

CONCEPTS & SYNTHESIS

EMPHASIZING NEW IDEAS TO STIMULATE RESEARCH IN ECOLOGY

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A REASSESSMENT OF GENETIC LIMITS TO EVOLUTIONARY CHANGE

MARK W. BLOWS^{1,3} AND ARY A. HOFFMANN²

¹*School of Integrative Biology, University of Queensland, Brisbane 4072, Australia*

²*Centre for Environmental Stress and Adaptation Research, La Trobe University, Melbourne 3083, Australia*

Abstract. An absence of genetic variance in traits under selection is perhaps the oldest explanation for a limit to evolutionary change, but has also been the most easily dismissed. We review a range of theoretical and empirical results covering single traits to more complex multivariate systems, and show that an absence of genetic variance may be more common than is currently appreciated. From a single-trait perspective, we highlight that it is becoming clear that some trait types do not display significant levels of genetic variation, and we raise the possibility that species with restricted ranges may differ qualitatively from more widespread species in levels of genetic variance in ecologically important traits. A common misconception in many life-history studies is that a lack of genetic variance in single traits, and genetic constraints as a consequence of bivariate genetic correlations, are different causes of selection limits. We detail how interpretations of bivariate patterns are unlikely to demonstrate genetic limits to selection in many cases. We advocate a multivariate definition of genetic constraints that emphasizes the presence (or otherwise) of genetic variance in the multivariate direction of selection. For multitrait systems, recent results using longer term studies of organisms, in which more is understood concerning what traits may be under selection, have indicated that selection may exhaust genetic variance, resulting in a limit to the selection response.

Key words: *genetic correlations and variance; G matrix; heritability; life-history studies; natural selection; response to selection; selection experiments; species distributions; trade-offs.*

‘Few believe that a straightforward lack of genetic variance for a target trait is likely to strongly constrain a response to natural selection’.

—P. M. Brakefield (2003)

INTRODUCTION

Ecologists and evolutionary biologists often use optimality approaches to predict the phenotypes that may evolve when there are different fitness costs and benefits associated with these phenotypes (Maynard Smith 1982, Grafen 1991, Seger and Stubblefield 1996). Optimality models assume that the genetic details underlying trait variation do not matter, and are compatible with genetic variation controlled by many loci with small effects, unlimited mutation rates, and no constraints on trait evolution as a consequence of pleiotropy. However, biologists are also acutely aware of evolutionary limits. While limits to species distribution

and abundance can be due to a variety of factors such as habitat spacing and dispersal ability that prevent colonization of environments (e.g., Case and Taper 2000, Holt et al. 2004), they can also reflect evolutionary limits. Species often do not utilize the full range of hosts or environments they encounter, and where survival and persistence is possible (Hare and Kennedy 1986, Perlman and Jaenike 2003). Treatises on the distributions of plant and animal groups contain many examples of sharp contrasts between related species, where one has a highly restricted distribution or narrow host range, while another uses a range of hosts or is more widely distributed.

Consistent with the assumption that genetic variance is limitless, a large body of empirical literature indicates substantial levels of genetic variation in a range of traits. Decades of single-trait heritability experiments and artificial selection have led to the conclusion that genetic variance will be present in almost any trait (Barton and Partridge 2000, Brakefield 2003), even to the point where testing for the presence of genetic variance has been suggested to have little biological relevance (Lynch and Walsh 1998: 561). Similarly, there

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³ E-mail: mblows@uq.edu.au

is a large body of theory indicating that high levels of genetic variance can be maintained in natural populations through a number of mechanisms (Barton and Turelli 1989, Barton and Keightley 2002, Turelli and Barton 2004).

What then might limit the response to selection in natural populations, and account for the narrow environmental range of many species? Over 10 years ago, we reviewed a range of processes that might contribute to limiting species distributions (Hoffmann and Blows 1994), and more recently a number of authors have addressed various aspects of limits to the response to selection (Barton and Partridge 2000, Merilä et al. 2001, Hansen and Houle 2004). Here, we reassess just one of these possible causes: the absence of genetic variance. We focus solely on this issue, as a recent special issue of *Ecology* (Volume 84, Issue 7) outlined the potential of selection experiments and other quantitative genetic approaches to address limits to evolutionary change in the context of species distributions and trade-offs (Brakefield 2003, Conner 2003, Fry 2003), suggesting that such approaches may become more common in evolutionary ecology.

In this paper, we review evidence for genetic limits to evolutionary change, and critically assess common approaches to the study of genetic limits. We first assess genetic limits to the response to selection in single traits, and highlight that some traits in some populations/species can display undetectable or very low genetic variance. Second, we detail how genetic limits in two-trait systems have been investigated, and highlight methodological limitations in approaches applied to the analysis of genetic constraints as a consequence of trade-offs. Third, we show how a multivariate definition of genetic constraint can be achieved that provides a unified framework for the analysis of genetic limits. Finally, a program for further research is outlined that may yet determine that genetic limits are more frequently encountered in natural populations than is currently appreciated.

QUANTIFICATION OF GENETIC VARIATION

There are a number of ways that the level of genetic variation has been quantified. The genetic variance of a trait is the fundamental measure of genetic variation, and is central to many applications in quantitative genetics. Since the genetic variance remains in the units of the original measurements, it is of little use for comparing, for instance, levels of genetic variation across traits. To determine the effect of selection on a trait in a single generation, the genetic variance is often represented as a proportion of the total phenotypic variance, the heritability:

$$R = h^2S \quad (1)$$

where R is the response to selection, h^2 is the heritability, and S is the selection differential. Since heritability is a ratio, differences between two populations

in heritability may be a consequence of a change in any constituent part of the phenotypic variance; additive genetic variance (usually the target of interest), nonadditive genetic variance, and any form of environmental variance.

Houle (1992) proposed that proportional differences in the ability of traits to evolve (termed evolvability) could be represented by the coefficient of additive genetic variance, CV_A , so that differences between populations or traits would not be a consequence of the environmental or nonadditive genetic variances. Another measure of evolvability is the additive genetic variance scaled by the square of the trait mean, I_A , (Hansen et al. 2003), which measures the proportional response to directional selection when a particular unit of selection strength is applied to a trait. The use of both these indices still needs to be approached with caution. Comparisons across traits are only generally useful if traits have similar original units (e.g., both have linear size measurements) (Simpson et al. 1960, Downhower et al. 1987), and if it can be safely assumed that proportional differences are likely between the variables (Sokal and Rohlf 1981).

GENETIC LIMITS TO EVOLUTIONARY CHANGE IN SINGLE TRAITS

There is a large literature on the quantitative genetic basis of single traits under natural, laboratory, and domesticated conditions, and we will not attempt a comprehensive review. Rather, our goal is to highlight zero or low levels of genetic variation in some traits or species/populations and why these might occur.

Traits with undetectable or very low genetic variance

Surveys of trait heritabilities originally suggested that genetic variance for morphological traits was higher than for life-history traits (Mousseau and Roff 1987). However, trait heritability differences may have reflected differences in environmental variance rather than genetic variance. Houle (1992) demonstrated that life-history traits have relatively greater levels of additive genetic variance. In addition, field studies indicated that life-history traits could have moderate heritabilities and evolvabilities, comparable to values from field studies of morphological traits (Sgrò and Hoffmann 1998). Similarly, male sexually selected traits that are thought to be under directional selection also display high values for coefficients of genetic variation (Pomiankowski and Møller 1995).

In light of these surveys demonstrating substantial genetic variance in different types of traits, is there any evidence that genetic variance limits the ability of natural populations to respond to selection? The classic example of such a trait is bilateral asymmetry. Bilateral traits are normally highly symmetric, and several attempts have been made to increase asymmetry on one side of an organism (directional asymmetry or DA), or

the level of asymmetry between sides that is not directional (fluctuating asymmetry or FA). For example, Breuker and Brakefield (2003) were unable to increase FA for eyespots on the forewings of a tropical butterfly. The heritability of FA for individual traits is zero or low in populations (Fuller and Houle 2003). However, given that single-trait FA (and probably DA) is usually not closely related to fitness (Clarke 1998, Woods et al. 2002), absence of heritable variation for single-trait FA is probably not interesting ecologically.

There are some ecologically important traits that appear to show no detectable genetic variance (Scharloo 1991), although in practice it is difficult to separate zero from low levels of genetic variance due to large standard errors of variance estimates. Examples include mass-specific metabolic traits, particularly when studied in wild populations of mammals. In the wild mouse *Phyllotis darwini*, foot length, but not metabolic rate, exhibited significant heritable variation (Nespolo et al. 2003). Mass-specific metabolic rate also has nondetectable heritability in other mammals including mice (Dohm et al. 2001) and voles (Labocha et al. 2004), although it may be higher in insects (Nespolo et al. 2003).

Floral components can have a low evolvability. In wild radish, most variation in floral traits is within plants, rather than between plants or populations (Williams and Conner 2001). This suggests that most of the variance in floral morphology is due to special environmental effects rather than general environmental or genetic effects. In the vine *Dalechampia scandens*, estimates of heritability for floral characteristics based on a diallel cross were often low and nonsignificant (<30%) (Hansen et al. 2003). Moreover, I_A values were extremely low, generally <1%, and there was a positive correlation between the I_A of traits and the extent to which trait means had diverged among populations.

The ability of thermal resistance to evolve has been investigated in a number of organisms. In *Drosophila melanogaster*, selection experiments suggest a plateau for increased heat resistance as measured by knock-down (Hoffmann et al. 1997, Gilchrist and Huey 1999). In a live-bearing fish, *Heterandria formos*, Baer and Travis (2000) were unable to select for increased thermal tolerance. In *E. coli*, Bennett and Lenski (1993) maintained populations for 2000 generations at temperatures of 32°, 37°, or 42°C. They then examined performance of the populations across a temperature range of 12°–44°C, and found that the upper and lower thermal limits of these populations had remained at 19° and 42°C for all groups regardless of thermal history and evidence of adaptation at the temperatures where they evolved. In later experiments with lines maintained at 20°C for 2000 generations, there was evidence of a small shift in the lower thermal minimum and a concomitant decrease in performance near the thermal maximum (Mongold et al. 1996). These results suggest

low genetic variance for upper thermal niche expansion in *E. coli*.

Differences in levels of genetic variance among species and populations

Striking differences can occur among species in their ability to evolve in response to specific stresses. Many pest species have not developed resistance to agricultural chemicals even when related species have developed resistance and when there is strong selection (Georghiou and Taylor 1986). Resistance to pesticides often involves highly specific genetic changes, which can be predicted from laboratory mutation studies and that involve alleles segregating in populations at a low frequency prior to selection being applied (McKenzie and Batterham 1998). In cases where resistance has not evolved, resistance alleles presumably do not occur in a population or may be masked from selection if they are recessive and at very low frequency. Resistance may only develop if favored alleles are introduced via gene flow, as appears to be the case for alleles responsible for organophosphate resistance in mosquitoes (Raymond et al. 2001).

In plants, selection for heavy metal resistance has often failed to produce tolerance because of the absence of genetic variability (Bradshaw 1991). For example, in plant species that have successfully colonized mine soil, tolerant individuals can normally be found at a low frequency in populations from uncontaminated areas, whereas these individuals are absent in species that have not colonized contaminated areas (Macnair 1997). In addition, where some populations have evolved tolerance but others have not, this may reflect the presence/absence of tolerance variation in the base population. For instance, *Agrostis capillaries* populations exposed to zinc contamination from coated electricity pylons differed in zinc tolerance; some had evolved tolerance, whereas others had not (Al-Hiyaly et al. 1988). Al-Hiyaly et al. (1993) found that, where tolerance had failed to evolve around pylons, there was no evidence for tolerance in plants near the pylons. In contrast, where tolerance had evolved around pylons, there was evidence for tolerance in plants near the pylons (but far enough away to exclude the possibility of gene flow producing tolerant individuals in the nearby population). This suggests that genetic variance for tolerance in populations surrounding the pylons was present before tolerance evolved.

Several studies have considered genetic variance in performance of insects on different host plants, including plants not used as field hosts. Often genetic variance for use of the novel host is not limiting (Rauscher 1984, Hare and Kennedy 1986, Ueno et al. 2003), but it can be in some cases. For instance, in the leaf-feeding beetle *Ophraella slobodkini*, Keese (1998) found that there was no detectable genetic variance for performance on an asteraceous plant where it performed

TABLE 1. Hypotheses about factors that contribute to low genetic variance in specific traits.

Hypothesis	Explanation
Persistent low population size	Drift decreases genetic variation, particularly in isolated populations or when breeding systems decrease effective population size.
Mechanistic/physiological constraint	When a constraint is encountered, a selection limit is reached, and mutation effects may become highly skewed in one direction.
Small mutation target	Lower levels of genetic variance occur when the mutation target is small.
Low mutation rate	This rate decreases allele variability and genetic variance, especially when only a few loci influence a trait.
Low recombination rate	This rate decreases variability in traits arising from allele combinations.
Canalization	Selection favors modifiers that decrease the expression of phenotypic variability.
Genetic correlations	These correlations can influence genetic variation when multiple traits are under selection.
Absence of genotype–environment interactions	When the same genotype has the highest fitness in all environments, genetic variation is reduced.
Directional selection leading to fixation	Continuous directional selection decreases genetic variance by fixing favored alleles, or by removing deleterious alleles.

poorly, even though this plant was used by a sister taxon.

Most quantitative genetic experiments have been undertaken on model systems or agricultural organisms, and often on species easily reared in artificial environments, rather than on species that are specialists with restricted distributions. It is possible that high levels of genetic variability commonly detected for traits on these organisms may not generalize to others. A recent example concerns the response to selection for desiccation resistance in a rain forest *Drosophila* (*Drosophila birchii*). This species is restricted to increasingly fragmented rain forest patches along the east coast of Australia. *D. birchii* is particularly sensitive to desiccation, and clinal variation in resistance suggests a pattern of past selection on this trait (Hoffmann et al. 2003). When the most resistant geographic population was intensely selected for increased resistance, there was no response even after 30 generations; the trait was at a selection limit. Parent–offspring comparisons indicated no significant additive genetic variance for this trait despite high levels of heritable variation in morphological traits and high levels of molecular genetic variation. These results contrast markedly with heritable variation and rapid responses to selection for this trait in other species, such as *D. melanogaster*. In fact, desiccation resistance is among traits with the highest heritability and evolvability in *Drosophila* (Hoffmann 2000).

Genetic variance in quantitative traits may vary between populations with different population sizes, although most of the evidence for an association between size and selection limits comes from experimental rather than natural populations. Several laboratory selection experiments have shown associations between the selection responses for a quantitative trait and population size, although in natural populations supporting evidence is limited (Houle 1989, Reed and Frankham 2001), and often no association is found (Podolsky 2001). Threatened populations can have lowered heterozygosity at neutral genetic markers, suggesting a

reduced evolutionary potential (Spielman et al. 2004). However, associations between population size and genetic variance are complex, and it is not possible to predict the effect of a reduction in population size on the genetic variance from knowledge of the variance components in the base population (Barton and Turelli 2004).

Possible causes of undetectable or very low genetic variance

There are a number of reasons why the genetic variance of traits being investigated by researchers may be low in natural populations (Houle 1998), and why genetic variance in the same traits differs between populations or species (Hoffmann and Parsons 1991) (Table 1). One hypothesis is that small population size decreases genetic variance because of genetic drift (Houle 1989). Persistently small population sizes should decrease genetic variation, as evident from the decrease in heterozygosity in small populations (Spielman et al. 2004). Genetic variance in populations can also be influenced by gene flow; when populations are close to a species border, the inhibition of gene flow into peripheral populations may decrease genetic variance (Case and Taper 2000). Another factor that influences genetic variance is the breeding system of a population; for instance, inbreeding is expected to decrease genetic variance and there is some supporting empirical evidence (Charlesworth and Charlesworth 1995). When genetic variance decreases due to drift, limited gene flow, and altered breeding systems, changes in genetic variation will occur at neutral markers as well as a range of quantitative traits.

Perhaps the simplest of the remaining hypotheses is that mechanistic or physiological constraints make it unlikely that genetic variation in one direction will be generated by mutation. For example, basal metabolic rate is closely tied to the size of organisms, and the relationship between size and metabolic rate in a wide range of organisms including mammals, invertebrates, and protists can be described by the same scaling func-

tion (Savage et al. 2004). This suggests that allometric relationships have a basic, mechanistic constraint, likely to limit evolutionary divergence. Nevertheless, deviations from this relationship in some groups of organisms might be related to phylogeny or diet in the case of mammals (Cruz-Neto et al. 2001), and cold adaptation in the case of insects (Addo-Bediako et al. 2002). Where a mechanistic constraint occurs, genetic variance will be reduced in the trait under consideration, but not in other traits or neutral markers.

One class of hypotheses in Table 1 deals with the generation of genetic variation by mutation. Traits may differ in the level of genetic variance if the mutational target size (the number of loci) varies and/or if the rate of mutation acting on loci affecting a trait varies. Life-history traits display higher coefficients of mutational variance than morphological traits (Houle et al. 1996), perhaps because of a higher mutational target, as traits closely associated with fitness, such as life-history traits, are themselves affected by numerous heritable morphological and behavioral traits (Price and Schluter 1991). In turn, the greater mutational target size may be enhanced by the fact that the expression of many life-history traits changes during the life span of the organism, and therefore such traits may display cumulative mutational effects. Hughes et al. (2002) demonstrated that the additive genetic variance in reproductive success in *D. melanogaster* increases with age, consistent with an accumulation of mutational variance. However, they also pointed out that selection can oppose the maintenance of deleterious mutations with an early age of onset, but not mutations with a later onset. These observations also provide support for the elimination of genetic variance by selection, which we discuss in more detail in the following paragraphs.

Low recombination rates might decrease genetic variance by decreasing the rate at which favored combinations of alleles arise. The results of some selection experiments suggest that selection responses can be inhibited when genes are tied up in large standard and inverted chromosome arrangements, and populations are polymorphic for these arrangements (e.g., Carson 1958). Recombination between inverted and standard arrangements is inhibited, preventing new combinations of alleles from forming. This can decrease genetic variance available for selection in a range of traits, but does not alter variation at neutral loci.

Another class of hypotheses involves the action of selection. Selection may favor modifiers that decrease the expression of phenotypic variability, resulting in genetic canalization (Wagner et al. 1997). Some experiments suggest that canalized traits can only evolve under some environmental conditions that allow cryptic genetic variation to be released, for example via changes in levels of Hsp90, one of the heat shock proteins (Rutherford 2003, Sangster et al. 2004). Traits that constrain the distribution and abundance of organisms have not yet been shown to evolve by an

Hsp90-related mechanism that can function in the genetic backgrounds of natural populations and/or in the types of environmental conditions experienced in the field.

Other hypotheses are that interactions between traits as well as between traits and the environment influence levels of genetic variance. The evolution of antagonistic pleiotropy between life-history traits is thought to increase genetic variance in life-history traits by resulting in balanced polymorphisms (Rose 1982). Although this process and its potential to generate trade-offs has been a central theme in evolutionary ecology (Lande 1982, Lynch and Walsh 1998), there is little evidence that negative genetic correlations among life-history traits frequently occur. Environmental variability can promote genetic variability for traits related to fitness (Turelli and Barton 2004); if a single genotype does not perform best across all environments, substantial levels of additive genetic variance can be maintained in a population, in contrast to the situation where environmental variability is absent. Environmental conditions can also influence the expression of genetic variation in traits (Donohue et al. 2000). These factors could contribute to differences in the genetic variance of traits in populations and species experiencing different environments, and may influence a range of quantitative traits but not genetic variation at neutral marker loci.

The final hypothesis concerns the effect of selection on genetic variance. Genetic variance can be increased by directional selection (selective sweep hypothesis, Houle 1992); if alleles increase from a very low frequency in the population and do not pass their symmetrical frequencies before equilibrium is reached (Barton and Turelli 1987), directional selection will increase genetic variance. Blows and Higgie (2003) demonstrated that natural selection on mate recognition increased the genetic variance in male and female pheromones of *Drosophila serrata* under both field and laboratory conditions. However, continued directional selection may be responsible for low genetic variance in some traits, as favored alleles become fixed. Surprisingly, the effect of sustained directional selection on the genetic variance has received infrequent treatment (Reeve 2000, but see Barton and Keightley 2002), and there has been little empirical evaluation (Barton and Turelli 1987, Keightley and Hill 1989). The notable exception is the special case of Fisher's fundamental theorem of natural selection, which predicts at equilibrium no genetic variance in total fitness (Fisher 1930).

The likelihood of the genetic variance being depleted under sustained directional selection will be determined by the genetic basis of the trait variation. Lande's original formulation of the multivariate response to selection (Lande 1979) and the quantitative genetic theory of life-histories (Lande 1982) modelled a large number of loci, each with a large number of alleles

with a Gaussian distribution of effects. Under such assumptions, the genetic variance changes little during the response to selection, perhaps only by as much as 20% (Reeve 2000). Turelli (1988) suggested that a more realistic genetic basis would be modelled by a leptokurtic distribution of allelic effects, as a consequence of the variance of mutational effects being larger than the variance of standing allelic effects. Under these assumptions, the genetic variance can change rapidly during the response to selection, although this is highly dependent on the genetic details of the selection response (Barton and Turelli 1987).

Unfortunately, few generalizations can be made concerning the genetic basis of adaptive transitions (Orr and Coyne 1992), and therefore whether most ecologically important traits may have a genetic basis that predisposes them to a loss of genetic variance under directional selection. QTL analyses are not precise enough (Barton and Partridge 2000, Barton and Keightley 2002), and currently are biased towards isolating genes of large effect (Lynch and Walsh 1998), making it difficult to empirically determine the size distribution and number of allelic effects that respond to selection. Orr (2000) showed that the optimum size of a mutational effect to speed adaptation is intermediate, and that the distribution of fixed factors during adaptation toward an optimum will be exponential (Orr 1998); many of the fixed factors will be quite small effects, but the initial steps may be quite large. Whether such a genetic basis to adaptive transitions will facilitate the depletion of genetic variance during adaptation has not been explicitly modeled to our knowledge. For example, fixation of a large leading factor would dramatically change the genetic variance during the increase in its frequency, but may leave little signature of its presence after fixation (Agrawal et al. 2001).

Empirical evidence for the depletion of genetic variance in single traits by selection is mixed. In long-term artificial selection experiments, limits are often reached. These include selection experiments on size in *D. melanogaster* (Robertson 1955) and in mice (Bunger and Herrendorfer 1994). However, in other experiments, selection responses seem almost continuous (e.g., Weber 1990). Limits were initially thought to be due to the exhaustion of standing variation in populations; however, theoretical work (Lande 1975) showed that mutation could maintain genetic variance and this could contribute to selection responses, as validated empirically (Frankham 1980, Mackay et al. 1994, Hill and Mbaga 1998). Nevertheless, selection can clearly affect the level of genetic variance in the short term, and decrease it even in natural populations of large size, as in the case of body size and condition in the Collared Flycatcher, *Ficedula albicollis* (Merilä et al. 2001).

Comparative approaches for determining if selection depletes the genetic variance have focused on a predicted negative association between the level of genetic

variance and the correlation of a trait with fitness. As discussed earlier, Mousseau and Roff (1987) surveyed estimates of heritability in morphological, behavioral, and life-history traits across a range of taxa, and suggested that life-history traits may have lower heritability as a consequence of their proximity to fitness, but coefficients of additive genetic variance can be higher for traits closely associated with fitness (Houle 1992). Within single species, there is a negative association between heritability of a trait and its correlation with total fitness (Gustafsson 1986, Kruuk et al. 2000), but this may simply reflect greater environmental or nonadditive genetic variance in fitness-related traits (Kruuk et al. 2000, Merilä and Sheldon 2000) rather than depletion of genetic variance.

The extent to which the different explanations apply to the cases of low levels of genetic variance discussed above is unknown. For the same trait in different populations or related species, it would be surprising if the mutational target had changed, because genomes are generally highly conserved and the number of genes affecting a trait in different species is likely to be similar. For different traits, it has now been firmly established that a trait's association with fitness is a poor predictor of the level of genetic variance. In general, however, given the large number of possible causes of variation in the genetic variance of single traits, it seems unlikely that broad generalizations concerning the relative importance of each mechanism in explaining this variation across trait types and populations will be possible in many cases.

GENETIC LIMITS TO EVOLUTIONARY CHANGE IN TWO TRAITS

Genetic correlations between traits are usually assumed to be a consequence of pleiotropy, and have played a central role in the development of genetical theories of life-history evolution (Lande 1982, Rose 1982). Although many studies have focused on bivariate genetic correlations, these have mostly been unsuccessful in demonstrating genetic limits to selection. Here, we outline the importance of bivariate genetic correlations and how they generate indirect selection on traits not under direct selection, and highlight a number of inherent weaknesses in focusing on bivariate genetic correlations as causes of genetic limits.

Genetic covariance between a trait and fitness

The most important bivariate genetic relationship is the genetic covariance between a trait and fitness. The response to selection of a single trait in Eq. 1 may be represented in an alternative form (Price 1970):

$$\Delta z = \text{cov}_A(w, z) \quad (2)$$

where w is relative fitness, z is the trait of interest, and $\text{cov}_A(w, z)$ is the additive genetic covariance between the trait and relative fitness. A heritable trait and fitness may covary at the phenotypic level (a significant se-

lection gradient, for example), but no evolution may occur if there is no genetic covariance with fitness.

The genetic covariance between a trait and fitness (or lack thereof) has been represented as a limit to the response to selection in two ways. First, selection may act on the environmental component of the phenotypic variance rather than on the genetic component (Merilä et al. 2001). As a consequence, means of some traits in vertebrate populations are static or even changing in a direction opposite to the one predicted by selection analyses. For example, Kruuk et al. (2002) found heritable variation for antler size in red deer (*Cervus elaphus*) as well as directional selection for increased size. However, there was no evolutionary shift in antler size over almost 30 years. It appears that only the environmental component of variation in antler size was under selection, and that this component was probably related to the nutritional state of the organisms.

Second, it is often the case that substantial genetic variance is maintained in selected lines because of the correlation between the trait under selection and fitness. Many limits in selection experiments appear to be reached because of counterbalancing natural selection as a consequence of negative pleiotropic effects of the alleles that have responded to selection (Barton and Turelli 1989, Tanaka 1998). In other words, the alleles underlying the selection response generate a negative genetic covariance with fitness as defined in the absence of the selection pressure. For instance, Hill and Mgeba (1998) described high levels of genetic variance remaining in lines of mice selected for 50 generations. In this case, low fitness of the selected individuals seemed to be responsible for the selection limit, because there was increasing natural selection against individuals with extreme phenotypes as artificial selection progressed.

These examples highlight the importance of considering the response to selection of a trait of interest in the context of a wider set of functionally related traits and pleiotropic effects if one is to understand the evolution of the target trait. In both cases, pleiotropic effects of the alleles underlying the genetic variance in the target trait appear to constrain further evolution. Dickerson (1955) emphasized that a set of individual traits could all display genetic variance, and yet a response to selection for increasing values of all traits ("total productivity") may not be possible as a consequence of the genetic covariance structure among traits, a view we develop further in later sections.

Genetic correlations among life-history traits

Often in evolutionary ecology, the genetic covariance between two life-history traits has been recognized as providing a potential genetic constraint on the response to selection. Life-history investigations have often sought to determine if negative genetic correlations exist between fitness components that would limit further evolution (Lynch and Walsh 1998). Compara-

tive analyses on genetic correlations from a range of traits have suggested that negative genetic correlations might be more prevalent between life-history traits than other types of traits, reflecting stronger underlying trade-offs between major fitness components (Roff 1996). Although negative genetic correlations are often considered the primary mechanism of trade-offs in natural populations, many life-history genetic correlations are positive (Roff 1996). Positive genetic correlations can also act as genetic constraints if selection favors small values of one trait, and large values of the other; genetic correlations in isolation represent only circumstantial evidence that a limit to selection may exist.

Genetic correlations between life-history traits can be difficult to measure with precision from breeding designs (Koots and Gibson 1996), and therefore selection experiments are commonly used to establish the presence of an evolutionary trade-off (Reznick 1992, Fry 2003). Selection experiments provide perhaps the most direct method for establishing evolutionary trade-offs, although phenotypic manipulations provide a complementary approach (Partridge and Harvey 1985). Nevertheless, selection experiments have failed to detect negative genetic correlations even between traits that are functionally related and share the same resource. For example, a selection experiment using a population of *D. melanogaster* recently derived from the field (Schwarzkopf et al. 1999) failed to find a negative genetic correlation between egg size (under direct selection) and egg number (the correlated response), even though overwhelming evidence for a trade-off between these traits exists at the among-population (Stamer et al. 1997) and among-species (Montague et al. 1981) levels in *Drosophila*.

Negative genetic correlations are not a reliable indicator of a trade-off between two traits for three reasons. First, Charlesworth (1990) and Houle (1991) emphasized that variation between individuals in the acquisition of resources, rather than simply the allocation of resources, could easily mask underlying functional trade-offs between traits with positive genetic correlations. Second, even when there is no variation in acquisition, hierarchical allocation of resources within individuals could again result in positive genetic correlations between traits that trade off, if there is variation between individuals in the allocation of resources to each level of the hierarchy (Worley et al. 2003). Therefore, without understanding the hierarchy of allocation of resources within individuals to a set of life-history traits, it is difficult to predict which bivariate combinations of traits may display negative genetic correlations.

Third, unmeasured traits can also be involved in the trade-off, resulting in a positive genetic covariance between any pair of traits involved (Pease and Bull 1988). It has long been appreciated that failing to include an important trait in a formal selection analysis can dramatically change the form and strength of selection on

other traits (Lande and Arnold 1983). Less attention has been given to the analogous problem of excluding genetically correlated traits in quantitative genetic experiments designed to detect trade-offs and genetic constraints (Pease and Bull 1988), but it is clear that leaving out traits of importance will dramatically affect predicted responses (e.g., Kruuk et al. 2002).

Genetic correlations and indirect selection

Information on bivariate genetic correlations may be combined with measures of selection to predict whether trait covariances inhibit selection response (Lande and Arnold 1983). The response of a single focal trait (i) may be subdivided into the response as a consequence of direct selection on that trait using an alternative form of Eq. 1,

$$\Delta z_i = V_A \beta_i \quad (3)$$

and that due to the indirect selection generated by selection on other traits transmitted through the additive genetic covariances (cov_{ij}) between the focal trait and the other traits (Lynch 1985),

$$\Delta z_i = \sum_{j \neq i} \text{cov}_{ij} \beta_j. \quad (4)$$

This approach showed that direct selection on a trait is often opposed by indirect selection transmitted through the bivariate genetic covariances (Grant and Grant 1995, Brooks and Endler 2001, Caruso 2004). Using a similar approach, Etterson and Shaw (2001) found that the univariate predicted responses to selection in three heritable functionally related traits associated with drought stress were consistently greater than when the genetic covariance structure was included in a multivariate predicted response.

Although Etterson and Shaw (2001) highlighted the fact that one of the three bivariate genetic correlations was antagonistic to the direction of selection in each of their populations, as we shall see, such obvious bivariate patterns are not essential in generating substantial genetic constraints. A lack of genetic variance in single traits, and genetic correlations between traits, are often discussed as separate mechanisms of genetic constraint (Mitchell-Olds 1996, Caruso 2004). Below, we show how a multivariate definition of genetic constraint can be achieved that provides a unified framework for the analysis of genetic limits.

GENETIC LIMITS TO EVOLUTIONARY CHANGE IN MULTIPLE TRAITS

Selection is unlikely to act on single traits in isolation (Lande and Arnold 1983), as correlational selection is common in natural populations (Brodie 1992, Schluter and Nychka 1994, Sinervo and Svensson 2002, Blows and Brooks 2003). Consequently, there is a critical distinction between the level of genetic variance in individual traits and genetic variance in multiple traits that together may form complex structures in mor-

phology, multicomponent signals, or complex life-histories. Here, we highlight that genetic constraints as a result of genetic covariance structure can be thought of as a lack of genetic variance in one or more multivariate directions.

Multivariate systems and the definition of genetic constraints

Lande (1979) showed that the response of a number of traits to selection will be influenced by the genetic variance-covariance matrix:

$$\Delta \mathbf{z} = \mathbf{G} \boldsymbol{\beta} \quad (5)$$

where \mathbf{z} is a vector of the response of individual traits, \mathbf{G} is the genetic variance-covariance matrix, and $\boldsymbol{\beta}$ is the vector of linear selection gradients. \mathbf{G} can have a dramatic effect on the response of individual traits to selection, and some traits may be predicted to respond in the direction opposite to that indicated by individual selection gradients (e.g., Grant and Grant 1995), a result that is analogous to the finding of opposed direct and indirect selection on a single trait using Eqs. 3 and 4.

The definition of a genetic constraint when multiple traits are involved is best approached by examining the properties of the genetic variance-covariance matrix (Pease and Bull 1988, Arnold 1992) or the genetic covariance function for infinite-dimensional characters such as reaction norms (Gomulkiewicz and Kirkpatrick 1992). Genetic variance-covariance matrices are natural subjects for diagonalization to determine their eigenvalues and eigenvectors (Lande 1979); because any $n \times n$ \mathbf{G} matrix is symmetrical (where n = the number of traits), it will always be possible to find n orthogonal axes (or eigenvectors), each explaining a proportion of the genetic variance that can be determined from the eigenvalues (λ_i) associated with each eigenvector. When all $\lambda_i > 0$, \mathbf{G} is positive definite and genetic variance is present in all multivariate directions. When one or more λ_i are zero, \mathbf{G} is semipositive definite and singular, reflecting the fact that there is a direction (or directions) in which no genetic variance exists, representing an absolute genetic constraint. In many cases, \mathbf{G} might be nonsingular but have one or more very small eigenvalues (i.e., the condition number of \mathbf{G} given by $\lambda_{\max}/\lambda_{\min}$ is large), and is called ill conditioned. An ill conditioned \mathbf{G} matrix predicts that a response to selection in some directions would be possible, but very slow (Pease and Bull 1988).

Adopting such a multivariate definition of genetic constraints clarifies the role of genetic correlations in limiting the response to selection, by rephrasing the question to, "is there genetic variance in the multivariate direction of selection?" For example, two recent selection experiments designed to test the ability of genetic correlations to act as constraints selected two traits simultaneously in directions orthogonal to the major axis of the bivariate genetic correlation between them (Beldade et al. 2002, Brakefield 2003, Con-

ner 2003). Responses were observed in both systems, and it was concluded that genetic correlations were ineffective in constraining the response to selection. Such results are not surprising when it is considered that the response to selection in these cases will simply depend on the size of the eigenvalue (i.e., the level of genetic variance) of the minor eigenvector in a two-dimensional system. A strong genetic correlation between two traits of 0.8, for example, still leaves 36% of the genetic variance in the two-trait system lying in the direction of the minor axis; consequently, a considerable response to selection in this direction is still possible. Such experiments do not invalidate the potential role of genetic correlations in constraining evolution; they simply validate the breeders' equation stating that the response to selection will be directly proportional to the level of genetic variance (the eigenvalue) in the direction of selection.

The important point here is that \mathbf{G} can act as a significant constraint even when the genetic basis of individual traits, or bivariate genetic correlations between traits, give no indication that a lack of genetic variance or a trade-off may be present. For example, \mathbf{G} may be singular even in the presence of genetic variance in all measured traits (Dickerson 1955, Amemiya 1985, Charlesworth 1990). Measuring heritabilities of single traits, or bivariate genetic correlations, potentially tells one little about the ability of the population to respond to selection if those traits are embedded in a larger set of functionally related traits, as will often be the case (Schluter and Nychka 1994, Blows and Brooks 2003). Just as the absence of genetic variance in individual traits is not necessary for \mathbf{G} to act as a constraint, a wide range in magnitude of genetic correlation among traits will allow \mathbf{G} to act as a constraint; neither perfect genetic correlations nor even the presence of substantial negative genetic correlations are necessary (Pease and Bull 1988, Charlesworth 1990). For example, Dickerson (1955) discussed the special case that when n traits have equal genetic variances, if all genetic correlations are equal to $-1/(n-1)$, \mathbf{G} will have one eigenvalue equal to zero, which represents the multivariate direction that increases all traits simultaneously (i.e., a response to selection for "total performance" will not eventuate).

The importance of the direction of selection in establishing genetic constraints

Although quantitative genetic experiments that enable an estimate of \mathbf{G} to be obtained can provide information on the availability of genetic variance in multitrait space, they will usually not be sufficient to determine if a lack of genetic variance limits the response to selection. This is clearly highlighted in the demonstration that all traits may show genetic variance, and yet \mathbf{G} may still be singular. Consequently, quantitative genetic experiments that estimate genetic variances and covariances in isolation are unlikely to elu-

cidate the role of genetic constraints in restricting species distributions, or the evolution of life-histories. Determining the direction of selection is essential in determining if a genetic constraint exists.

There are two main ways that information on the direction of selection can be associated with \mathbf{G} to determine if the availability of genetic variance may constrain evolutionary change. First, direct estimation of the vector of linear selection gradients ($\boldsymbol{\beta}$) allows the predicted response to selection to be estimated from Eq. 5. To illustrate why predicted responses may be small, even in the presence of substantial genetic variance in all traits, Blows et al. (2004) developed statistical approaches to the comparison of \mathbf{G} matrix orientation and linear selection gradients. By using matrix projection to explore the orientation of subspaces of \mathbf{G} , it was found that >99% of the genetic variance in male pheromones in two populations of *Drosophila serrata* was orientated >70° away from the direction of linear sexual selection under laboratory (Blows et al. 2004) and field conditions (Hine et al. 2004). Such an orientation of an ill-conditioned \mathbf{G} matrix with respect to the direction of selection suggested substantial genetic constraints on the evolution of male sexually selected traits, even though individual traits displayed the typically high coefficients of additive genetic variance found in other species for male display traits (Pomiankowski and Møller 1995).

Second, Schluter (1996) took a different approach and emphasized the potential importance of the dominant eigenvector of \mathbf{G} (termed \mathbf{g}_{\max}) that describes the direction in which most of the genetic variance lies, and how it may act as a genetic constraint. Here, it is assumed that multiple fitness peaks are present and the available patterns of genetic covariance may then influence which peak is eventually scaled by the populations under consideration (Arnold 1992). A number of studies have found associations between \mathbf{g}_{\max} and the direction of population divergence (Schluter 1996, Arnold and Phillips 1999, Begin and Roff 2003), suggesting that populations may be constrained to diverge in a direction in which substantial genetic variance is present.

The targeting of \mathbf{g}_{\max} in empirical studies searching for genetic constraints has at least two limitations. First, although \mathbf{g}_{\max} is the dominant eigenvector of \mathbf{G} , it will usually not be the only direction in which genetic variance exists. If a fitness peak lies in the direction of another eigenvector of \mathbf{G} , \mathbf{g}_{\max} will have little effect on the evolution of the population (Arnold et al. 2001: Fig. 5). Blows and Higgie (2003) proposed that an indication of genetic constraint on the evolution of populations in multivariate trait-space could be gained by a comparison of the eigenstructure of \mathbf{G} with that of the variance-covariance matrix of population means (Lande 1979), termed the \mathbf{D} matrix, and demonstrated that the level of divergence need not be proportional

to the level of genetic variance as is assumed in analyses restricted to \mathbf{g}_{\max} .

Second, the apparent success of studies in associating \mathbf{g}_{\max} with divergence needs to be interpreted with caution, as using the major axis of population variation as the measure of divergence confounds the potential influences of selection and drift (Phillips et al. 2001). The level of divergence as a consequence of genetic drift is predicted to be proportional to the amount of genetic variance (Lande 1979), and therefore \mathbf{g}_{\max} is expected to be closely associated with neutral divergence. To distinguish between drift and selection as potential causes of the association between \mathbf{g}_{\max} and divergence, McGuigan et al. (2005) demonstrated how \mathbf{D} could be decomposed into divergence vectors specifically associated with a particular selection regime.

G matrix evolution

Given the difficulty in generating a predictive theory for how the genetic variance changes under selection (Barton and Turelli 1987), it is perhaps not surprising that the issue of how the genetic variance–covariance matrix changes under directional selection remains unresolved (Turelli 1988, Jones et al. 2004). Under the Gaussian set of assumptions used by Lande (1979, 1982), \mathbf{G} will remain relatively constant, but as allele frequency change becomes more dramatic, genetic covariances will change rapidly (Bohren et al. 1966, Shaw et al. 1995). Nevertheless, comparative approaches to the question of \mathbf{G} matrix evolution have suggested that \mathbf{G} might remain similar until divergence reaches the level of between species (Steppan et al. 2002). Direct experimental approaches to \mathbf{G} matrix evolution are few, and in contrast to the conclusions drawn by comparative studies, suggest that \mathbf{G} might change during the first 20 generations of directional selection (Shaw et al. 1995, Blows and Higgie 2003).

Unfortunately, too few examples are available to determine if selection remains constant enough in the presence of spatial or temporal environmental heterogeneity under natural conditions to result in ill-conditioned \mathbf{G} matrices. Traits under sexual selection may be particularly good systems to determine the effect of directional selection on the orientation of \mathbf{G} , as selection may be more consistently applied in the same direction than occurs under other types of selection. Brooks and Endler (2001) reported an ill-conditioned \mathbf{G} matrix of male guppy sexually selected traits. A subsequent selection experiment was unable to increase guppy male attractiveness (Hall et al. 2004), suggesting that the ill-conditioned \mathbf{G} matrix of male display traits successfully predicted the lack of response to selection.

If sustained directional selection does generally result in ill-conditioned \mathbf{G} matrices, representing a depletion of genetic variance in the multivariate direction of selection, why can't mutation keep pace, supplying further genetic variance and resulting in a continued response to selection? The answer might lie in the com-

plexity of adaptation represented by multiple traits responding to selection. Fisher (1930) first noted, and Orr (1998, 2000) has subsequently confirmed, that the rate of adaptive change slows as the complexity of the adaptation increases. Fisher (1930) proposed a geometrical view of an organism composed of orthogonal traits, in the same fashion as the diagonalization of a \mathbf{G} matrix discussed above establishes the effective dimensionality of the genetic basis of a set of traits. As the number of dimensions increases in an adaptation, the slower the response to selection, as mutation finds it increasingly difficult to supply allelic variants that satisfy all the selective constraints imposed by the complex solution natural selection has found. The probability that a mutation will be favorable is roughly inversely proportional to the number of dimensions. Therefore, genetic variance in multitrait systems under directional selection may be depleted faster than mutation can supply.

CONCLUSIONS AND FUTURE DIRECTIONS

An empirical reliance on single-trait heritability and bivariate genetic correlations studies, and the frequent use of cosmopolitan species as laboratory models, have influenced how genetic limits to evolutionary change are viewed. Contrary to the common opinion that genetic variance will be present in any trait (Lynch and Walsh 1998) or any direction in trait space (Barton and Partridge 2000), we suggest that a lack of genetic variance may be an underappreciated cause of limits to selection. Many critical experiments that might elucidate the importance of an absence of genetic variance in limiting evolutionary change are yet to be conducted.

As a consequence of the logistical limitations of many quantitative genetic experiments, \mathbf{G} in practice is often nonpositive definite because of sampling (Hill and Thompson 1978), reflecting the fact that at least one negative eigenvalue exists. The frequency of occurrence of nonpositive definite covariance matrices is a statistical problem that plagues any application of between-group covariance matrix estimation. Determining the rank (i.e., how many nonzero eigenvalues exist) of any covariance matrix is difficult, and many maximum likelihood algorithms in common use may perform poorly if the true covariance matrix is singular (Shin and Amemiya 1997). It is a critical, but virtually unexplored, empirical issue to determine if eigenvalues of \mathbf{G} are small as a consequence of sampling, incorrect estimation, the inclusion of redundant (highly correlated) traits, or a real lack of genetic variance (D. Houle, *personal communication*) if we are to understand the availability of genetic variance in complex multitrait systems. Such investigations should ideally be placed in the context of understanding the direction of selection acting on those traits included in the analysis.

Ecological genetic investigations need to consider a wider variety of species and to contrast selection responses in related species with very different ecologies.

Only by undertaking quantitative genetic experiments in restricted or specialized species can we assess the generality of these results and whether sharp differences between species exist in the ability to respond to environmental challenges. Of particular interest will be experimental systems involving a manipulation of an environmental challenge that is not currently experienced by one species, but has been overcome by a close relative. Selection experiments are needed to determine whether species with restricted ranges of hosts or restricted distributions display low levels of genetic variance in ecologically important traits in comparison to more widespread closely related species. Selection experiments can be used to test whether species at ecological limits are also at evolutionary limits. A simple but underutilized approach is to hold organisms under conditions that mimic those factors that restrict their distributions (Magiafoglou and Hoffmann 2003). Determining the role of genetic constraints on evolutionary change will require more detailed ecological and genetical knowledge of natural populations than we currently possess.

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