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## Natural selection and its limits: Where ecology meets evolution

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**Abstract:** Natural selection [Darwin 1859] is perhaps the most important component of evolutionary theory, since it is the only known process that can bring about the adaptation of living organisms to their environments [Gould 2002]. And yet, its study is conceptually and methodologically complex, and much attention needs to be paid to a variety of phenomena that can limit the efficacy of selection [Antonovics 1976; Pigliucci and Kaplan 2000]. In this essay, I will use examples of recent work carried out in my laboratory to illustrate basic research on natural selection as conducted using a variety of approaches, including field work, laboratory experiments, and molecular genetics. I also discuss the application of this array of tools to questions pertinent to conservation biology, and in particular to the all-important problem of what makes invasive species so good at creating the sort of problems they are infamous for [Lee 2002].

The evident complexity and specificity of living organisms has always generated curiosity and a search for explanations to account for it. During the early 19th century, William Paley [1831] proposed a close analogy between structures such as the human eye and artifacts such as watches, concluding that living organisms must have been intelligently designed. Paley's was the last historically significant attempt at explaining the natural world in theological terms, in the tradition of what at the time was appropriately known as "natural theology". David Hume [1779] had already dealt a mortal philosophical blow to the argument from design during the 18th century, essentially pointing out that natural theology actually provided no explanation at all: to invoke a mysterious and unknowable designer to "explain" nature was an unnecessarily fancy way of admitting our ignorance in the matter. However, Hume did not actually have an alternative explanation available, and the dispute remained unsettled until the work of Charles Darwin [1859]. He was the first one to propose a reasonable alternative explanation for what we now call adaptation: the action of natural selection. Darwin reasoned that the natural tendency of populations to increase exponentially in number, had to clash with the obviously limited resources available at any particular time and place (something first noted by Thomas Malthus). Since living organisms

are different from each other, and some of these differences are heritable from one generation to the next, Darwin expected those variants that had the highest fitness to leave more offspring because of competition for the available resources. This was, in essence, the principle of natural selection.

In the remainder of this essay, I will explore the idea that the study of alleged adaptations is in part a historical question and in part an experimental one [Pigliucci and Kaplan 2000], and represents one of the best examples of convergence between the disciplines of ecology and evolutionary biology. In this area, hypothesis testing must be conducted by the combination of a series of approaches, each one of which by itself may not be sufficient to shed light on any particular case of interest. Members of my laboratory use this varied toolbox during their investigations, though additional ones are obviously available to evolutionary biologists in general [Freeman and Herron 2001].

### 1. HETEROPHYLLY IN AQUATIC PLANTS: MEASURING NATURAL SELECTION IN THE FIELD

I will illustrate the historical-experimental study of adaptations and their limits by way of four examples of recent or ongoing research in my laboratory. The first instance involves a classic case of allegedly adaptive phenotypic plasticity

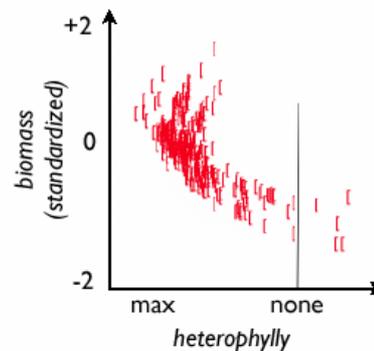
(the response of genotypes to changing environmental conditions: Pigliucci 2001): heterophylly in semi-aquatic plants [Cook and Johnson 1968]. This is work that I conducted with Carolyn Wells [Wells and Pigliucci 2000]. If one considers the gradient across a shoreline (say, of a lake or pond), one moves from completely aquatic plants to completely terrestrial ones, with an intermediate zone populated by plants that have to be able to live alternatively under and above water, depending on the season. Some species seem to be able to cope with these changing conditions by altering the structure and shape of their leaves so that the underwater ones have a much higher surface/volume ratio, and are therefore better adapted to the slow gas diffusion typical of their environmental conditions.

We identified a convenient system to study heterophylly: three very closely related species of the genus *Proserpinaca* occasionally live in the same pond and show variation from a high degree of heterophylly (*P. palustris*) to no change in leaf shape (*P. pectinata*), through the existence of intermediate hybrids (*P. intermedia*). These hybrids can also be produced in the laboratory, thus allowing genetic research to be conducted.

While heterophylly is usually considered an adaptation to changing water conditions [Cook and Johnson 1968; Wells and Pigliucci 2000], there have been very few instances of actual quantitative measurement of natural selection acting in natural populations to maintain plasticity for leaf shape in response to water levels. Indeed, one of these few exceptions has failed to detect any selection on heterophylly, although the particular system used was not a semi-aquatic plant [Winn 1999]. We therefore set out to measure the type and intensity of natural selection for heterophylly in *Proserpinaca* using the standard quantitative approach proposed by Lande and Arnold [1983], which uses multiple regression analysis of the relationship between phenotypic traits and measures of fitness.

The results of two field seasons were indeed consistent with the hypothesis that heterophylly in this system is maintained by natural selection [Wells and Pigliucci, in prep.]. Using several transects, we showed that plants with high degrees of heterophylly do have a significantly higher vegetative (growth rate) and reproductive (flower and fruit production) fitness than plants with lower

levels of divergence in leaf morphology (Figure 1).



**Figure 1.** Combined data from several transects in a field study of heterophylly in *Proserpinaca* [Wells and Pigliucci, in prep.]. Each segment represents an individual. Notice how vegetative fitness (measured as biomass production) is highest for plants with the maximum level of heterophylly.

These findings, however, have spurred an additional question: if heterophylly is advantageous under the conditions experienced by all three species of *Proserpinaca*, why is it that one of them shows no change in leaf shape while the season progresses? A partial answer may be provided by the fact that *P. pectinata* (the non-heterophyllous species) may have adopted alternative means to facilitate gas exchange underwater, since we found that these plants collapse many of the cell walls inside their roots when compared to the closely related *P. palustris*. This phenomenon is known to aid several aquatic species in their gas exchange underwater, and it is a particular illustration of the general principle that living organisms can achieve a better fit with their environment in a variety of not necessarily mutually exclusive ways. They can alter their external morphology, as in the case of heterophylly, but also their internal anatomy, or even their physiology. Which particular path is taken may depend as much on chance (the sort of genetic variation that happens to be available at a particular time) as on the specific selection pressures experienced by a population.

## 2. LIMITS TO SELECTION IMPOSED BY THE GENETIC ARCHITECTURE

The second example that I wish to briefly present deals with potential limits to selection, in this case imposed by the genetic variance-covariance ( $\mathbf{G}$ ) structure underlying a set of traits [Stephan et al. 2002].  $\mathbf{G}$  is a mathematical construct that

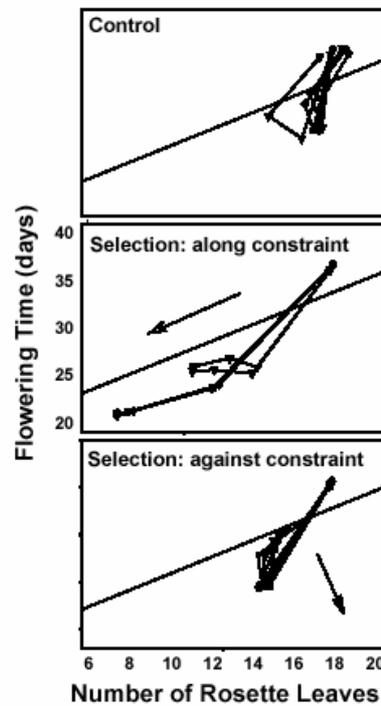
quantifies the set of genetic variances of a given number of characters, together with the genetic covariances connecting each pair of characters. In theory [but see Houle 1991],  $\mathbf{G}$  can be used to quantify the ability of certain traits to respond to selection (which depends on their genetic variance), as well as how much each trait affects the evolution of other ones (through their covariances). Mark Camara and I [Camara and Pigliucci 1999; Camara et al. 2000] were interested in empirical investigations of the role of  $\mathbf{G}$ , within the general theory of evolutionary quantitative genetics.

Studying  $\mathbf{G}$  is not easy because of both theoretical and logistical problems. To complicate things further, Gunter Wagner [Wagner and Altenberg 1996] has proposed that we should really be concerned not just with  $\mathbf{G}$ , which is a snapshot of the genetic architecture of a population at a given time, but with what he referred to as the  $\mathbf{M}$ -matrix. This is an extension of  $\mathbf{G}$  that incorporates information about new genetic variation (via mutation and recombination) that may appear over a given (usually unspecified) temporal horizon. The idea is that the apparent constraints on evolution inferred from  $\mathbf{G}$  may not be as strong if one considers  $\mathbf{M}$ , which is supposed to be characterized by higher degrees of genetic variance and lower degrees of genetic covariance. Camara and I wished to approach this problem experimentally, by carrying out selection along and against an observable genetic constraint in natural and mutagenized (i.e., characterized by augmented genetic variation) populations of the weed *Arabidopsis thaliana*.

The results (Figure 2) showed that a set of replicated base and mutagenized populations did not move in phenotypic space if left to their own devices, as expected from quantitative genetics theory. On the other hand, the same populations responded very rapidly to directional selection along the genetic correlation, again following expectations. Indeed, the mutagenized populations responded much more quickly and went farther than the non-mutagenized populations, presumably because of the increased genetic variation for the selected characters. This confirms Wagner's intuition that  $\mathbf{M}$  may speed up evolution when compared to  $\mathbf{G}$ . However, selection away from the constraint proved completely unsuccessful: after an initial strong response, all populations bounced back and behaved essentially as the unselected controls. The constraint relating the two traits is

apparently difficult to overcome, despite the addition of fresh mutations to the base population.

And yet, the constraint between leaf production and flowering time is most certainly not universal, since species closely related to *A. thaliana* do not show it. Therefore, we are currently investigating the genetic architecture of close relatives of *A. thaliana* to address the question of how the constraint evolved to begin with (since molecular genetics and phylogenetics tell us that the constrained *A. thaliana* are a recent result of evolution in this group: Simpson and Dean 2002).



**Figure 2.** Results of a selection experiment conducted by Camara and Pigliucci [in prep.] showing the effect of standing and new genetic variation and covariation on the ability of two traits to respond to selection in *Arabidopsis thaliana* (see text for details).

### 3. SELECTION IN *ARABIDOPSIS*: THE COMPLICATIONS OF REAL ECOLOGY

A third example of multiple approaches to the study of adaptation comes from research I have done with Courtney Murren [in prep.] and Hilary Callahan [Callahan and Pigliucci 2002], again on flowering time and related traits in *Arabidopsis*.

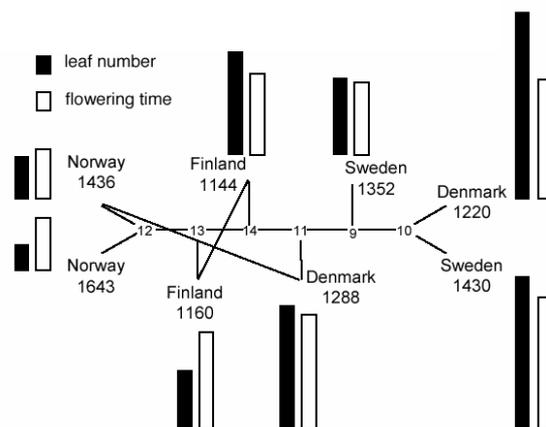
When we conducted two years of field studies at two different localities, we were able to show strong and consistent natural selection favoring early flowering in two natural populations by conducting a reciprocal transplant experiment. This is consistent with the idea that *A. thaliana*, not being a good competitor, would be selected for a short life cycle. However, a separate experiment in a different year and location yielded completely different results, suggesting instead the action of strong stabilizing selection for flowering time. We were able to figure out what was going on because of constant checking of the plants in the field: our experimental plot got hit by two waves of herbivores (fungus gnats and aphids) at the beginning and end of the flowering season, which selected for plants that flowered in the middle of the two herbivores' invasions. This is a good example of how intensive field monitoring is necessary to make sense of patterns of selection that may otherwise be suggestive but difficult to interpret.

As a follow-up on that study, we are now analyzing Quantitative Trait Loci data [Murren and Pigliucci, in prep.] relative to the population that experienced herbivory. This will allow us to pinpoint some of the genomic regions involved in the selective episode, and possibly to match some of them with known candidate genes affecting flowering time in this species [Stratton 1998].

In order to test adaptive hypotheses one also has to have information about the historical (genetic) relationships among populations [Templeton et al. 2000; Martins et al. 2002], since there is always the possibility of phylogenetic "constraints" (though the latter represent a rather heterogeneous

category, which may include the historical outcome of past selection as well). To this end, together with Mitch Cruzan and Heidi Pollard [Pollard et al. 2001; Pigliucci et al. 2003], I have begun working out the intra-specific "network" phylogenies of populations of *A. thaliana*.

The idea is to use network (as opposed to standard bifurcating ones, more appropriate for inter-specific studies) phylogenies as a baseline to track the evolution of ecologically interesting traits and their co-evolution with other characters, as it is shown in Figure 3. Here, the height of the bars is proportional to the average phenotypic value of each trait (leaf number and flowering time) in each population. While the analysis can be carried out statistically, it is clear that some closely related populations have very similar trait values (e.g., the two Norwegian accessions, as well as Denmark 1220 and Sweden 1430). It is also clear that the two traits (the same that we have shown so far to be genetically correlated to each other) tend to co-evolve across this phylogenetic network. (Note that the phylogenies were derived with a variety of algorithms, which yielded essentially congruent results, and are based on chloroplast DNA sequences.) Research in my laboratory is currently being conducted to expand this approach to several dozens populations collected from a large longitudinal span in the natural habitat of *Arabidopsis* in Europe. The goal is not only to reconstruct the intraspecific phylogeny of these accessions—which will be of great value to the entire *Arabidopsis* community for comparative studies—but to test specific hypotheses about the evolution of responses to photoperiod and other environmental conditions.



**Figure 3.** Co-evolution of leaf number and flowering time in a set of *Arabidopsis thaliana* Scandinavian populations connected by a network phylogeny (numbers on the network indicate genetic differences; Pigliucci et al. 2003).

#### 4. APPLYING EVOLUTIONARY ECOLOGY: INVASIVE SPECIES

The final example to be briefly considered here concerns research that I am conducting with Norris Muth on the role of phenotypic plasticity in invasions [Muth and Pigliucci, in prep.]. We are taking again a comparative phylogenetic approach, this time to compare pairs of invasive and non-invasive species belonging to two distinct but relatively closely related genera of Asteraceae, *Centaurea* and *Crepis*.

The preliminary results are exciting because they clearly show that traits can be grouped into two broad categories. On the one hand, all invasives in our group seem to behave similarly (and distinctly from the respective non-invasives) for certain traits, like branching and inflorescence production. This is regardless of the phylogenetic relationship of the different species, so that if one combines the data from all species the general pattern still holds.

However, a second group of traits (e.g., biomass and plant size) behave very differently between the two genera of invasives, reflecting their peculiarities in life history and growth habit. In this sense, it appears that not all invasives are created equal, and our research points out that the quest for elusive universal hallmarks of “invasiveness” may have to be rethought [Lee 2002].

Similarly, at the multivariate (i.e., many traits simultaneously) level of analysis, a relative simple picture of the “perfect invasive” that would emerge if one were to pool data regardless of the phylogenetic relationships of the different species, turns out to be misleading. The reality is that different genera of invasives are characterized by their own unique combination of traits that aid their reproductive fitness and—indirectly—their ability to invade.

#### 5. CONCLUSIONS

Getting now back to the “big picture,” what I tried to convey is the idea that the study of adaptations and their limits is a fascinating mix of historical and experimental work, which has a lot in common with the activities of a detective. Historical events (such as the process of adaptation) leave incomplete traces, rarely a clear-cut “smoking gun” [Cleland 2002]. As a result, the best approach is to attack the problem from a variety of perspectives simultaneously, hoping for a “consilience” [Whewell 1840] of different

results, i.e. a convergence toward the same conclusion.

We are clearly far from a complete understanding of the mechanisms and limits of natural selection in natural populations, despite the fact that Darwin [1859] formulated the basic idea more than 140 years ago. Yet, tremendous progress has been achieved during the last few decades, thanks to both conceptual and technical advances in evolutionary ecology. There is no reason to think that our progress will not continue in the immediate future, benefiting from a unique convergence of biological disciplines to elicit the fundamental mechanism that creates the apparent fit between organisms and environments.

#### 6. ACKNOWLEDGMENTS

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