Chapter 10
Considering Intra-individual Genetic Heterogeneity to Understand Biodiversity

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Abstract In this chapter, I am concerned with the concept of Intra-individual Genetic Hetereogeneity (IGH) and its potential influence on biodiversity estimates. Definitions of biological individuality are often indirectly dependent on genetic sampling -and vice versa. Genetic sampling typically focuses on a particular locus or set of loci, found in the the mitochondrial, chloroplast or nuclear genome. If ecological function or evolutionary individuality can be defined on the level of multiple divergent genomes, as I shall argue is the case in IGH, our current genetic sampling strategies and analytic approaches may miss out on relevant biodiversity. Now that more and more examples of IGH are available, it is becoming possible to investigate the positive and negative effects of IGH on the functioning and evolution of multicellular individuals more systematically. I consider some examples and argue that studying diversity through the lens of IGH facilitates thinking not in terms of units, but in terms of interactions between biological entities. This, in turn, enables a fresh take on the ecological and evolutionary significance of biological diversity.

10.1 Introduction to Intra-individual Genetic Heterogeneity

> These days we have become beguiled with diversity: how animals, such as insects, and plants, such as angiosperms, have produced so incredibly many species. In the origins of multicellularity we see a most primitive example of diversification. In some ways, it is almost an ideal case because we can make an argument for its basis. (Bonner 1998)

Intra-individual genetic heterogeneity (IGH for short) is a characterisation that applies to multicellular biological entities. Simply put, it describes a state in which the cells of the biological entity under consideration contain divergent genomes. Some have argued that (similarity in) genome structure and content can give indications about the expected balance between cooperation and conflict between and

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even within the cells (Queller and Strassmann 2009, 2016; Strassmann and Queller 2010) – in other words, about ecological interactions between genomes. This idea is a major rationale behind investigating biodiversity in the light of IGH.

Another rationale is that IGH highlights the fundamental elusiveness of some key concepts included in definitions of biodiversity. For example, the UN Convention on Biological Diversity defines biodiversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems".[1] The introduction to this volume already mentioned that the ambiguity surrounding terms such as "organisms", "species" and "ecosystems" has already been discussed for a longer time. Technological advances now enable philosophers of biology and biologists to consider in detail how actual patterns of genetic diversity match or clash with these concepts. Studying IGH is part of this effort, in the sense that patterns of genetic diversity within and between biological entities do not always coincide with our perception of these entities as biological, ecological or evolutionary units. To stay with the analogy of diagnosing and treating a patient, as was elaborated in the introduction, understanding IGH is important to better diagnose a patient. The consequences for treatment will be discussed at the end of this chapter.

Here, I am concerned with how the concept of IGH can influence our perception of biodiversity, considering that in biodiversity studies, our definition of biological individuality is often dependent on genetic sampling -and vice versa. Genetic sampling is often concentrated on a particular genome, such as the mitochondrial, chloroplast of nucelar genomes. However, if ecological function or evolutionary individuality can play out on the level of multiple divergent genomes, as I shall argue is the case in IGH, our current genetic sampling strategies may miss out on relevant biodiversity.

I will proceed as follows. As more and more examples of IGH become available, it is becoming possible to investigate the positive and negative effects of IGH on the functioning of a multicellular individual (sect. 10.2). I argue that considering diversity through IGH facilitates thinking not in terms of units, but in terms of interactions between biological entities (sect. 10.3). This, in turn, may enable a fresh take on the ecological and evolutionary significance of diversity. From the examples proposed in this chapter, we can consider how IGH as an unexplored dimension of biodiversity may help us understand what diversity is *relevant* to our research goals.

10.2 Examples of IGH

To make the concept of IGH more concrete, I will start off with a number of familiar and possibly less familiar examples of IGH. Common resolutions to genetic conflict within the organism, such as the separation of germline and soma and genetic

[1] https://www.cbd.int/doc/legal/cbd-en.pdf

bottlenecks, are only applicable to a narrow range of multicellular organisms -mostly metazoans. Organisms that consist of easily regenerating parts (plants, algae) or hyphal networks (fungi) seem often more genetically heterogeneous. In this context, it is interesting to note that metazoans that regenerate from body fragments (medusa, sponges, urochordates) are also more often reported to be genetically heterogeneous (Rinkevich 2004, 2005).

Cases of intra-individual genetic heterogeneity can be divided in chimeras and mosaicisms (Pineda-Krch and Lehtila 2004a; Santelices 2004a), of which examples will be discussed separately below. In the case of mosaic entities, genomes are divergent but homologous in the sense that they share a recent common ancestor. In the case of chimeras, the genetic heterogeneity is nonhomologous: cells may have originated from evolutionary highly divergent lineages. From this definition, it becomes apparent that the distinction between a mosaic and a chimeric biological entitity is ultimately a judgment on evolutionary divergence, which in itself is often based on genome similarity.

10.2.1 Mosaic Individuals

What kinds of individuals are mosaic? As mentioned above, genetic differentiation between somatic cells is often found within plants, animals and fungi that propagate by cloning of body parts. The prevalence of mosaicism in clonally reproducing plants and animals is easy to explain if mosaicism correlates with mutation rate and longevity (Gill et al. 1995). Of course, the mosaic state will be cut short when a single cell bottleneck occurs in the reproductive cycle. Still, this does not seem to stop mosaicism from occurring in multicellular entites that pass through a single cell bottleneck, i.e. metazoans such as fish (Matos et al. 2011) and corals (Schweinsberg et al. 2015).

Mosaicism in humans is a burgeoning field, since much of present-day cancer research relies on assessing genetic heterogeneity between tumor cells, which in turn determines (in some cancers) much of the treatment and prognosis. This approach relies on the argument that the genetic heterogeneity in the tumor is governed by different evolutionary dynamics as the rest of the body (see for example Jacobs et al. 2012; Laurie et al. 2012; Vijg 2014 and references therein). Mosaicism can also have much less dramatic influences in humans, and increasing reports on mosaicism in humans (Youssoufian and Pyeritz 2002; Erickson 2014; Spinner and Conlin 2014) and even the germline (Samuels and Friedman 2015; Jónsson et al. 2017) seem to underline the varying evolutionary outcomes for IGH in a multicellular individual: positive, negative or neutral.

Another example demonstrates how mosaicism can be deeply integrated in the life history of a biological entity. Arbuscular mycorrhizal fungi (AMF) are an ancient phylum of heterotrophs that form symbioses with the majority of land plants (Wang and Qiu 2006). AMF were reported to contain hundreds or thousands of genetically differentiated nuclei within the same cytoplasm (Kuhn et al. 2001; Hijri

and Sanders 2005; Boon et al. 2010, 2013b, 2015). The exact number of genetically differentiated nuclei is not clear, since genetic variation has never been exhaustively sampled for any locus in these fungi. No sexual stage has been observed, although possible recombination has been reported in AMF (see Riley et al. 2016 and references therein).[2]

It is possible that a single nucleus is not a viable entity in AMF –only populations of nuclei are (see Boon et al. 2015; Wyss et al. 2016 and references therein). For example, spores of *Rhizophagus irregularis* do not germinate under a certain volume, which is positively correlated with the number of nuclei within the spore (Marleau et al. 2011). Some authors have proposed that genetic differentiation between nuclei within the AMF cytoplasm is maintained through the fusion of related hyphae, or anastomosis (Giovannetti et al. 2015 and references therein), and is lost at sporulation (Boon et al. 2013b). This means that AMF are both mosaic individuals in the sense that their nuclei share the same genealogy, and chimeras in the sense that at least some of this variation is the result of hyphal fusion between related hyphal systems.

The positive correlation between anastomosis rates and level of relatedness between hyphae supports the view that AMF do form genetically delineable entities –although maybe not on a genome level, but on that of the genome population or pangenome. This has also been suggested by Boon et al. (2015). The propensity to fuse within cultures of the same strain seems diminished by drift (Cárdenas-Flores et al. 2010). This may indicate that the nuclei within an AMF hyphal system show self-nonself recognition *as a population*. Finally the composition of the genome population has an influence on the genotype: a change in nuclear population through anastomosis changes the (symbiotic) properties of a strain (Sanders and Rodriguez 2016 and references therein).

To summarise this example, the AMF phenotype seems determined by not a single, but multiple coexisting genomes. Anastomosis, or a lack thereof, can change the AMF phenotype, which is in turn selected upon by its environment (Roger et al. 2013b, a; Angelard et al. 2014; Wyss et al. 2016; Sanders and Rodriguez 2016). AMF form symbioses with almost all land plants (Wang and Qiu 2006), and the age of their evolutionary lineage (an estimated 500 million years, coinciding with the rise of land plants (Corradi and Bonfante 2012)) testifies to the potential ecological impact and longevity of IGH as an evolutionary strategy. The presence of mycorrhiza in the soil confers a inestimable fitness advantage to plant communities: most plant taxa form symbioses with AMF in which posphorus is exchanged for plant-produced sugars, thus stimulating plant growth and overall community biodiversity (van der Heijden et al. 2016)

In this example on AMF, IGH seems to be an essential to understand AMF life history, ecology and evolution. At the same time, the precise effects of IGH on AMF life history are hard to estimate since this IGH is often not taken into account in experimental setups and field studies due to technical and conceptual challenges (Sanders and Rodriguez 2016). Still, we can add yet another layer of complexity. If

[2] Note that some recombination estimates may not be reliable if Glomus is indeed multigenomic.

we consider that AMF are obligate plant symbionts and themselves are associated with particular microbial communities, a picture emerges of another set of interactions. AMF are functionally dependent on their plant hosts, and probably gain major fitness benefits from their associated microbial communities (Bonfante and Anca 2009; Herman et al. 2011). Thus, since the ecological function and evolutionary longevity of the mosaic AMF is dependent on nonhomologous lineages (i.e. plants and microbes), we may also consider AMF and their associated plants and microbes as chimeras. This is not only theoretically relevant: the growth benefits that AMF confer to their plant hosts can potentially confer huge benefits to sustainable agriculture.

10.2.2 Chimeric Individuals

As in the AMF example above, most mutualisms can also be considered chimeras. This depends on the criteria for individuality that are being used, and to some extent on the degree of genetic divergence one is willing to accept between the component genomes of the chimera. For example, in the case of lichen and corals the mutualism is so tight that the historical and most intuitive view is to see the chimera as a single entity. Only with the advent of molecular techniques have scientists started to distinguish different genomes and consider the partners as separate 'individuals'. With the following two examples, I would like to illustrate how broad the definition of 'chimera' can be, and highlight how considering IGH has consequences for ecological and evolutionary inference in these examples.

My first case is chimerism in macroalgae, which is a nonmonophyletic group that encompasses brown, green and red algae (Santelices 2004b). Here, I will focus on red algae or Rhodophyta, since IGH in this taxon has been most extensively documented. The algae germinate from a disk, which can originate from multiple spores. These spores may fuse, or form individual cell walls that are subsequently surrounded by a thickened common wall. This process, called coalescence, occurs often in red algae (Santelices et al. 1999) but not between different species (Santelices et al. 2003). Coalescing disks, or crusts, may or may not fuse with each other or with new algal sporelings. Various fitness advantages were demonstrated for coalescencing disks compared to unitary disks (which have originated from a single spore). Fusion decreases the probability of mortality in early growth (Santelices and Alvarado 2008), improves erect axis formation and growth (Santelices et al. 2010) and confers an advantage later in the life cycle through differences in branching and fertility (Santelices et al. 2011). Coalescence and a fitness advantage for coalescing disks have also been reported, although less extensively, in brown (Wernberg 2005) and green (Gonzalez and Santelices 2008) algae.

Thus, like in AMF, we encounter a population of genotypes (from multiple haploid spores) which together creates a polyploid phenotype that is selected upon as a single entity (Monro and Poore 2004). In red algae, these polyploids break up again at sporulation. Possibly, selection (for cooperation) between haploid genomes occurs at the formation of the disk. How this selection takes place is unclear. It

seems reasonable to suppose that selection occurs for compatibility between particular loci or even entire genomes. It is important to note that, like in the previous AMF example, it is not possible to associate the phenotype of a red alga with a particular unchanging set of spore haplotypes. The phenotype of interest, i..e the disk and the thalli that grow from it, is based on a *varying* population of multiple haploid nuclear genomes. Therefore, the phenotype of the macroalga cannot be reduced to its component genomes. It is in the *interaction* between these varying genomes that a unique phenotype is established.

The ecological and evolutionary consequences of the chimeric state in macroalgae are not easy to disentangle, even more so because seaweeds can reproduce clonally as well (Fagerström and Poore 2000; Collado-Vides 2001). Nevertheless, the above description makes clear that the life history and evolutionary constraints of coalescing red algae cannot be described accurately without taking chimerism into account. Again, in the light of the ecological and agricultural importance red algae this is not a merely academic preoccupation. For example, multisporic coalescing recruits have higher survival rates (Santelices and Aedo 2006). Thus, IGH as a state can be manipulated by farmers to increase higher yields.

My second example of chimerism is the case of microbial multi-species consortia or communities. With the advance of molecular techniques it has become possible to study microbial communities in more detail than ever before, even though passing from the fase of amassing vast quantities of data to that of interpreting them has proven to be a challenge. Here, I would like to highlight a few patterns that have emerged with respect to microbial diversity and function and that are relevant to the concept of IGH. The following three points are discussed in more detail in Boon et al. (2013a).

First, microbial taxa are hard to circumscribe precisely, for a number of well-described ontological and epistemological reasons.[3] For the purposes of this chapter, it suffices to state that microbial taxonomy is heavily dependent on the molecular biology toolbox. This toolbox, although indispensible, has a number of limitations. The most relevant limitation for my point is that especially early conceptions of microbial taxa heavily relied on the assumption of genome stability. And there's the rub: in many microorganisms, genomes can change rapidly through gene loss, gene duplication, and the acquisition of genes from distant lineages via lateral gene transfer (LGT).

A second pattern is that taxonomic or phylogenetic thresholds (e.g. 3% genetic differentiation) for taxon delineation fail to adequately delineate ecologically cohesive units. Even though a unifying species concept is not strictly needed for ecological analysis, also a pluralist stance needs a sound rationale and consistent approach (or set of approaches) to define 'units'. Unfortunately, microbial diversity and community function do not always correlate. Microbes rarely act alone and are often interdependent. It is possible that less than 1% of all known microbes can be successfully cultured on their own (Staley and Konopka 1985), an observation also known as 'Great Plate Count Anomaly'. It is now clear that many microbes depend on the activity of other microbes to successfully grow and reproduce via mecha-

[3] Doolittle (2013) has written an extensive review on the history and challenges of microbial ontology and of course O'Malley (2014) is an invaluable resource here as well.

nisms including acquisition and exchange of metabolites (references in Boon et al. 2013a).

A final tendency is that microbial function may be a property of communities as well as of cells. Particular metabolic capacities might not be encoded within a single microbial cell, or even within a single type of microbial cell. Instead, there is increasing evidence that many microbial functions are encoded by gene networks in which genes may be easily replaced by functionally equivalent but phylogentically distant alternatives. These gene networks may be found in varying sets of microbial taxa, without a single taxa being characterised by a particular set of genes or functions. We face the same situation as in the previous examples, in which no single community genotype codes for a single community phenotype.

If microbial communities can be understood as 'chimeras', it might not be possible to lead a community function (for example, a particular metabolic product or process) back to a single taxonomic group (but see Inkpen et al. 2017). The diversity of microbes is now being explored using surveys that draw on hundreds or thousands of samples and controlled experiments, with rapid genetic assessment techniques providing much of the evidence for taxonomic and functional diversity. Since microbial interactions span all taxonomic ranks, from strain to superkingdom, understanding microbial diversity then seems to necessitate a community-centric approach (Zarraonaindia et al. 2013).

Mechanisms for the evolution of interdependence within microbial communities have been proposed in the form of a Public Goods Hypothesis ((McInerney et al. 2011) and the Black Queen Hypothesis (Morris et al. 2012). The authors of the ITSNTS model ('It's the song, not the singer') even propose 'casting metabolic and developmental interaction patterns, rather than the taxa responsible for them, as units of selection' (Doolittle and Booth 2016): in other words, microbial interaction patterns are stabler units of selection than the microbial cells that produce these patterns. For a more in-depth discussion of the evolutionary and ecological implications of seeing microbial communities such as biofilms as evolutionary individuals, see Boon (in preparation).

10.2.3 Mosaic vs. Chimeric Individuals

The reader might wonder by now whether the distinction between mosaic and chimeric individuals is at all relevant. Is the difference between the two not just a matter of degree of relatedness between individuals, rather than a difference in kind of individual? The answer to this ontological question is not at all straightforward. However, from an epistemic point of view, the differences between these two types of intra-individual genetic heterogeneity are relevant to the practise of evolutionary inference.

For example, fitness calculations between mosaic entities and chimeras are performed differently. If genetically differentiated but related cell lines work together, as in mosaic individuals, a case could be made for a special sort of kin selection. After all, there is a considerable chance that gene variants between related lineages

are shared. However, if unrelated cell lines become integrated in a single entity, the balance of costs and benefits that ultimately decides between competition and cooperation cannot be explained by a more than average chance to transfer one's own genes as present in the other.

Some might disagree that there is even an epistemic difference between chimeras and mosaic biological entites. Multilevel selection theory (see Okasha 2006 and references therein) stresses that kin selection is really just a special case of group selection. Interestingly, this discussion is also highly relevant within microbial community ecology and evolution, in which the question whether microbial communities can evolve is a hot topic of debate. Boiled down to its essence, this question is really about whether entities that are composed of nonhomologous lineages can evolve as a single unit–and whether is this is a useful question to ask (Boon in preparation).

10.3 The Importance of IGH in Ecology and Evolution

Above examples lead me to two main themes for the relevance of IGH in biodiversity research. The first is that multiple *varying* genotypes can lead to a single phenotype. Since the phenotype is the actual set of traits that is selected upon, or that is ecologically relevant, extreme caution should then be exercised when a single genome (or genotype, or even a simple barcode) is taken as a proxy for the phenotype. If the more complicated genotype-phenotype relationship that is implied by IGH is ignored and genotypes that are associated with a particular phenotype are inadequately sampled, it will be difficult if not impossible to find reproducible patterns and predict community composition or ecosystem function. Second, if one of the aims of measuring biodiversity is to predict or at least understand ecosystem function, it is vital to note that while community ecology considers interactions among entities, the inference of these interactions depends critically on the level at which entities are defined.

These two themes may be made more concrete with an example: while it may be possible to describe a microbial community as performing a single ecosystem function, it may not be possible to find a specific genotype or even set of genotypes stable enough (i.e. reoccurring consistently) to characterise this functional unit. Instead, one may want to consider whether instead particular interactions between units, such as a particular exchange of metabolites or another shared fitness benefit, may be the most stable component of the interaction.[4]

This situation is a radical departure from a more traditional view, in which a one-to-one relation is assumed between genotype and phenotype. In other words, once we look away from our metazoan bias it may no longer be possible to explain phenotype and its ecological role by measuring the genotype, since this genotype, even as an amalgation of multiple component genotypes, is simply not stable enough.

[4] See also the ITSNTS argument of Doolittle and Booth (2016).

10.3.1 The Metazoan Bias

The examples in the previous section might seem "atypical" in the context of biodiversity. In fact, when speaking of biodiversity there is often a bias towards species that are relatively easy to identify and delineate, such as animal species. Yet vast diversity, however measured, can be found in groups such as algae, fungi, and the many phylae of microbes and virusses, which are often at the basis of ecosystem function (e.g. Wagg et al. 2014).

Still, one should wonder whether it makes sense to describe above examples as instances of IGH. In other words, how permissive can a definition of biological or evolutionary individuality be without losing its use? The term 'Intra-individual genetic heterogeneity' ultimately pivots on the definition of the 'individual'. To determine an ecological function or identify an evolutionary process, one needs to distinguish the entities that perform these functions or processes.

The discussion on biological delineation and individuation has been conducted in different contexts already and has taken a central place in recent philosophy of biology discussions (see for example Queller and Strassmann 2016; Clarke 2016; Pradeu 2016 and references therein). It becomes clear from these recent considerations that there are valid reasons to consider biological organisation from many different viewpoints. In other words, different research goals justify the use of divergent concepts of biological or evolutionary individuality and thus warrant a pluralist approach.

In this context, it does make sense to describe different kinds of biological entities as instances of IGH. For example, when we consider a system with AMF, we could choose to look at a single AMF nucleus, at a population of nuclei, or at an entire hyphal system. Enlarging our scope even more, we could choose to include the plant partners as well as the surrounding microbial communities. I propose that it is in this choice that the real point of discussion lies: how to decide on the relevant unit of diversity?

10.3.2 Biological Organization, Hierarchy and Relevance

A genotype, or even the entire genome, is often for practical purposes employed as a unique identifier for 'the biological individual'. Of course, this biological individual cannot be fully described by only its genetic code. If this were so, we would consider human identical twins to be one and the same biological individual. However, although the individual is not defined by a unique genome, a unique genome seems to havebeen a convincing criterion for assigning individuality. Why?

One possible reason is that the organization of biological diversity is considered to be hierarchical. In this view, DNA is organized in cells, cells in bodies, bodies in populations and populations in species. It is implied that without cells competing or cooperating, the body would not exist, and without bodies competing or cooperating,

a population would not exist, and so forth. Leo Buss, for example, stated that "[An] explicitly hierarchical perspective on evolution predicts that the myriad complexities of ontogeny, cell biology and molecular genetics are ultimately penetrable in the context of an interplay of synergisms and conflicts between different units of selection" (Buss 1987).

This idea of hierarchy is also prominent in the literature on major transitions in evolution. Maynard Smith and Szathmáry proposed that complexity in evolution increases with time, which is achieved through a series of major transitions. They also described this complexity as mostly hierarchical (Maynard Smith and Szathmáry 1995). Others have continued or varied on this view of evolution of life on earth, yet all agree that cooperation and competition takes place at definable 'levels' (Clarke 2016 and references therein). A formalisation of this view can be found in multilevel selection theory. Proponents of this theory aim to develop and formalize the tools we need to describe and quantify the relative importance of different levels of selection (e.g. Wilson and Sober 1994). Ultimately, the interactions between these levels are proposed to lead to the diversity we observe among biological entities.

Much discussion in the major transitions literature is then about finding out how conflict at a particular level of organization is resolved, in order to explain the evolution and diversity of another level of biological organization. In this manner, a hierarchical view on biological diversity can offer a perspective with the scope to explain a large number of observations. However, it can also lead to misleading assumptions or obscure similarities. For example, the link of genome homogeneity with the delineation of the biological individual is based on the assumption that IGH leads to conflict within that individual (Michod and Roze 2001; Strassmann and Queller 2004). Yet it is not clear whether genome heterogeneity always leads to conflict. It is possible that there are cases where IGH can actually confer an advantage to the multicellular community it is part of. Some of these examples were already discussed above. The question then becomes more nuanced: when is IGH relevant?

The simple answer may be: when there is a significant effect of IGH on the possible evolutionary trajectories (sometimes referred to as 'evolvability') and ecological range that a biological unit can follow or occupy as a result of its IGH. These latter two concepts are exactly what is at stake in many biodiversity investigations. Moreover, some of the ways in which biodiversity is understood are based on taxonomic or ecologic hierarchies (e.g. Sarkar 2002). Red algae and arbuscular mycorrhizal fungi are two fairly well-documented organisms in which intra-individual heterogeneity plays an important role in understanding of both evolvability and ecological range. Furthermore, even though IGH is sparsely documented, reviews are available with more examples (Santelices 1999; Pineda-Krch and Lehtila 2004b, a), as well as a range of suggestions on how IGH could affect life history (Pineda-Krch and Lehtila 2004b; Folse 2011; Folse and Roughgarden 2012). Finally, the importance and relevance of IGH should be decided on a case-by-case basis –without assuming or dismissing its potential role off-hand.

10.4 Conclusions

I argued that the shortcut one genome-one individual has closed our eyes to the possible importance of IGH in evolution and evolution –and thus to its role in for biodiversity estimates. Arguing from the examples in this chapter, I propose that IGH can help us understand what diversity is *relevant* to our research goals. To maintain the analogy from the introduction: which characteristics of the patient and her symptoms are relevant to a diagnosis and treatment?

By expanding our practical and conceptual tools to facilitate the study of genetic heterogeneity at many different levels of biological organisation, we can start to understand diversity by focusing on interactions between entities –however defined.

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