11

Studying Mutational Effects on **G**-Matrices

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Variation versus Variability, G versus M

Wagner and Altenberg (1996) introduced the concept of variability as distinct from variation in the study of the evolution of complex phenotypes, a concept that has parallels in complexity theory and that concerns the "genetic neighborhood" of a given genotype and its role in evolution on rugged adaptive landscapes (Kauffman and Levin 1987; Kauffman 1993). Their basic idea is that evolution depends not just on the extant genetic *variation* as studied by classical quantitative genetics, but also on the underlying genetic *variability* that quantifies the range of immediately possible genotypes that can occur in a given population in the not too distant future.

In evolutionary theory, the **G**-matrix, which summarizes the additive variances of and covariances among different traits of interest, quantifies the amount of extant genetic variation. **G** enters into the multivariate version of the "breeders' equation," which is taken to be a sufficient description of evolution by natural selection, given certain assumptions:

$$\Delta\overline{\mathbf{Z}} = \mathbf{G}\mathbf{P}^{-1}\mathbf{S}$$

where $\Delta\overline{\mathbf{Z}}$ is the vector of change in trait means, **G** is the matrix of additive genetic variances and covariances, **P** is the matrix of phenotypic variances and covariances, and **S** is a vector of selection differentials. One problem with the use of the breeders' equation is that there are both theoretical (Turelli 1988) and empirical (Roff and Mousseau 1999) reasons to think that **G** does not stay constant or even proportional over evolutionary time (see also Hansen and Houle, Chapter 6, this

volume, for a different angle), contrary to what is assumed by common evolutionary quantitative genetics theory. Hence, the breeders' equation can work only approximately, on the short run of a few generations (despite the common practice of projecting it over tens of thousands of generations: e.g., Via 1987).

Wagner and Altenberg's, as well as Kauffman's, idea is then that we need to conceive of an expansion of **G**, often referred to as **M** (for mutation, the ultimate source of new genetic variation), which estimates the *variability* potentially harbored by a population, and that can give us a better idea of the medium-capacity for evolutionary responses. I have conceptualized the difference in Fig. 11.1: suppose we measure selection in a natural population using the standard multiple regression approach (Lande and Arnold 1983; Rausher 1992) and find that there is a push to shift the position of the population in phenotypic space from where it currently is to the area identified by the star in the diagram. However, from a study of the quantitative genetics of the situation, we also uncover a fairly strong genetic correlation between these same two traits (a component of **G**, indicated by the narrow ellipse). Our conclusion, based on the breeders' equation, would be that the resultant of the two forces (natural selection and the existing genetic constraint) will actually deflect the evolutionary trajectory pretty far from the optimum favored by selection. This conclusion, of course, depends on usually unstated assumptions concerning **M**, which is normally not estimated empirically and cannot be derived from first principles. For example, it is possible that the genetic architecture of the traits in question is such that, if we allow for a sufficient amount of time to pass so that mutation and recombination will translate some of the underlying *variability* into actual *variation*, the resulting genetic correlation will be much less tight (larger ellipse in the figure). This, in turn, may allow the population to get significantly closer to the selected optimum than we might at first have surmised.

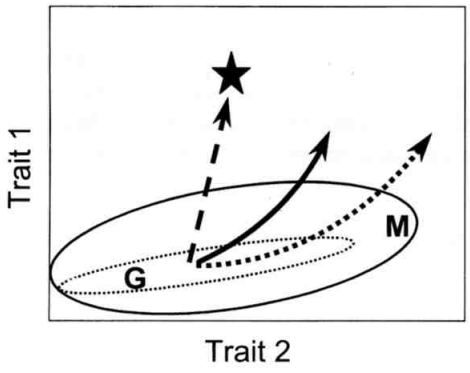

Figure 11.1 The difference between a **G**- and an **M**-matrix and the impact it can have on evolutionary change. The scenario considers the simplified situation of just two traits related by a genetic correlation, represented by the small ellipse. Assuming that natural selection pushes the population in the direction of phenotypic space identified by the star, the presence of a genetic correlation between the two traits instead generates a highly deflected evolutionary trajectory. However, if the **M**-matrix (represented by the larger ellipse) allows for more leeway in the relationship between the two characters, then the balance between selection and genetic constraints may move the population in an intermediate area of phenotypic space (solid arrow).

This view of the evolutionary process seems to open up several avenues of research. In the first place, it provides us with a new conceptual handle on the complex problem of the relationship between natural selection and genetic constraints, something that has been the focus of a sustained series of efforts (e.g., Antonovics 1976; Cheverud 1984; Maynard-Smith et al. 1985; Kirkpatrick and Lofsvold 1992; van Tienderen and Koelewijn 1994; Shaw et al. 1995; Schlichting and Pigliucci 1998; Barton and Partridge 2000). Second, considering **M** and not just **G** appears to offer a more realistic view of the evolutionary process itself, one that can be more comfortably extended to stretches of time beyond which the breeders' equation is of little use, and that are actually most interesting to evolutionary biologists. Third, the concept of variability readily allows us to see how to extend current techniques to empirically estimate components of **M**, just as we already do in the case of **G**. Furthermore, studies of the mutational properties of individual quantitative traits (e.g., Houle et al. 1994; Mackay et al. 1994; Fry et al. 1995, 1996; Fernandez and Lopez-Fanjul 1997; Keightley and Ohnishi 1998) can provide the starting point for analogous research into the effect of mutations on genetic covariances.

However, thinking in terms of **M** also immediately carries a slew of difficulties. First, despite Wagner's referring to the new matrix as **M**, it is obvious that in the short run the translation of variability into variation will more likely be the result of other genetic mechanisms, such as recombination (at least in outbreeding species), than of mutations. This brings into the equation complications related to the enormous natural variation in breeding systems, not to mention the necessity of estimating the frequencies of crossing-over events at the molecular level. Second, while **G** is clearly temporally defined (it can be observed *now*), it is not quite as clear over what time interval one needs to study **M**, since the more time passes, the more recombination and mutation will affect its structure. Third, at the moment, and despite Kauffman's valiant efforts in exploring the properties of *N*-*K* models (where *N* is the number of loci underlying a trait and *K* the number of interactions among them), we have very little theoretical grounding for studying the properties of the **M**-matrix or, more importantly, for making any predictions about its relationship to the more easily observable **G**. Finally, while it is cumbersome enough to estimate components of **G** via what often become logistically challenging experimental designs, it is even more difficult to obtain empirical estimates of components of **M**, since one has to take into account the effect of recombination and of new mutations on the currently existing **G**.

In the following pages I shall concentrate on the results of some attempts at studying components of **M** carried out in my laboratory during the last few years using the weedy herbaceous plant *Arabidopsis thaliana* as a model system (Pigliucci 1998; Alonso-Blanco and Koornneef 2000; Mitchell-Olds 2001; Simpson and Dean 2002). These examples are meant to provide an appreciation for both the insights yielded by this approach as well as the difficulties inherent in it. I then summarize the common threads emerging from our efforts in the hope of stimulating other students to engage in similar research in this and other systems, and conclude with a somewhat provocative discussion of the very conceptualization of selection and constraints as "forces" (as is done, for example, in Fig. 11.1).

Evolution from a "Lab Rat"

Several of the *A. thaliana* genotypes used in genetic research have evolved under highly artificial conditions for a considerable amount of time, and have been more or less consciously selected for a very short life cycle (Redei 1992). Such selection has reshaped several aspects of the phenotype of this plant, not limited to its life history. For example, the Landsberg "lab rat" version of *A. thaliana* not only flowers and senesces much earlier than field-collected conspecifics, but produces many fewer leaves, tends to be smaller, is significantly less branched (if at all), and has a markedly reduced reproductive fitness (measured by fruit and seed production).

An interesting question, therefore, concerns the possibility and limits of reevolving a normal-looking *A. thaliana* from the starting point of the highly specialized Landsberg genotype, which is what I set out to do as a way to begin to study the characteristics of **M**-matrices in this species. It is important to note at the outset that Landsberg, properly speaking, does not *have* a **G**-matrix. This is because **G** is a population-level concept, and Landsberg is a single genotype, for which genetic correlations are simply undefined. This, of course, does not mean that Landsberg's characteristics are not genetically constrained, but simply that such constraints cannot be quantified as components of **G**. Nevertheless, the first question we asked was if a mutation-selection protocol could allow descendants of Landsberg to evolve a higher fecundity and, if so, by means of what changes in the phenotype of the starting genetic background (Pigliucci et al. 1998).

We used EMS- (ethyl methane sulfonate) mutagenized seeds of Landsberg, which we grew for two generations, the first of which experienced an episode of selection imposed on the now genetically variable population. Instead of selecting directly on fecundity, we favored an increase in leaf production because we wished to see if a commonly observed genetic correlation between leaf number and flowering time in *A. thaliana* (Mitchell-Olds 1996) would appear in the descendants of our isogenic line. Leaf production in our mutagenized populations turned out to be highly positively correlated with both fecundity and time to senescence. The results of the mutation-selection protocol were astounding, considering that we applied selection for only one generation (choosing plants with a mean leaf production more than three standard deviations from that of the baseline strain): the number of leaves produced by the mutants varied from around 6 (the average for Landsberg) to 39; correspondingly, the time to flower was extended from about 33 days to as much as 86. Interestingly, however, the mutants and Landsberg lined up pretty tightly to show a genetic (mutational) correlation of $+0.86$ between these two traits (Fig. 11.2a), essentially reproducing the known association between the same characters that has been described in many early-flowering populations of *A. thaliana*. Perhaps of equal interest was the fact that other traits were not genetically correlated in the descendants of Landsberg, as they usually are not in other populations of this species: for example, time to senescence and size of the main inflorescence (measured as plant height) were completely unrelated among the mutants, even though all plants senesced much later than the baseline strain (Fig 11.2b).

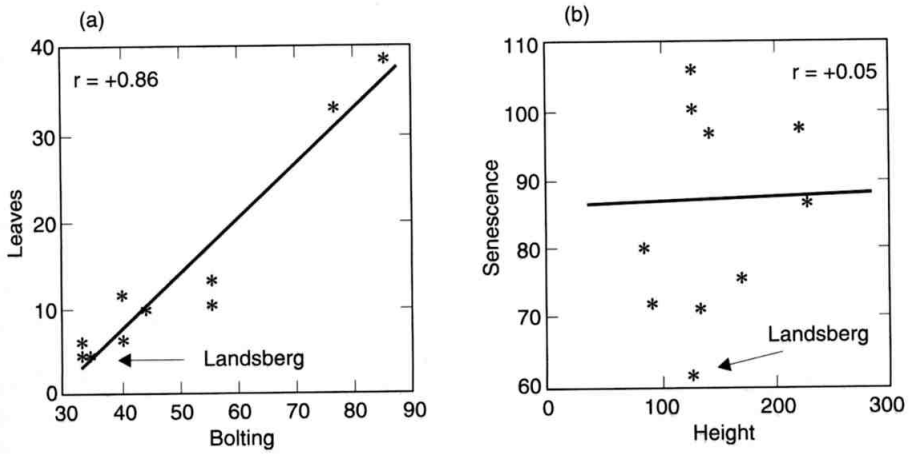

Figure 11.2 Experimental evolution of new genotypes of *A. thaliana* from a baseline inbred line. (a) High genetic correlation between leaf production and bolting (flowering) time in the baseline and mutants. (b) Low genetic correlation between height and time to senescence in the same population. Data from Pigliucci et al. (1998).

One can think of this experiment as a successful attempt at expanding the initial range of genetic variation of a base population (which in this case happened to be essentially zero, given that we started with an inbred line) and exploring the limitations intrinsic in the structure of the underlying **M**-matrix. It was possible to obtain the evolution of phenotypes far removed from the initial one, but for some combinations of traits this was clearly restricted to only certain preferential directions. It seems that some characters in *A. thaliana* can evolve only along "lines of least resistance" (Schluter 1996).

The Importance of Genetic Background and Mutation Pressure

While the previous experiment provided a first glimpse into components of the **M**-matrix constraining multivariate trait evolution in *Arabidopsis thaliana*, one obvious follow-up question is how much the particular genetic background of the baseline population matters. This is a rather difficult question to answer because there are so many possible backgrounds, some of which are representative of populations with markedly distinct ecological niches (e.g., winter annual versus spring annual populations of *A. thaliana*: Donohue 2002). Furthermore, this is a question that opens up a logistical Pandora's box, since it is not clear what the consistency of mutagenic results is even for the same genetic background: it is possible that a given mutagenic correlation between two traits, like the one discussed above concerning flowering time and leaf production, will actually manifest itself only some of the times in which one attempts to bring out the variability of a population using the experimental approach advocated here.

In part to address these questions, Camara and I (Camara and Pigliucci 1999) examined the effect of EMS mutagenesis on three genetic backgrounds, one of which was the same as used in the first experiment: Landsberg, Dijon, and Wassilewskija. We found that the mutagenic treatment did not alter significantly any trait mean (which it was not expected to do, unlike the case of the previous experiment, which used a mutation-selection protocol). However, it did dramatically affect trait covariances, as was clear from a series of principal components analyses (Fig. 11.3). The detailed constitution of each eigenvector (i.e., the weights or loadings of each original variable on a given principal component) were highly heterogeneous among the three genetic backgrounds, indicating that the underlying **M**-matrix was different, or at least that the patterns of variability we were able to translate into actual variation in our mutant populations depended on the particular genetic background used. Notice in particular what happened to the genetic correlation between flowering time (vegetative period) and leaf production discussed above: in the Landsberg background it was still prominent, thereby confirming it as a major feature of the **M**-matrix of this line (consider the two tallest bars on the positive side of PC2 in Fig. 11.3 for the Landsberg genotype). However, the same relationship was much less marked in the Wassilewskija and Dijon backgrounds, where it showed up to a reduced extent only on the third principal component. Moreover, this relationship appeared in a different association with other traits; the negative relationship between the two focal traits and number of nonelongated inflorescences—a measure of potential additional

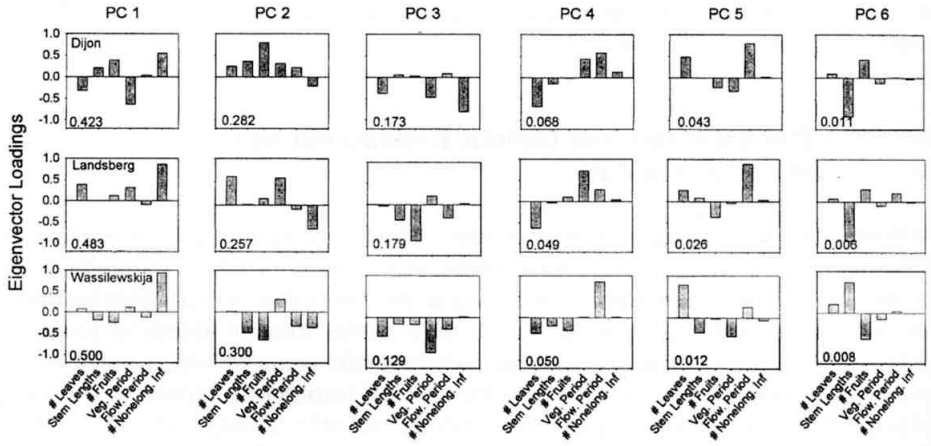

Figure 11.3 The six principal components summarizing the mutagenic covariation among traits in three genetic backgrounds of *A. thaliana*. For each component the bars indicate the weight (loading) of each original variable on that eigenvector, and the numbers in the lower left corner of each plot represent the standardized amount of variance explained by the corresponding eigenvector. Notice how the distribution of weights changed dramatically among genetic backgrounds, indicating that the **M**-matrix may have significantly different structure in these three populations. Data from Camara and Pigliucci (1999).

reproductive output—was negative in Landsberg, positive in Dijon, and null in Wassilewskija. While a formal statistical test using common principal components analysis (see Steppan, Chapter 15, this volume) did suggest commonality among the principal components of our three backgrounds, it also rejected both the hypotheses of equality and proportionality among the full trait matrices. It is instructive to compare this result with the common finding of similar or identical **G**- (or **P**, phenotypic) matrices among closely related populations of the same species sampled from the field (see Roff and Mousseau 1999 for a review). Even if **G**-matrices do not diverge significantly among closely related populations, they seem to have the potential to do so once enough mutations have accumulated. If the latter conclusion should be confirmed for natural and mutagenic populations of the same species, they will be an important piece of the puzzle in locating the balance between genetic constraints and selective pressures (see Hansen and Houle, Chapter 6, this volume).

In a different study, Camara, Ancell, and I (2000) also examined another complicating factor of studying **M**-matrices via induced mutagenesis: the effect of different numbers of mutations. The obvious advantage of generating mutations artificially is that one can compress evolutionary time and study attributes of **M** in the laboratory, but an equally clear limit to this is that if one uses high doses of mutagen, the number of mutations that result are likely to greatly diminish the organism's fitness through a panoply of pleiotropic and epistatic effects. In other words, this is another incarnation of the well-known tradeoff between experimental convenience and empirical realism: we do not want to give up the first one, but cannot afford to push it as far as to make our results completely irrelevant to what happens in nature (recently there has been a flurry of studies on the limits and advantages of laboratory experiments in evolutionary ecology: Matos et al. 2000, 2002; Sgrò and Partridge 2000, 2001; Hoffmann et al. 2001; Matos and Avelar 2001).

We then used four different levels of EMS mutagen (plus the nonmutagenic control) on a single genetic background (the Kendalville natural population) to assess the effects on characters' means, variances, and covariances of various numbers of induced mutations. The results were sharply different for the distinct measures: trait means were unaffected by the number of mutations (Fig. 11.4), even at high levels of mutagen. Trait variances, on the other hand, were markedly different, with a general tendency toward an increase in variance with higher mutagenic effects (Fig. 11.5), though several morphological traits actually peaked at intermediate dosages for as yet unclear reasons. When we used common principal components to compare the genetic covariance matrices among mutagenic treatments (details in Camara et al. 2000), we found that similar doses produced matrices with a few common principal components, while more diverging dosages yielded matrices with no common structure at all. Overall, these results suggest that mutations have very different effects on means and variance/covariances, and that—somewhat surprisingly—one can subject *A. thaliana* to considerably high levels of mutagenesis without causing any apparent decrease in the mean fitness of the resulting plants.

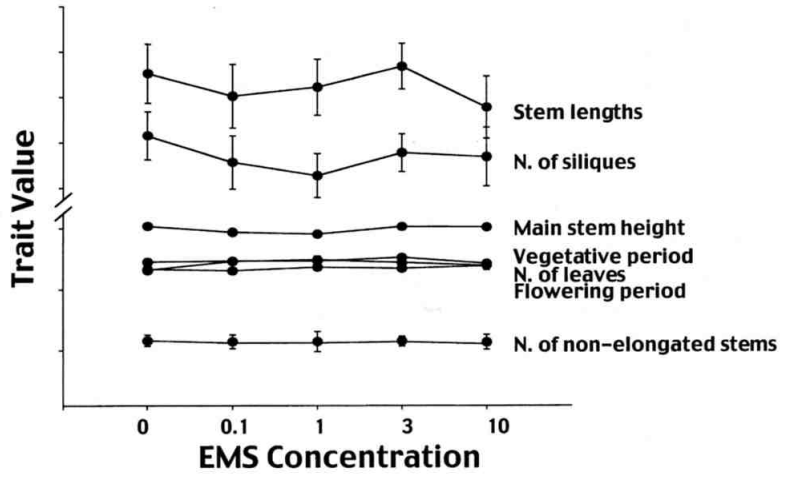

Figure 11.4 The effect of different concentration of mutagen (and increasing number of actual mutations) on the means of quantitative characters in *A. thaliana*. In fact, trait means remained remarkably stable regardless of the number of hits. Data from Camara et al. (2000).

Selection Along and Away from the Line of Least Resistance

The first two experiments described above found that a well-known genetic constraint linking flowering time and leaf production in *A. thaliana* was present in the mutagenized progeny of the Landsberg genetic background, and the first experiment also found that it channeled the response to selection along the area of phenotypic space identified by the genetic correlation. However, the second experiment also revealed that other genetic backgrounds might not be subject to as strong a constraint as the one found in Landsberg. So, Camara and I (in prep.) set out to repeat the original mutation-selection experiment with a larger sample and using a different genetic background, the Kendalville population. We first established that this population does in fact show natural genetic variation for both traits (i.e., it is not an inbred line), and that the characters in question are linked to each other by a measurable, strong genetic correlation. We then applied the mutagen, thereby increasing the available range of phenotypic variation for both traits, and conducted three generations of selection to shift the population mean of both characters in predetermined directions.

As a control, we grew replicates of both mutagenized and base populations simply propagated by selfing, and verified that these did not in fact wander far from the original population centroid in the phenotypic space identified by flowering time and leaf production (Fig. 11.6a). We also applied selection along the observed genetic correlation, that is, along what should be the line of "least resistance" from an evolutionary standpoint. Both mutagenized and non-muta-

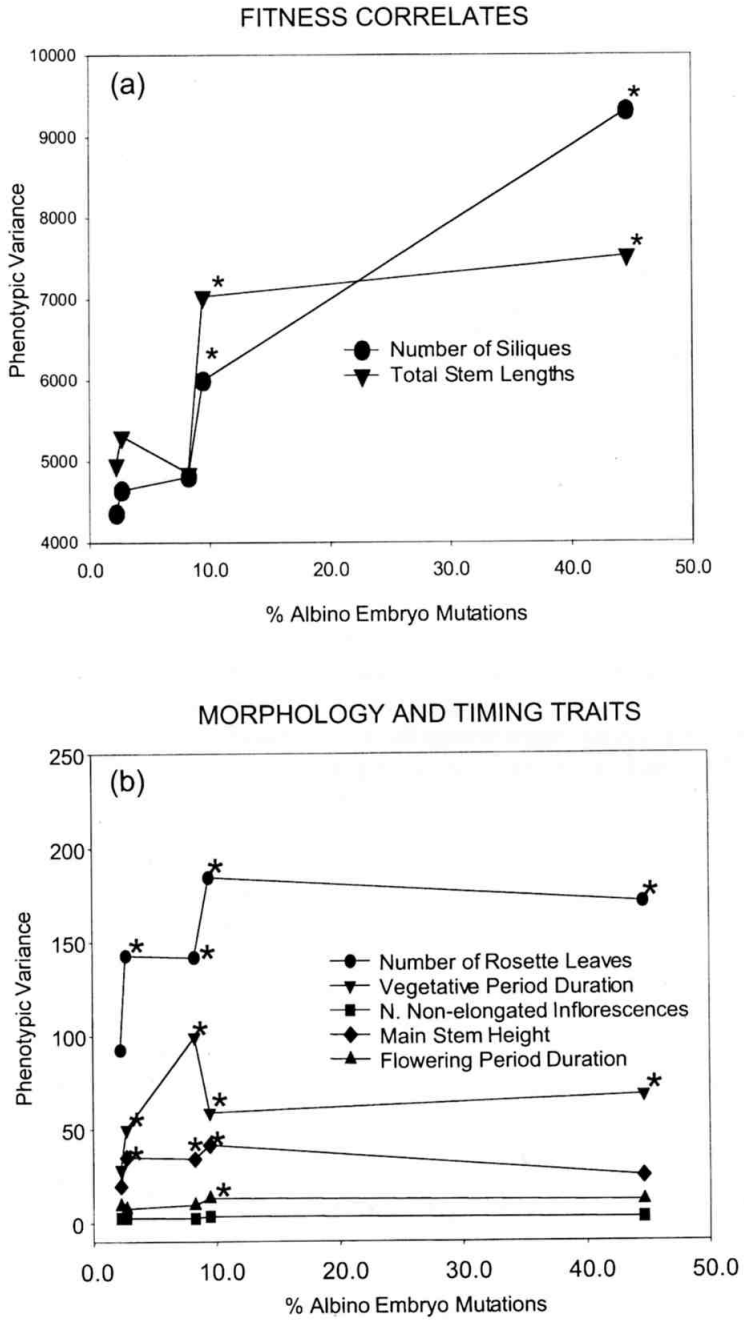

Figure 11.5 The effect of different concentration of mutagen (measured as the increasing number of actual albino mutations) on the variances of quantitative characters in *A. thaliana*. Contrary to the trait means (Fig. 11.4), variances were greatly affected by the number of hits, though not always in a monotonic fashion. Data from Camara et al. (2000).

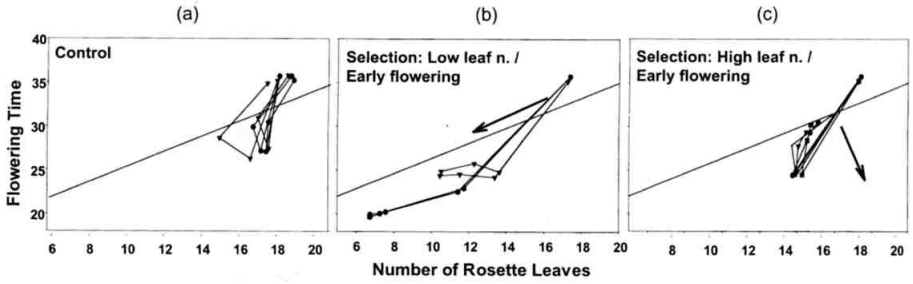

Figure 11.6 Results of a mutation-selection experiment to break a genetic constraint between leaf number and flowering time in *A. thaliana*. Control populations (a) did not move appreciably from their initial position in phenotypic space. Populations selected to move along the diagonal identified by the constraint (b) responded quickly to selection (arrows indicate direction of selection), the mutagenized ones (circles) more so. However, when selection was attempted in a direction orthogonal to the constraint (c) the results were no different from the unselected control. Data from Camara and Pigliucci (in prep.).

genized populations responded quickly to selection (Fig. 11.6b), though the former showed a much prompter response and extended the original phenotypic space significantly more. Indeed, in only three generations we had essentially produced an *A. thaliana* that looked like the Landsberg "lab rat," with only a few leaves and a very early flowering schedule. However, when we attempted to select against the diagonal representing the genetic correlation, we hit a wall with both the mutagenized and nonmutagenized populations (Fig. 11.6c): the selected populations simply did not move from the original region of phenotypic space, as if they had not been selected at all.

It seems clear, therefore, that certain constraints in *A. thaliana* are fairly common, and furthermore, that they are not just a reflection of the observable **G**-matrix (i.e., a matter of variation), but extend to the underlying **M**-matrix (i.e., they are a problem of limited variability). This, of course, cannot be considered a general conclusion (the situation may be different not only for other species, but even for other constraints in *A. thaliana* itself), but it shows the way to an empirical investigation of the **M**-matrix by mutagenesis analyses, thereby significantly extending our experimental grasp of the question of genetic variability.

It May Be Called M, But Recombination and the Environment Play a Role, Too

Following Wagner's introduction of the idea, the matrix of genetic variability (as opposed to **G**, the matrix of variation) has been referred to popularly as **M** for mutation, although it is obvious that plenty of other evolutionary phenomena actually influence the structure of **M**, for example recombination. It is therefore important to explore the role of these additional factors in shaping and limiting the amount and type of genetic variability that can be available over the short and

medium term for a given population. One attempt in this direction is exemplified by my collaboration with Karen Hayden (Pigliucci and Hayden 2001) exploring variability in phenotypic integration in *Arabidopsis thaliana* linked to both genetic recombination and environmental variation.

We used five genotypes of *A. thaliana*, one of which was the standard laboratory line Landsberg, while the remaining four were samples from natural populations from Germany (two accessions), Norway, and Russia. All of these were so-called "early flowering" populations, which probably means that under natural conditions they behave as spring annuals (i.e., they germinate in the spring and go directly to flower, without vernalization). We began by crossing eight individuals of each of the natural accessions with Landsberg, using the latter always as the maternal parent. We planted the seeds obtained from the crosses and grew three generations of progeny, each time propagating the lines by single-plant descent (via selfing). We then used the F_4 generation as the material for the actual experiment, having allowed sufficient time for genetic reshuffling to generate new genetic variation (i.e., to translate underlying variability into variation). Given the crossing design, each F_4 population was genetically heterogeneous both as a result of recombination and because it originated from eight distinct founder lines used in each of the original crosses. The F_4 was grown with the respective parental genotypes under two environmental conditions: 2 ml of standard Hoagland solution administered twice weekly, or the same treatment but with only 10% of the nitrogen concentration.

The results were surprising in that environmentally induced changes in the observed patterns of phenotypic integration were much more marked than differences due to the degree of genetic differentiation among the recombinant lines. For example, population Eil-0 from Germany was characterized by a different arrangement of its first two principal components when the measurements were conducted under low and high nutrients. In particular, the two crucial life-history characters of bolting (flowering) time and length of the reproductive period were independent of each other under high nutrients (the corresponding vectors in PC-space were orthogonal), but negatively correlated to each other (diametrically opposite vectors) under low nutrients. This suggests that the more stressful environment (low nutrients) generated a tradeoff between vegetative and reproductive phases in this population of *A. thaliana*. Even more interestingly, this tradeoff was present *regardless* of the environmental conditions in all three remaining recombinant lines.

When we compared the patterns of phenotypic integration among all line/environment combinations, the emerging picture was remarkably clear (Fig. 11.7): both when measured by overall degree of matrix similarity and by the formal tests provided by common principal components analysis, matrices split first along environmental lines (with most of the low nutrients on one branch and all the high nutrients in the other), and only later by genetic dissimilarity. Therefore, even though the reshuffling of genetic material had contributed to distinct realized patterns of integration, the plasticity of character correlations (Schlichting 1989) was the overwhelming determinant of the observed differences.

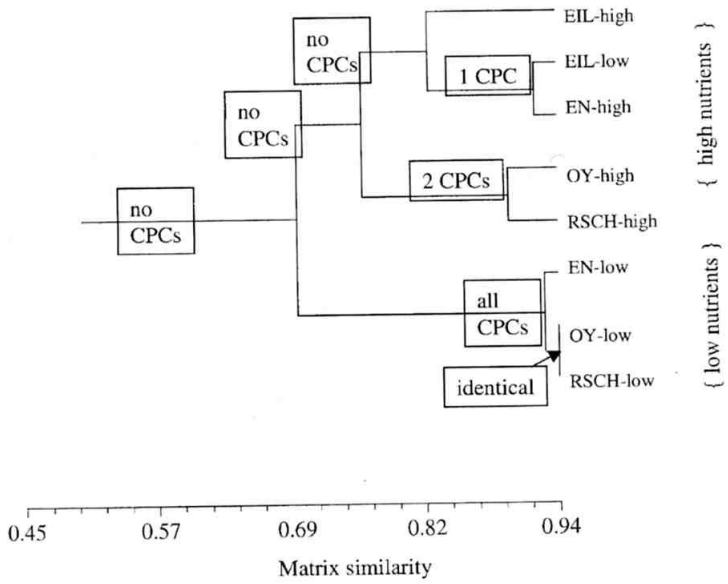

Figure 11.7 Dendrogram depicting the similarity of covariance matrices among four recombinant lines of *A. thaliana* exposed to two different nutrient environments. The boxes at each node indicate the results of formal statistical tests using common principal components (CPCs) analyses to compare the degree of similarity of the matrices on the two branches of a given node. Notice how the two major branches do not share any CPC, and that the major differences in the pattern of phenotypic integration are induced by environmental, not genetic, changes. Data from Pigliucci and Hayden (2001).

Are Constraints Positive "Forces" in Evolution?

The experiments briefly discussed above begin to address the fundamental question of how it is possible to study the relationship between variation and variability from an experimental point of view. As I have tried to emphasize, they also show the many perils and pitfalls of any empirical research on these matters, which will offer a set of challenges to researchers interested in this topic for years to come. As we have seen, these include the logistics of large experiments, the difficulty of estimating components of **M** given the often overwhelming effect of the environment, as well as the problem of the repeatability of mutagenic and mutation-selection experiments.

All of the above notwithstanding, I should like to conclude this chapter by going back to the big picture of the relationship between (genetic) constraints and natural selection, and in particular to a debate that has been articulated now for some time: should we think of constraints as limitations on natural selection, or as "opportunities" channeling selection in particular directions? I shall argue that recent work in philosophy of science may offer a third alternative: neither selection nor constraints should be thought of as forces at all, contrary to the popular imagery as evoked by Fig. 11.1.

Traditionally, and perhaps most widely, constraints are conceptualized by evolutionary biologists pretty much in line with what the word implies: as *limitations* on the possible evolutionary outcomes for populations subjected to natural selection (Maynard-Smith et al. 1985). Gould, however, has repeatedly argued (most recently in Gould 2002) that constraints should be thought of as *opportunities* for evolutionary change, albeit opportunities that tend to be channeled along preferential routes. The literature on phenotypic integration engages this debate with work showing that evolution does (Schluter 1996) or does not (Merilä and Björklund 1999) in fact occur mostly along lines of "least resistance" as defined by trait correlations.

To some extent this may seem a matter of "semantics," an appellative to which biologists tend to be allergic and that philosophers of science relish. However, at its core the debate is about limits and opportunities for natural selection to shape evolution and to yield adaptation, hardly a secondary matter for evolutionary biologists. It seems to me that the experiments briefly summarized above, as well as anything else we can learn from the ample published literature on the study of genetic correlations, clearly point to the conclusion that constraints (thought of in this case as intercharacter correlations) do both *limit* and *channel* evolution along certain pathways. Just consider the genetic correlation between leaf production and flowering time in *A. thaliana*: on the one hand, it precluded short-term evolution of the population in directions of phenotypic space away from the diagonal represented by the correlation itself. On the other hand, it accelerated the response to selection along the diagonal of those populations characterized by a higher degree of expressed genetic variation for either trait.

This said, it is still not clear at all to me how one can consider, as Gould does, such preferential directions of evolutionary change as "creative" in any sense comparable to the way we understand "unconstrained" natural selection to be. It seems that the problematic idea of constraint as creative force may have arisen from confusion between the *number of directions* available to evolution in a given phenotypic space and the *speed* with which evolutionary change occurs along any one of these directions. If there is no genetic correlation between two traits, then all directions of phenotypic space are available for the population to move toward, with a speed proportional only to the available genetic variation for both traits. If, on the other hand, a genetic correlation exists, the number of directions toward which selection can push the population is greatly diminished (proportionally to the strength of the correlation itself), but the speed of change along the remaining directions may in fact increase because such change is now fueled also by the covariation between the two traits. Another way to think of the latter case is this: the genetic covariation between characters, which is what acts as a brake against evolution away from the "line of least resistance," also acts as an accelerator *along* the same line. But the rate and direction(s) of evolution are distinct concepts, and only confusion between the two may lead somebody to claim that an increased speed in one direction is a "creative" element in the evolutionary process: no matter how one slices it, a genetic correlation is still a (more or less strong) *limit* to what selection can do with the phenotype of a population.

Perhaps a more fundamental challenge to the whole standard way of thinking about selection and constraints has recently come from an external source:

philosophy of science. Throughout the above discussion and presentation of empirical results I have tacitly assumed the validity of the metaphor that casts selection and constraints as "forces" that act independently on populations, sometimes in concert, other times in opposition. This metaphor is in fact very common in technical papers written on selection and constraints, and has been formalized most completely by Sober (1984). For Sober, evolutionary theory can be cast as a theory of forces analogous to Newtonian mechanics, complete with zero-force states, which can be studied through the Hardy-Weinberg equilibrium (the biological equivalent of Newton's statement about bodies on which no forces are acting).

More recently, however, the conception of natural selection as a force has gotten into some trouble, for example when the time comes to distinguish it from the action of other alleged "forces" such as genetic drift (see, for example, a discussion of this in Millstein 2002). The problem has been directly tackled by Matthen and Ariew (2002), who have made the radical proposal that evolutionary "forces," including selection, are not forces at all. Indeed, they claim that the picture of interacting forces is misleading and encourages wrongheaded thinking in both theoretical and empirical evolutionary research. A complete discussion of Matthen and Ariew's argument would lead us too far from the topic of this chapter, but it is instructive to consider their main points briefly and to begin at least to think about the sort of impact they may have on our understanding of the evolution of phenotypic integration.

The first wedge into the "force" conception of evolution is that drift in particular certainly cannot be regarded as a force. For one thing, it does not have predictable and constant direction, as a force is supposed to. Second, and more subtly, since drift is a causative agent that is probabilistic in nature, evolutionary change may have the same cause as no change at all, a notion that would be nonsensical in a framework analogous to Newtonian mechanics.

A second problem for the idea of forces acting during evolution is that forces are, by definition, expressed in the same units of measurement (think of the various laws of Newtonian mechanics). That is obviously not the case for, say, natural selection and genetic constraints, which are literally incommensurable causative agents. They can, of course, both affect gene frequencies over time, but such change is exactly that, an *effect*, not a measure of the cause itself.

Perhaps the most convincing objection to conceiving natural selection and genetic constraints in particular as forces, however, comes from Matthen and Ariew's discussion of what they call the "substrates" of selection. Darwin's original formulation of the theory of evolution by natural selection famously ignored, for lack of knowledge, the genetic bases of the inheritance necessary for selection to produce cumulative change over generations. We now know not only what such substrate is (Mendelian genetics); we also know that evolution *would not work* if different substrates were in place. For example, Fisher (1930) showed that the "blending inheritance" imagined by Darwin would in fact lead to a quick exhaustion of genetic variation, which means that selection could not operate in the long run. As Matthen and Ariew put it, then, it is not natural selection that is responsible for adaptive evolution, but selection within a Mendelian substrate, where the latter is *integral* to the process of evolution, not

an obstacle to it. This brings us full circle to the question of the relationship between selection and constraints, which is central to much of this book. To quote Matthen and Ariew (2002, p. 66): "What would happen if selection were to act *by itself* [i.e., without 'constraints']? ... It is clear that natural selection acts in certain underlying causal media, and the so-called constraints are features of these media. Since natural selection cannot act without such a medium, it is not at all clear what sense can be made of the idea of natural selection acting 'without the intrusion of constraints.' "

If taken seriously, this analysis implies much more than even Gould dared to put forth: not only are constraints not necessarily an obstacle to natural selection, but in general selection cannot work at all *unless* there is a particular genetic substrate that also provides the "constraint." This idea may take a while to sink in, but once one has processed it, one quickly sees all sorts of implications for the way we think about phenotypic evolution. If we abandon the "force" metaphor, then the genetic architecture underlying phenotypic traits, which we can quantify as **G** or **M**, is an integral causal agent of evolutionary change, one that interacts with natural selection in an intimate fashion to yield whatever evolutionary trajectory a particular population takes.

This new paradigm for thinking about selection and constraints also casts the breeders' equation and its multivariate extension with which I started this chapter in a completely different light. While it may be mathematically convenient to think of selection as the vector **S** and of the genetic architecture as the matrix **G**, in reality the two are not that easy to separate. Perhaps a more familiar way to put this is in terms of the uneasiness that at least some biologists (this author, for example) feel when thinking alternatively of **G** as either the result of past selection or as the constraining force limiting future response to selection, depending on the objective of a given study. In reality, of course, **G** and its changes measure the continuous dialectic between past and future evolutionary events, without any clear breaking point, despite the fact that the researcher happens to observe **G** at a particular point in time. Just as there is no clear-cut partitioning of phenotypic variance into genetic and environmental (Pigliucci 2001), so there is no meaningful separation between selection and constraints in explaining changes in phenotypic means.

If abandoning our thinking of selection and constraints (and of other evolutionary causative agents) in terms of forces has the potential to reshape our thinking about how we model long-term phenotypic evolution, then it surely has implications for the way we conceive, carry out, and interpret our experiments too. In particular, it seems to me that this means that we should no longer see **G** as a static attribute of populations or species, but rather we should focus on its *dynamic* as an indispensable partner to natural selection. The coupling of mutational studies of **M** and comparative phylogenetic analyses of **G**, then, becomes a promising venue for empirical research into what we can begin to think of as the continuous coevolution of selective pressures and genetic architectures.

Of course, all of the above will become relevant only if evolutionary biologists are able to work these conceptual suggestions into their empirical research and theoretical models. The proof, so to speak, is in the pudding. However, my goal here has been simply to bring the philosophical discussion to the attention of the

evolutionary community: without awareness of the contribution of others, without food for thought as it were, one cannot even begin to think about how to change the direction of one's research program.

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