History of Symbiosis*. New York: Oxford University Press

[329] Ho, M. W. and Saunders, P. T. (1979). “Beyond neo-Darwinism – an epigeneticapproach to evolution”. *J. Theor. Biol.* 78, 573-591.

[330] Lima-de-Faria, A. 1988. *Evolution without Selection: Form and Function by Autoevolution*. Netherlands: Elsvier.

human body a ‘superorganism’, but this is probably a little misleading as all animals and even plants are composed of multiple organisms as well. One is, nonetheless, as Turney states, in possession of an inner ecosystem.

In many ways, every organism is a unique composite of many parts. The problem underlying classification is that all inner ecosystems will vary, and it follows that for the production of RNA and proteins the genetic input will also vary. To use an analogy, one is like a captain of a large ferry who chooses a direction but is unaware of how all the passengers and staff on board interact, while the passengers have only a crude idea of how the ship’s engines and dynamics work. The captain appears to be in control, but she or he is not only completely dependent upon the members of staff on board but also on the external vagaries of the environment.

The difficulty is, how can one speak of species if every organism is unique? And yet, an agreed classification is necessary for shared consensus. This is not the only difficulty; there are serious problems that concern inheritance and the symbolic ‘tree of life’. Since the revelation of at least five mass extinctions in the past where on one occasion up to 90% of species were eliminated, the tree of life has always been a troublesome representation because it was severely pruned by those extinctions. Moreover, the shapely branches of the tree represent distinct species and generally fail to represent the important symbiotic relationships that exist between some of these species. And this is not all. This work has demonstrated that hybridisation and lateral genetic exchanges are major factors in the process of evolution, but how can this be represented on the tree? For one, not only can new species emerge from crossbreeding, but there also needs to be a representation for introgression and its consequences. The tree of life should therefore only be considered of historical interest as an early and rather simplistic representation of evolution. It has been suggested that a mosaic diagram would be a better representation. Although this would be an improvement, it would, however, fail to represent symbiotic relationships.

The following concerns the issue of inheritance and the original idea of ‘descent with modification’. The word descent conjures up a linear and vertical line from one generation to the next, but this too is an oversimplification. Again, this depiction fails to accommodate lateral gene transfers and symbiotic liaisons. Furthermore, recent research has demonstrated that many organisms are chimeric after inheriting and harbouring more than a single genotype within one body. It is our conclusion, therefore, that the existing theory of descent with modification is insufficient to explain evolution and the diversity of biological life.

In addition, the question of what it is that constitutes the ‘modifiers’ is now an area of keen debate. Evolutionary biology has progressed from simply recognising small mutations and Mendelian inheritance. That is not to say that these mechanisms are not present, but there are many other processes that affect the evolution of form, and some of them transcend genetics. The new science of systems biology is an example of a wider, integrative approach that has moved away from the gene-centric model. The desire here is to combine a mathematical basis of complexity theory with the many factors that influence the form and changing form of an organism. Genetics are necessary conditions to understand what 'modification' entails, but genetics alone are not sufficient. Other factors need to be taken into account such as epigenetics, evo-devo, the proteome, the microbiome, the direct effects of the environment including such things as bio-tensegrity, photoperiodism, photosynthesis, temperature, nutrition, gravity, size and scaling. These are just some examples. All of these factors must be considered within the framework of constraints, which come from chemistry, physics and topology.

The factors that impinge upon the form of organisms and their evolution are many and are often connected. To look for a singular cause or law that encompasses all would be at best optimistic but probably foolhardy. Enclosing that many variables, one would end up with something so generalised, it would not have a scientific backbone. Natural selection has often been employed as the fundamental explanation or 'law' that explains or 'drives' evolution but unfortunately, the term is used in many contexts and in so many contrary relationships that it is difficult to know exactly what it is saying. The challenge for biologists and philosophers of science is to give natural selection a formal scientific definition, but that has so far proved elusive. Until a formal definition is provided, the debate over the 'levels of selection' will continue, with questions whether it is the gene, the individual, the group, or all of the above that selection is acting on. If it is 'all', can it be applied to particles, atoms, molecules or even universes as well? Does natural selection create anything original or does it just eliminate and preserve changes that already have occurred? Where does genetic drift end and selection begin? Without a formal definition, how does one demarcate? What does the word 'selection' mean? Does it have a different meaning to natural selection or is it just a shorthand term? Can the term also be applied to psychology, or to eugenics and the fascist movements of the 1920s and 30s? There are over a hundred different usages of the term selection, all with contrary attributes, so what is the baseline? Is the reformulation of the term 'natural selection' in the 1930s no more than a tautology?

These are difficult questions, some of which have been discussed off and on since the Victorian times, others are more recent. The creationists and the 'intelligent designers' would have one believe that, because there are many problems with natural selection and with neo-Darwinism, the theory of evolution is false. This is, however, a fallacious argument and one should not be discouraged, for there are ways of solving these issues without compromising evolutionary theory. If one cannot resolve a persistent problem, one can still avoid it. In evolutionary theory, one can simply exchange the terms employed with others that are less contentious. Dennis Noble is correct in saying that there is a need for a new language, though it goes deeper than that. Let us banish, as he suggests, such metaphors as 'selfish' or 'cooperative' genes and just talk of interactive genes or even active transcription regions. Let us replace 'genetic programme' or 'genetic blueprint' with genetic effects or influence instead. But more than that, let us recognise that evolutionary change occurs in many other ways than just small mutational changes. To reflect this pluralism, one can employ the general term of 'natural processes'. This term reflects the empirical research of biologists and proscribes against any attempt of the creationists to invoke supernatural explanations. Furthermore, the problems of the term natural selection can be overcome by using a term first recommended by the English author Samuel Butler (1835 - 1902) in the late 19th century. Butler suggested that natural selection can be replaced with the term 'meeting the conditions for existence' –

> '………..the conditions for existence' is a less misleading term than 'natural selection': 'I have said that there is a practical identity of meaning between 'natural selection' and the 'conditions of existence' when both expressions are fully extended'. (Butler 1882 p9)[331]

Whatever the change, be it from hybridisation, small mutation or polyploidy, to name a few, in order to survive, any organism must still meet whatever the prevailing environmental conditions are. The term 'meeting the conditions for existence' also diffuses the tension of having to demarcate between 'drift' and 'selection', for both mechanisms are embraced under this term. No doubt, creationists will continue to scrutinise any new term and look for inconsistencies and weaknesses, but this new term will lend itself less to equivocation and ambiguity than the term 'natural selection'. One could also say environmental pressures rather than 'selective pressures' or 'evolutionary pressures'. The last two terms have now become conflated. Moreover, the term 'evolutionary pressure' is a misnomer. It gives the misleading impression of teleology and that there is some kind of 'force' called evolution. This is a mistake, for evolution is merely a series of changes where the form is altered or population sizes increase or decrease over time. 'Statis', on the other hand, describes periods in history where form has not changed and populations are fairly static, hence there is no evolution.

How then does all this correspond with what has been written thus far? Early on, some fundamental principles were identified that concern the evolution of the universe. Simply said, things began very small and then, through combinations, increased over

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[331] Butler, S. 1882. *Evolution, Old and New; or the Theories of Buffon, Dr Erasmus Darwin and Lamarck, as compared with that of Charles Darwin*. London: D. Bogue.

time in mass and complexity. It appears that what is true for physics and the evolution of the universe is also true for the biological life that ensued thereafter. Whether it is called combination or hybridisation or symbiosis, the principle is basically the same: New forms are created by combination and re-combination, both in the pre-biological and the biological world. When two or more entities that can exist independently come together to produce a new form, these combinations and liaisons lead generally over time to greater mass and complexity. However, objects can also divest mass and complexity, which again may add to the overall diversity. Hence, the direction is not always toward greater complexity or greater mass and size; some forms can become less complex or smaller or disappear altogether. Here are some examples: In physics, the 'weak interaction' recognises that decay is present and in biology, it was seen that polyploidy was followed by rearrangement and disuse of some of the cells' components. Likewise, on the macro scale and throughout Earth's long history, disappearances have occurred not only of many forms of megafauna but possibly also megaflora. (Beckley 2014)[332]

From the perspective of biology, the importance of some fundamental chemicals was observed, in particular of carbon, which forms the basis for carbohydrates, lipids and nucleic acids. As to the amino acids, all of them, for instance, have the same basic atomic arrangement, which is built upon and extended[333]. More complex structures resulting from endosymbiosis and multicellularity evolve by combination, with organisms becoming larger over time and in some instances demonstrating greater diversity. Electrical engineer Libb Thims (2005)[334], who has an interest in human thermodynamics, offers a novel perspective of the earliest stages of cosmological evolution through to the evolution of humans, which is illustrated in a molecular evolution table. Molecules accrue over the course of time and become more complex, demonstrating that the phenotype is more complex than simply a reflection of the genotype. Here are some examples from the table -

RNA:
$C_{10}H_{12}O_6N_5P$

Pro-Bacteria:
$C_{E7}H_{E7}O_{E6}N_{E6}P_{E4}S_{E4}Ca_{E4}K_{E3}Cl_{E3}Na_{E3}Mg_{E2}Fe_{E2}$

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[332] Beckley, C. 2015. *The Hidden Hand of Gravity,* Milton Keynes: Think Logically Books.

[333] Amino acids are seen as building blocks of an organism's body, but one must remember that there are not only the products of genes but also the essential amino acids that are only provided through nutritional intake.

[334] Thims, L. 2005. In *Encyclopedia of Human Thermodynamics*.

Pre-aquatic worm:
$\mathrm{C_{E16}H_{E16}O_{E16}N_{E15}P_{E14}S_{E14}Ca_{E14}K_{E12}Cl_{E12}Na_{E12}Mg_{E12}Fe_{E11}F_{E11}\ Si_{E10}Cu_{E9}Mn_{E8}Se_{E8}Co_{E7}}$

Fish:
$\mathrm{C_{E22}H_{E22}O_{E22}N_{E21}P_{E20}S_{E19}Ca_{E20}K_{E18}Cl_{E18}Na_{E18}Mg_{E18}Fe_{E17}F_{E17}}$
$\mathrm{Zn_{E16}Si_{E16}Cu_{E15}I_{E14}Mn_{E14}Se_{E14}Mo_{E13}Co_{E13}V_{E12}}$

Human:
$\mathrm{C_{E27}H_{E27}O_{E27}N_{E26}P_{E25}S_{E24}Ca_{E25}K_{E24}Cl_{E24}Na_{E24}Mg_{E24}Fe_{E23}F_{E23}}$
$\mathrm{Zn_{E22}Si_{E22}Cu_{E21}B_{E21}I_{E20}Sn_{E20}Mn_{E20}Se_{E20}Cr_{E20}Ni_{E20}Mo_{E19}Co_{E19}V_{E18}}$

These are approximations based on the available data from a cross section of academic compilations.[335] The highlighted parts show common origins of atomic structure. What is not clear, though, is whether this analysis includes the human microbiome, which, without a doubt, must complicate results. Nevertheless, the rationale of an evolving accumulation of all molecules, not just those derived from genetics, is an important recognition and a contribution to a better understanding of the diversity of life. One might even consider this approach to become a possible foundation for a new taxonomic classification system.

Overview

In many ways, a turning point has now been reached in evolutionary biology, which bears some resemblance to the one that revolutionised physics at the beginning of the 20th century. Then, the discovery of new phenomena changed the theories of physics and chemistry and overturned the assurances of the 'clockwork universe'. With the introduction of the theories of relativity and quantum mechanics, the Newtonian world became seen only as a close approximation of how nature interacts. This did not mean, however, that the Newtonian method was abandoned completely, nor that Newton was regarded with less esteem. Indeed, astronomers still navigate through space using Newtonian mechanics. Nonetheless, Newton and the scientists of the 19th century knew nothing of radiation and, accordingly, were unable to calculate, for example, the correct age of the Earth.

Similarly, in the 21st century, the field of biology has witnessed its own host of new discoveries. Some are novel and surprising, others are re-discoveries of phenomena that were, at an earlier time, ignored or seen as marginal in importance and simply

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[335] Thims, L. 2016. http://www.humanthermodynamics.com/Evolution-Table.html

dismissed. This work has tried to capture and illustrate both, the recent and the revised. The combination of the overlooked and the new puts pressure on the theories and changes are necessary. As in the case of Newton, this does not mean that Darwin or Wallace should be revered less. Given the limited empirical evidence available to them, their achievements are remarkable. Nevertheless, one must not forget that, when these two great minds devised their theories of evolution, they were influenced by prevailing ideologies that stemmed from the ideas of the English cleric and scholar Thomas Malthus (1766 – 1834) and *laissez-faire* economics. Looking back, the element of the 'struggle for existence' or 'survival of the fittest' must have presented some sort of scientific justification and convenience to the proponents of the industrial revolution and unfettered capitalism. However, these same ideas harboured yet another, darker side, which was exploited to promote and justify the excesses of eugenics and fascism in the 1920s and 30s. It was not until that period in time that biologists began a reform of the concept of 'natural selection' and based it on differential reproduction and the unfortunate tautological 'survival of the best reproducers'. Regretfully, no formal definition of natural selection was ever agreed upon, which left the door wide open to the many problems mentioned earlier.

When one reads scientific journals these days, one will invariably come across new discoveries of the mechanisms or driving 'force' behind evolution. In one journal, one may read, for instance, about gene regulation rather than the traditional gene mutation. Another journal might argue for epigenetic quantum effects. In yet another journal, one might discover that the driving 'force' is 'selfish' ribosomes and in another one that it is differential methylation. Some authors might see evolution being determined by external factors such as changing nutrition, the influence of invasive viruses or microbes and even return to pre-Darwinian explanations of climate change. Some of the proponents see their discoveries as compatible with natural selection or subsumed under it, while others see their mechanisms as independent of natural selection. These are just a few examples, for the list is long and, indeed, whatever one believes, all of these processes probably have some merit.

In this work, we have focussed principally on symbiosis and hybrisisation as factors of evolution but in addition, we have also given consideration to polyploidy, chimerism and lateral transfers. The empirical evidence for symbiosis and hybridisation that we encountered in our research and have documented in part is much greater than we imagined but is not universally featured within the existing theory of evolution. These processes are not alone in lacking recognition of their importance; the different forms of polyploidy, chimerism and lateral gene transfer are additional casualties.

We have reached the conclusion that there is no single 'driving force' to explain evolutionary change and the amazing diversity of the forms of life and there is no single explanation. Instead, there are many different natural processes involved that bring about change. When we began our research, we were surprised by the amount of empirical evidence for hybridisation that we encountered. As always, the more one concentrates on looking in one direction, the more information one finds, though one must always remember that there is not just one direction. There are, indeed, many directions that manifest themselves and we believe that all of the discoveries of scientific researchers should be respected and examined. We, therefore, advocate a tolerant attitude toward a pluralist explanation of the causes of evolution.

The important, underlying trend that has emerged from this work is the occurrence of biological combinations that lead to more complex forms, which, in turn, bring about more complex forms and so on. This is apparent not only in biological life, but it is a process that has continued since the origins of the Universe. It should not, however, considered to be a law of nature. Combinations do not occur necessarily; sometimes simplicity ensues. Indeed, if some cosmologists are correct, the Universe will eventually expand to such an extent that matter will be ripped apart, leaving only fundamental particles.

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