Constraints Evolve: Context Dependency of Gene Effects Allows Evolution of Pleiotropy

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Abstract

Evolutionary constraint due to pleiotropy refers to a situation in which mutations in genes shared among traits generate trait covariance; therefore, traits that are not directly exposed to selective challenge show a correlated response. When such a correlated response is deleterious, it may constrain the trait from evolving. Here, we argue that the idea of absolute constraints draws from the perception that gene effects are inherent to alleles and thus invariant across genetic and environmental backgrounds. However, evidence from studies involving genetic effects on multiple traits, observed across different genetic backgrounds and environments, supports the notion that genes' effects on traits change. Consequently, pleiotropy also varies across backgrounds. We argue for a stronger emphasis on interaction effects when describing a trait's genetic basis and its evolutionary potential. By discussing different cases of trait individuation, we demonstrate how this approach can lead to new insights.

INTRODUCTION

As the joint evolution of multiple traits has become better conceptually delineated and more technically accessible over the past 50 years (Hansen & Houle 2008, Lande 1979, Riedl 1978), Darwin's idea of correlation of growth (Darwin 1859) has played an increasingly important role in evolutionary biology and has led to a large body of work on the phenotypic, genetic, and evolutionary relationships between traits. This has often been organized around the concept of morphological integration (Berg 1960, Olson & Miller 1958) and genetic constraint. In a wide array of biological taxa and over a broad array of traits, the phenotype and its genetic basis have a modular structure; strong genetic, phenotypic, and evolutionary relationships exist between some traits, which often share underlying developmental processes and/or participate in a common functional process (Cheverud 1982, Ehrich et al. 2003). Observations in developmental biology underscore established population patterns: Even newly arisen mutations tend to affect very specific trait combinations, whereas mutants that affect other combinations of trait values do not occur (e.g., Alberch 1983).

The directions in phenotypic space associated with most genetic variation introduce bias into the response to selection (Schluter 1996). Indeed, comparative studies of trait interrelationship patterns have shown broad similarities in modular trait structures across large taxonomic groups (Marroig & Cheverud 2001) but have also highlighted evolutionary change in the extent and pattern of trait interrelationships, especially at higher levels of taxonomic diversity. For example, studies of mammalian cranial morphology (Hallgrimsson et al. 2004, Porto et al. 2009, Sanger et al. 2013) and vertebrate fore- and hindlimbs (Kelly & Sears 2011, Young et al. 2010) have documented divergence in trait covariation.

The standard view on the evolution of intertrait relationships, as measured by genetic or phenotypic variance/covariance or a correlation matrix, is that allele frequency change at pleiotropic gene loci is shaped by stabilizing selection, so that the trait variance/covariance structure approaches the structure favored by stabilizing selection (Lande 1980). The specific level of genetic correlation between traits arises as the variance-weighted average of positively pleiotropic, negatively pleiotropic, and nonpleiotropic loci (Cheverud 1984). Allele frequency changes alter the variance weights for these three classes of gene effects, resulting in changes in genetic and phenotypic covariation. Pleiotropy, by causing covariation among traits, is thought to direct evolutionary change by enhancing the response to selection in some directions and constraining it in others.

In this model, the pattern and size of gene effects on multiple traits are fixed—they either show positive pleiotropy, negative pleiotropy, or no pleiotropy—whereas allele frequencies at those loci change in response to selection. However, it has long been understood that the effects of genes themselves also vary and can evolve under epistasis, as in Wright's (1968) shifting balance theory and Mayr's (1970) concept of the unity of the genotype and the genetic revolution that results from a founder effect. In both cases, the effects of genes themselves evolve not only because of changes in allele frequency at a locus but also as a result of allele frequency changes at other loci epistatically interacting with the locus in question. The effect of an allele is not a property of the allele itself but depends critically on genetic background, including alleles at other loci (epistasis) and alleles at sex-determining loci (SDL; sexual dimorphism), and on the environment [gene by environment ($G \times E$) interactions and phenotypic plasticity]. Changes in context can thereby cause variation in allelic effects, so that pleiotropy itself is variable and evolves. Through epistasis, or other forms of interaction, alleles can shift between positive pleiotropy, negative pleiotropy, and null pleiotropy. Such structural change in the genotype-phenotype (GP) map has been less commonly considered in evolutionary studies than changes in allele frequency at pleiotropic loci. Consequently, the

molecular and population processes underlying changes in the range and strength of pleiotropy have received little attention.

In this review, we describe the concepts and empirical data supporting genetic variation in pleiotropy, the molecular and developmental mechanisms underlying pleiotropy and its variation, and the evolution of pleiotropy under directional selection on multiple trait combinations. In studies of phenotypic evolution, we argue that greater emphasis on specific interactions, as the embodiment of context dependency, will enhance the connection between evolutionary patterns and their mechanistic bases. We then apply our model to three topics in evolutionary biology that can substantially profit from the conceptualization: evolution of trait individualization, sexual dimorphism, and allometry.

PLEIOTROPY: WHAT DO WE MEAN?

To study pleiotropy and its evolution, we must first address some of the conceptual uncertainties about pleiotropy. Several authors have pointed out the difficulties in assessing pleiotropy (e.g., Hill & Zhang 2012, Paaby & Rockman 2013; reviewed in Wagner & Zhang 2011), some of which are conceptual and others technical. We address some of these difficulties below. The distribution of pleiotropy across genes and the effects of pleiotropy on variation have been a subject of multiple recent contributions and reviews (Solovieff et al. 2013, Stearns 2010, Stern 2000, Wagner & Zhang 2011) and are not discussed here.

From Variation to Mechanisms

Pleiotropy is a gene-centered description of the property of variation rather than a depiction of a mechanism. It describes an association between genetic and phenotypic variation in which polymorphisms in one gene affect phenotypic values of multiple traits. Similar variational patterns can be generated by multiple mechanisms, and in the short term, similar variational patterns lead to similar short-term evolutionary consequences (Cowley & Atchley 1992); thus, only pattern appears relevant. Long-term consequences, however, may depend on the developmental details (Gromko 1995). To address these questions, understanding how molecular mechanisms translate into variational patterns is crucial.

Inferring developmental and functional processes from a variational pattern is not straightforward, if possible at all. One well-established complication is hidden pleiotropy, in which the combined effects of pleiotropy across loci cancel each other out and thus pleiotropic effects are not reflected in genetic correlations (Cheverud 1984). Another complication is pleiotropy arising as a result of indirect effects on traits (e.g., Li et al. 2006, Welsh et al. 2010). For example, mandible shape is a result of developmental negotiation between tooth and bone; a change in genes shaping teeth only will also indirectly affect mandible shape. Another example of indirect effects involves circulating factors, such as hormones, which can have broad effects despite local expression. In this case, genes that cause variation in a trait are not necessarily expressed in that trait's tissue at any developmental stage, although they contribute to its development.

How Many Traits?

How much independent variation must traits manifest to be considered different? To answer this question, most approaches measure the number of uncorrelated dimensions between traits (e.g., Mezey & Houle 2005). In the next section, we show that this question is less important when discussing the evolution of pleiotropy than when considering pleiotropy itself.

How Do We Define Traits?

One difficulty in defining pleiotropy comes from the difficulty in defining phenotypic traits themselves (Wagner 2001). This difficulty becomes particularly interesting when we observe traits that only occur in a subset of individuals in the population. This is the case for sexually dimorphic traits, in which male and female realizations are often treated as separate traits (Lande 1980). Analogously, in microorganisms, the same mutant growing under different conditions is described as having different phenotypic traits. In both cases, the alternative to referring to multiple traits, and thus invoking pleiotropy, is to consider the observations context-dependent realizations of the same trait. In sexually dimorphic traits, the realization depends on the individual's sex [gene-gene ($G \times G$) interaction, epistasis]; in the example of microorganisms, the realization depends on the environment, such as different drugs in the medium ($G \times E$ interaction). Indeed, pleiotropy and interaction are mathematically equivalent. In terms of the mathematics, the choice is a question of convenience (Falconer 1952).

Is there a biological rationale for focusing on pleiotropy rather than interaction? This question is best considered at the individual rather than the population level. Not only can context-dependent traits among individuals be considered different traits, traits within an individual—traits that we usually think of as sharing many genes—can be seen as context-dependent realizations of gene effects. For example, repeated traits, such as vertebrae or segments, share many developmental pathways and are influenced by many underlying, pleiotropic genes. However, these traits are expressed in different parts of the body, in different developmental-genetic contexts. Focusing on pleiotropy addresses why traits are (or remain) similar. Alternatively, focusing on how pleiotropic effects are modified across traits, such as different developmental contexts, may be useful when studying trait differentiation and the evolution of constraint.

This perspective is at the core of our argument: Considering genetic effects on traits as a consequence of interactions with other genes rather than as a consequence of invariant attributes of genes enables us to think of the GP map as evolvable. This conceptualization of traits does not resolve the question of how many traits are affected by a mutation. Rather, it emphasizes a different question: How can traits sharing genes become individualized?

Is Fitness a Trait?

Some difficulty is introduced when fitness is treated as a trait. Much insightful experimental evolutionary work on pleiotropy has been performed on various microorganisms and viruses (Barrick & Lenski 2013, Burch & Chao 2000, Turner & Chao 1999, Wiser et al. 2013). In these studies, mutational effects are assessed with respect to fitness rather than component organismal traits, such as metabolic pathways or gene expression. Pleiotropy then represents fitness effects in different environments (e.g., in the presence of drugs, parasites, or different hosts). These effects can also be conceptualized as $G \times E$ interactions, consistent with the interchangeability of pleiotropy and interaction discussed above. Although fitness is arguably the most relevant measure for population dynamics, the implicit mapping from traits to fitness has the disadvantage of removing us further from the mechanistic basis of the evolutionary process. Many different combinations of features may have the same fitness. We thus focus predominantly on organismal traits.

Pleiotropy of a Gene

An important distinction has been made between the pleiotropy of a mutation and the pleiotropy of a gene (Stern 2000). Because multifunctional genes possess different functional domains, they can

harbor mutations that affect single traits, contributing to the traits' variance rather than covariance among traits. With respect to genes, therefore, pleiotropy is a dispositional property, describing their potential to generate pleiotropy by mutation. We are concerned here with pleiotropic mutations, which generate covariance. Thus, we refer to single mutations rather than genes.

Assessing Variation in Pleiotropy Is Less Problematic Than Assessing Pleiotropy Per Se

Identifying changes in pleiotropy is not dependent on defining traits or pleiotropy. This is illustrated in **Figure 1**. The mutational vector in **Figure 1*a*** is drawn between the origin, A, and B (B′). The letters A, B, and B′ represent the mean two-trait phenotypes of individuals before (A) and after (B, B′) mutation. In this model, a vector of pleiotropic mutation is any vector not aligned with either trait axis. Even if the position of the trait axes may be arbitrary (discussed in Mitterocker et al. 2012, Wagner & Zhang 2011), and hence the trait can be defined to be parallel to a mutation, the change in the angle of the mutation vector changes pleiotropy. Thus, whereas the number of traits affected by a mutation depends on the definition of traits, the change in the angle of this vector does not. Although the arbitrariness of trait direction sometimes makes it difficult to precisely quantify pleiotropic effects, it does not diminish their presence; this can be easily shown by plotting the effects of mutations at multiple loci into the same plot. The effects form a distribution rather than falling along single dimensions (see examples in **Figure 1*c***), reflecting the presence of widespread variation in pleiotropy across loci, regardless of the definition of trait axes.

EVIDENCE FOR VARIATION IN PLEIOTROPIC EFFECTS

Few studies have explicitly addressed changes in pleiotropic effects of particular loci. However, in studies that have recorded genetic effects on multiple traits in different backgrounds or environments, the evidence for such variation is striking. A few examples illustrate this point (for more detail, see Pavličev & Wagner 2012).

Microorganisms and viruses provide particularly detailed information on variation in the GP map and the evolutionary potential it confers. Particularly well-worked-out examples of variable pleiotropy can be found in *Escherichia coli* (Lenski 1988) and budding yeast (Kim et al. 2009). A detailed example of variation in pleiotropy comes from a study of the phage ϕX174. Pepin et al. (2006) studied mutants that differed from each other in either one or two codons. The mutations affected host attachment rate and viral reproductive success. The authors found that the two traits did not vary coordinately across genotypes at the two codons, which would be the case if each substitution affected both traits. Rather, a mutation at one locus affected one or both traits depending on the allele at the second locus. Importantly, not only was the covariance between the two traits genotype dependent, it depended on the environment.

Although microorganisms offer particularly convenient study systems because of their fast, simple development and small size, the effects of genetic and environmental context on pleiotropy are not limited to this group. For example, Templeton et al. (1985) studied the *abnormal abdomen* mutation, which results in a juvenilized abdominal cuticle and a range of life history defects in laboratory *Drosophila mercatorum*. In a wild population of the same species, however, the authors observed that the allele lacks an effect on the abdominal cuticle, although it retains its effects on life history traits. They thus demonstrated that this pleiotropic effect depends on genetic background.

Another detailed example comes from the butterfly *Bicyclus anynana* (Monteiro et al. 2007). Here, the authors studied the combined effects of two mutations, *missing* and *spotty*, on the wing eyespot pattern. They found that whether *missing* affects the fore- and hindwing or only the

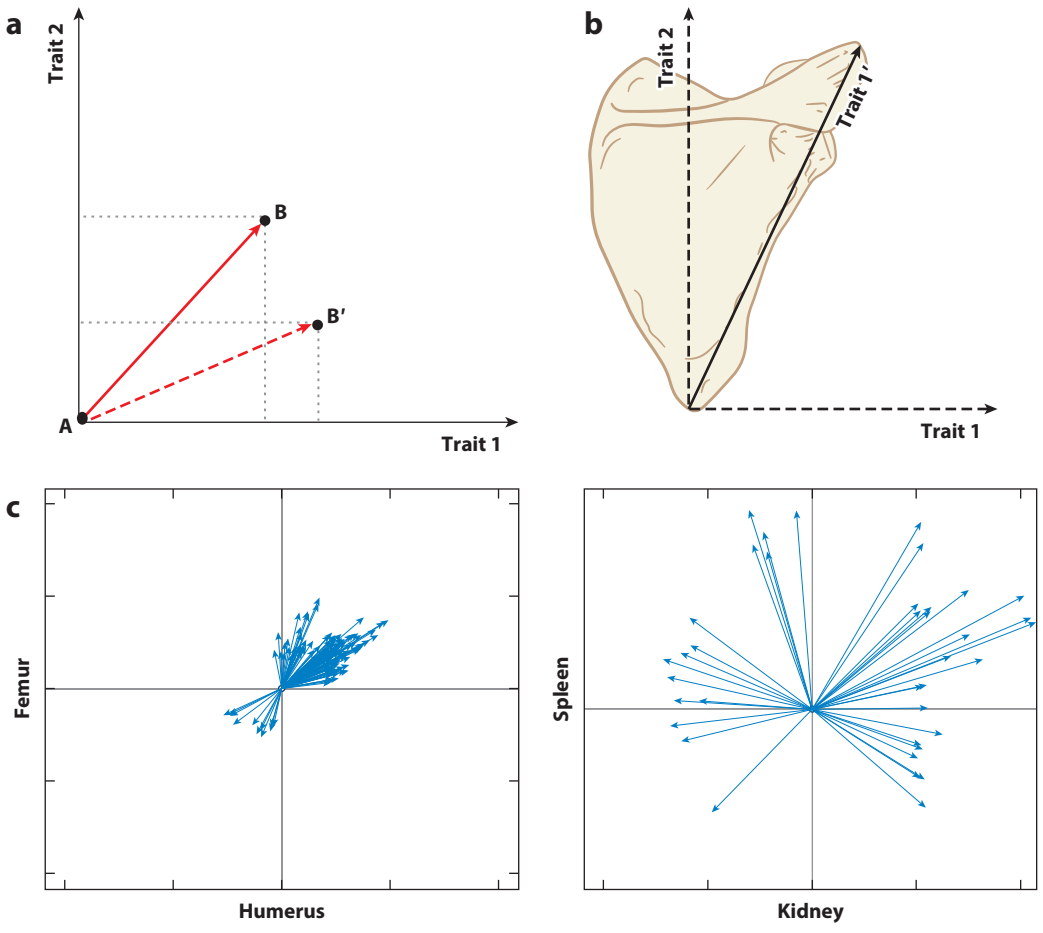

Figure 1

Variation in pleiotropic mutational effect. (*a*) Let us consider two trait axes and a mutation vector in a two-dimensional phenotype space. The mutational effect is represented by the vectors drawn between the points A and B (or A and B′). A and B (B′) are the mean two-trait phenotypes of individuals before (A, origin) and after (B, B′) mutation. In this model, a vector of pleiotropic mutation is any vector not aligned with either trait axis. A change in the pleiotropy of a mutation is a change in the relative effects of the mutation on two traits. This is shown as a change in vector angle (from the *solid line* to the *dashed line*), leading to a phenotype with a new combination of trait values (B′). The trait axes are often hard to define unambiguously and thus could be redefined to align with a mutational vector. As a consequence, pleiotropy is dependent on the definition of trait axes. For example, in panel *b*, a simultaneous effect on the width (trait 1) and height (trait 2) of the scapula may be reduced to a measurement along a single dimension (trait 1′). This would justify the claim that the mutation is not pleiotropic. However, a change in the angle, such as that from the solid to dashed vector in panel *a*, always changes pleiotropy regardless of the trait axes. (*c*) The distribution of mutational vectors in two-dimensional space for two pairs of traits (data from an LG/J × SM/J mouse intercross; M. Pavličev & J.M. Cheverud, unpublished data). When mutational effects of multiple loci on two traits are considered simultaneously, shown here as a distribution of vectors in two dimensions, we see that these also show variation in direction. This underlines the fact that, although redefining the trait axes may explain a single mutation in terms of a new trait, there is often abundant variation around this direction, supporting pleiotropy and its variability. The two plots also show that the combinations of traits differ in degree of consistency of mutational effects. Whereas most effects are of the same sign in femur and humerus, this is not the case for spleen and kidney, enabling a range of very different trait combinations in the latter.

forewing depends on whether the *spotty* allele is present at a second genetic locus. The pleiotropy of the *missing* locus hence depends on the *spotty* genotype.

Plant breeding offers another clear example of variation in pleiotropy (Gibbon & Larkins 2005). When plant breeders were seeking to increase the lysine content of corn, they found a mutation (*opaque-2*) that doubled lysine levels in the endosperm. Unfortunately, the mutation simultaneously resulted in low seed density and a soft texture, resulting in corn with increased bitterness and insect susceptibility. Further research identified epistatically interacting modifier alleles that squelched the undesirable effects of *opaque-2*; these alleles were bred into *opaque-2* lines to produce elite lines with high lysine content and hard, vitreous endosperm. These have been used in hybrids and synthetics worldwide. We see a similar process at work in nature, where variation in pleiotropic effects caused by interacting loci can lead to the evolution of pleiotropy.

The effect of environmental context on the pleiotropy of a mutation is also well documented. Using recombinant, inbred *Drosophila* lines, Bergland et al. (2008) found heritable variation in ovary size relative to body size that depended on diet as well as genetic background. Lawson et al. (2011) demonstrated that the heritable associations between risk traits for metabolic syndrome varied across dietary regimes (and between the sexes) in mice. Barrett et al. (2009) demonstrated that the pleiotropic effects of the *Ectodysplasin* locus (*Eda*) on body growth and armor in the threespine stickleback differed between marine and freshwater environments. In freshwater, only fish with the low *Eda* allele and reduced armor achieved large body size, whereas in the marine environment, this trade-off was absent, allowing fully armored fish with the high allele to reach a large body size. Thus, *Eda* affects size and armor in freshwater environments but only armor in marine environments.

The examples above represent cases of large phenotypic effects due to changes in pleiotropy at single loci across contexts. But are these rare events, or is there widespread, continuous variation in pleiotropy? The first systematic study to address this question was conducted by Cheverud et al. (2004) on the mouse mandible. They used a modified quantitative trait locus (QTL) mapping technique to identify genetic loci associated with phenotypic variation. Whereas conventional QTL mapping tests genotypes at polymorphic genomic locations for differences in the mean phenotypic value of a trait, this modified mapping technique tests for an effect on the regression slope between two traits. The genotypes of the resulting relationship QTLs (rQTLs) differ in the variational relationship (e.g., regression, covariance, correlation) between two traits (**Figure 2**). By using this technique, the authors found multiple loci affecting the relationship between mandibular traits.

Subsequent applications of rQTL mapping have revealed ample variation in pleiotropic effects for a range of systems. For example, Leamy et al. (2009) demonstrated genetic variation in the association between physical activity and body weight; Pavličev et al. (2008, 2011b) detected rQTLs associated with variation in the allometric relationships between limb lengths and organ sizes, as well as rQTLs for forelimb-hindlimb relationships (Pavličev et al. 2013). Recently, Maxwell et al. (2013) used this approach to analyze factors that modify the relationship between human blood lipid traits and heart disease. They found that the relationship depends on genetic background and differs between European and African-American populations.

Ample genetic variation in trait covariances is interesting because it reveals the potential for pleiotropy and covariances to evolve, as discussed in the Models of Selection on Pleiotropy (see below). But what is the genetic basis of this phenomenon? In the examples above, the genotypes differ with regard to the covariance between traits because pleiotropic gene effects depend on genetic background (epistasis). When a pleiotropic locus is dependent on genetic background (i.e., interacts with the genetic background), pleiotropic effects on single traits may be modified differently by the interaction. This is referred to as differential epistasis (Cheverud et al. 2004). Environmental interaction with a pleiotropic locus may also modify genetic effects in a way that

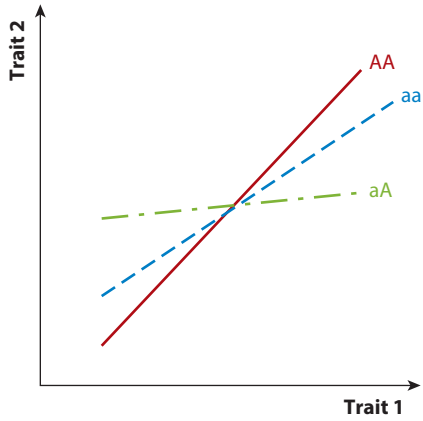

Figure 2

Genotypes at a relationship locus (AA, aA, aa) differ with regard to the relationship, or regression slope, between two traits rather than the mean of a single trait. This is the basis of relationship quantitative trait locus mapping.

differs across single traits, resulting in differences in the trait covariance structure between environments.

In the experimental populations in which most rQTLs have been mapped, it is unlikely that environment varies significantly. Therefore, most of the context dependency of pleiotropy probably originates within the genome. Indeed, we have demonstrated that the pleiotropy in the intercross population of LG/J and SM/J inbred mouse lines varies as a result of differential epistatic effects (Pavličev et al. 2008). In other populations, environment may be as important as genetic background.

Addressing the interdependence of traits and loci requires consideration of the effects of multiple loci on multiple traits. Methods for systematically detecting loci at which genotypes differ not only with regard to a trait's mean but also with regard to its variance are increasingly important (Cao et al. 2014, Ronnegard & Valdar 2012). Some of this attention should also concern loci at which genotypes differ with regard to trait covariance. The rQTL method allows very efficient mapping of genetic effects on covariance, as it requires a lower number of tests than a conventional epistasis scan; one maps the epistatic interactions involving the rQTL rather than all pairs of loci.

Interaction is not the only possible source of the variable pleiotropy of mutations. Alternative alleles at a locus can themselves differ in the number of traits they affect (see Hansen 2006). This possibility has been considered less than interaction, possibly because it is less approachable with conventional biallelic mapping. To compare the additive effects of two alleles at the same locus (in the same background), a third allele must serve as a reference; this type of variation in pleiotropy therefore cannot be studied in classical biallelic systems using the mapping approach discussed above. It may be feasible to study it by using larger assemblies, however, such as diallel crosses.

MODELS OF SELECTION ON PLEIOTROPY

The presence of variation in pleiotropy reveals the potential for evolution, but can this variation be selected upon, resulting in the evolution of pleiotropy? Multiple groups have modeled the evolution of the structure of GP maps, primarily in the context of the evolution of modularity (Clune et al. 2013, Kashtan & Alon 2005, Le Nagard et al. 2011). Their studies have not necessarily

explicitly addressed variation in pleiotropy but have identified conditions and processes likely to be important in its evolution, such as fluctuating modular selection, the cost of interactions, and gene duplication and differentiation. Under these conditions, restricted gene pleiotropy (genes at which mutations affect only a subset of traits, rather than having universal effects) may evolve to generate modular variation. Here, we turn our attention to studies that focus on microevolutionary processes involving variation in pleiotropic effects.

To the best of our knowledge, the first explicit model of the evolution of pleiotropy was a one-locus, two-allele model that focused on a relationship locus (rQTL), a signature of the effect of allele substitutions at this locus on pleiotropy being the change in trait relationship between genotypes at rQTL, as explained above (Pavličev et al. 2011a). We assumed in this model that the relationship locus itself had no direct effect on trait means. Its mean effect stemmed only from linkage disequilibrium with other mean effect loci. Given such variation in pleiotropy, genetic correlations between traits will evolve: Over time, concordant directional selection on two traits will increase their positive correlation, whereas opposing directional selection will decrease their negative correlation. Decoupling (i.e., a decrease in absolute trait correlation toward zero) evolves in this model when directional selection acts on one trait and stabilizing selection on another. Our work on this model also showed that even when the direction of selection on mean values is simultaneously switched for both traits (fluctuating selection), as long as the sign of correlation is maintained across the selection regimes, the correlational structure evolves.

Watson et al. (2014) extended this model to complex GP maps. Instead of representing the multitrait GP map as a one-step transformation of genetic variation into phenotypic variation and covariation, the authors conceptualized a multitrait GP map with multiple steps connecting genotype with phenotype. The initial step comprises (*a*) genes encoding embryonic traits and (*b*) genes encoding a matrix of developmental interactions between embryonic genes. These two sets of gene effects generate the phenotypic correlational structure at a particular stage and act as the input for the next developmental stage. Each developmental step adds an additional layer contributing to the structure of the adult phenotype, enabling nonlinearities in the process to occur. When subject to mutation and selection, the model shows great flexibility in adapting to complex, multivariate selection patterns. The correlations between the traits increase or decrease readily as a consequence of selection, so that the variation pattern mimics that of selection pressures on single traits, even when those pressures conflict with the original correlations between traits. What evolves in this model is the developmental interaction matrix, which determines (*a*) the correlations between traits and therefore (*b*) the phenotypic directions of the adult phenotype, into which variation is funneled during development. We hypothesize that many relationships, such as those between the bat fore- and hindlimb and different segments in arthropods, evolve in a similar manner. Moreover, in this model, phenotypic trait combinations can be recapitulated quickly in recurrent environments, even after adaptation to distinctly different environments. This means that developmental interactions, particularly those not conflicting with successive selection regimes, are maintained in the population and enable an accelerated selection response. In addition, some covariation patterns can be extrapolated to novel selection regimes, thus manifesting high adaptability to new selective challenges. The more often traits are selected together, the greater proportion of their genetic bases are wired together and able to maintain functionality in the face of new combinations of environmental conditions. In summary, this model avoids the assumptions of a linear GP map, as well as that of the lack of direct effects on traits, and increases the number of traits beyond two.

Guillaume & Otto (2012) have also presented an explicit model for pleiotropy evolution. The authors model pleiotropy as the allocation of a gene product to two traits; allocation to one trait diminishes the product's availability to another. The trait relationship is determined by the

proportion of the gene product allocated to each trait, a parameter determined by a modifier gene. The variances of traits change simultaneously in opposite directions as a result of changes in this parameter. The authors have explored various types of trade-offs, including one in which an increase in allocation to one trait strongly negatively affects the functionality of the other (a strong trade-off) and one in which this relationship is only marginal (a weak trade-off). The selective advantage or disadvantage of pleiotropy is derived from the contribution of both traits to fitness. The authors found that genes become specialized if the loss in fitness due to diminished allocation to one trait is smaller than the gain due to increased allocation to the other. In contrast, genes maintain pleiotropy if the allocation to multiple traits does not significantly interfere with the performance of either trait. In this model, selection on the variation in pleiotropy is highly dependent on the character of the trade-offs. As the authors point out, the caveat is the symmetry of benefits conferred by allocating product to each trait. For example, if an increase in allocation to one trait is advantageous but an increase to another trait is neutral or even deleterious, as in many of the cases discussed above, the results will presumably change. Whereas the trade-off in this article is at the level of the gene product, the existence of a trade-off with respect to the genetic variance available to traits has also been proposed (Hansen 2003, Pavličev & Hansen 2011). In short, Hansen (2003) has proposed that the evolution of genes specialized for single traits decreases the interference between traits but also diminishes other traits' potential for variation, and thus their evolutionary potential.

In the above models, the pleiotropy of a mutation is affected by a single interaction. Realistically, multiple loci across a genetic background will affect the effect size and pleiotropy of a mutation (Carter et al. 2005, Hansen & Wagner 2001, Pavličev et al. 2010). For background loci to contribute to the evolution of genetic effects, their interactions with the locus must be concordant with each other in terms of whether they increase or suppress the genetic variation for a particular trait, the phenomenon called directionality of epistasis (Hansen & Wagner 2001). With multiple traits and a pleiotropic locus, the result will similarly depend on how the interactions with this locus combine among themselves with respect to the traits they affect; that is, it will depend on the multivariate effect of the directionality of epistasis. The pattern of the multitrait directionality of epistasis is an exciting empirical question, but to the best of our knowledge it remains unaddressed (Jones et al. 2014).

MECHANISMS OF VARIATION IN PLEIOTROPY

Ultimately, we would like to understand the molecular changes associated with changes in variational patterns. However, rooting population-level variational patterns in their underlying mechanisms is not a trivial undertaking (Lehner 2011, Olson-Manning et al. 2012). Genetic interaction means that the combination of two mutations results in a phenotypic effect that is different from the sum of their individual effects. Such a population-level pattern does not necessarily imply a physical interaction between the underlying genes or their products. Conversely, variation in gene products involved in real physical interactions need not result in nonadditivity at the population level. Indeed, in spite of the ubiquity of physical interactions between genes, gene products, and even tissues during development, variance at the level of the population manifests appreciable additivity (Gjuvsland et al. 2011). The reasons are manifold, from the effects of allele frequencies to the structure of regulatory networks to the nature of developmental interactions (Cheverud & Routman 1995, Olson-Manning et al. 2012, Tyler et al. 2009). Focusing on the relationship between molecular variation, the structure of physical interactions during development, and population patterns of variance will allow us to better integrate the rich knowledge existing in each of these fields.

Even if studying the mechanisms underlying the microevolution of pleiotropy is difficult, insight can be attained starting from each side of the GP map. First, it is likely that evolutionary changes in multifunctional molecules such as hormones, hormone receptors, or enzymes will affect population covariances as well. Molecular evolution of multifunctional molecules can thus provide insight into the evolution of pleiotropy on a longer time scale. Second, we can use gene mapping to target the mechanisms causing population variation in pleiotropy.

Evolution of Multifunctional Factors

Comparisons of molecules such as transcription factors, splicing factors, enzymes, hormones, cytokines, and hormone receptors across taxa can provide interesting insights into the evolution of multifunctionality. Often, the ancestral state can be inferred even if the exact processes underlying change are unknown. As gene effects are mediated by physical interactions, pleiotropy likely evolves via changes in existing interactions, including loss or gain. The mutations involved have a range of effects, from enabling the coexpression of interacting molecules to altering interaction sites on proteins or nucleic acids. The rich work in this field is a source of insight into the evolution of multifunctionality. Here, we consider a few examples.

Instances of *cis*-regulatory evolution provide the best-established examples of gene pleiotropy evolution (Prud'homme et al. 2007, Wray 2007). *Cis*-regulatory regions regulate gene expression by interacting with *trans*-acting transcription factors and various cofactors, themselves products of other genes. These interactions can be tissue specific. Thus, the gain or loss of *cis*-regulatory region changes the potential of the regulated gene, as well as that of the gene encoding the transcription factor, to encounter pleiotropic mutations (Barriere et al. 2012, Kuo et al. 2010, Landry et al. 2005). Despite the presumed constraint on the multifunctional coding regions, both *cis*-regulatory binding sites and transcription factor coding regions evolve, although coding regions likely evolve at lower rates (reviewed in Wagner & Lynch 2008).

Hormones and their cofactors and receptors are appealing systems for studying the evolution of multifunctionality (e.g., Flatt et al. 2005, Ketterson et al. 2005). Hormones coregulate whole suites of characters, thereby mediating integrated responses to the internal and external environment. In spite of the high potential for pleiotropy, hormone systems have undergone rapid evolution. For example, a large group of growth hormones and placental lactogens has diversified following multiple gene duplications in different clades (Forsyth & Wallis 2002, Niall et al. 1971). These changes were accompanied by functional specialization (i.e., a change in pleiotropy). Protein coding sequence analysis in this group of hormones reveals changes in the hormone-receptor interface as well as in cofactor binding. For example, Wallis (2008) found a surge of positive selection in growth hormone coding sequences in multiple mammalian lineages, including primates. Interestingly, these changes are located primarily in the parts of the hormone facing away from the receptor interaction site, suggesting evolution of the cofactor interaction sites; such changes likely alter the number or specificity of hormonal interactions.

With respect to hormone receptors, changes in the number of ligands that trigger a response alter the number of pathways that the receptor is involved in and consequently the pleiotropy of mutations in the receptor. Detailed work on steroid receptors provides an example of this. The group of extant vertebrate steroid receptors evolved from the ancestral estrogen-like receptor via a series of duplications and differentiations. Thereby, some of the newly acquired receptor-ligand relationships have arisen by recruitment of an intermediate product of ligand production, becoming a ligand to a duplicated estrogen receptor (such as progesterone, an intermediate product of estrogen production and its receptor), resulting in androgen and progestin receptors (Thornton 2001). In other cases, a duplicated receptor was recruited into interaction with newly

arisen ligand, such as in the case of aldosterone and mineralocorticoid receptors (Bridgham et al. 2006). This later diversification between mineralocorticoid receptor binding aldosterone and glucocorticoid receptor binding cortisol led from ancestrally multifunctional receptor to loss of the ancestral sensitivity for one ligand, aldosterone, in tetrapods. In each duplication and divergence, the downstream targets affected by the newly evolving interactions have changed.

These examples illustrate the evolution of multifunctionality through interdependent mutations, either mutations in two interacting molecules or separate mutations within a single molecule. Although they shed light on past changes, it is still hard to imagine what variational patterns these changes in functionality cause and how they spread in the population, in part because changes in functional interactions must arise in single individuals, before they become fixed in a population. An approach that addresses this aspect of the evolution of pleiotropy starts from population variation and then searches for molecular changes (see below).

Connecting Patterns from Systems Biology with Patterns in Population Genetic Variation

With the recent expansion of technology, systems biology has been able to access a rich body of detailed knowledge on the developmental and physiological processes involved in constructing the phenotype, including developmental pathways and, within these, interaction patterns of genes and gene products. We cannot even broadly review the insights gained. Instead, we suggest how this knowledge, if connected to population-level phenomena, may illuminate the evolution of pleiotropy.

One particularly interesting result arising from systems biology is the enrichment of for particular interaction patterns between small subsets of genes (so-called motifs, e.g., Milo et al. 2002). If we assume that interaction patterns readily change, such recurrence suggests that particular motifs may be enriched because of their specific functionality (although constraint may offer an alternative explanation; see Cordero & Hogeweg 2006). The range of gene regulatory dynamics that a particular motif can generate is easily modeled if the effects of interacting molecules on one another are known; a famous example is spatiotemporal patterning in development, in which heterogeneous morphologies, such as stripes or segments, develop from continuously expressed factors (Gierer & Meinhardt 1972, Turing 1952). Modeling transcriptional regulation shows that single enriched motifs tend to generate distinct temporal dynamics of gene expression, such as pulses or robust delayed-expression bouts (e.g., Alon 2007, Widder et al. 2012).

In addition to the presence of particular motifs, their transitions also offer important insights. Motif enrichment changes spatially and temporally during development, resulting in a succession of motifs as an individual organism develops (Kim et al. 2012). At the level of cell type differentiation, such developmental succession involves the induction of core transcriptional regulators by an upstream gene. This is followed by implementation of a feedback loop among the induced genes, enabling downstream genes to achieve regulatory autonomy and independence from induction (Holmberg & Perlmann 2012). The importance of this newly introduced feedback topology is supported by its frequent occurrence in networks maintaining differentiated cell fate (Neph et al. 2012). This suggests that undifferentiated stages and individualized stages are associated with specific regulatory topologies and that development consists of the transitions between these types of topologies.

What does all of this have to do with pleiotropy and its evolution? The developmental transition between topologies described above allows the inductive genes to be involved in phenotypically distinct parts of the organism. The question arises: Do the separate units produced during this process also acquire autonomous variation and thus the ability to respond to selection

independently? If so, principles similar to the ones that govern the decoupling of downstream subnetworks from upstream inducers shared with other traits may be at work over an evolutionary time scale (Pavličev & Widder 2015). Furthermore, different regulatory topologies may enhance or hinder the evolvability of different developmental stages.

Thus, characterizing (*a*) the kind of variation in gene expression that perturbations (mutations) in particular motifs generate (Gjuvsland et al. 2011, 2013; Omholt et al. 2000) and (*b*) variational properties during the transition between motifs (Pavličev & Widder 2015) may be highly informative regarding a motif's evolutionary potential and the evolution of pleiotropy.

Molecular Basis of Single Nucleotide Polymorphism–Derived Variation in Pleiotropy

Insight into mechanisms of pleiotropy evolution can also be gained by exploring the genetic basis of extant variation in pleiotropy. Recently, we analyzed the variation in pleiotropy between fore- and hindlimbs in mice (Pavličev et al. 2013). As mentioned, the fore- and hindlimbs can be seen as reoccurring developmental mechanisms in two different contexts. Many genes involved are therefore pleiotropic, although the two limbs are morphologically divergent. We used the rQTL approach to map genome-wide interactions involved in this divergence. By using our knowledge of polymorphic locations in this particular mouse intercross population, we were able to identify polymorphisms and their functional annotations in the mapped regions. Interactions associated with limb divergence probably occurred between single nucleotide polymorphisms (SNPs) in coding regions (rQTLs) and SNPs in limb type–specific regulatory regions (their epistatically interacting partners). As coding region mutations tend to be more pleiotropic than regulatory region mutations, this result supports the notion that divergence between traits relies on morphologically local modifiers of the shared genes. Such interactions not only create a novel phenotype but also enable the forelimb to vary independently from the hind limb. To the best of our knowledge, this represents the first attempt to directly connect population genetic variation in pleiotropy to its mechanistic basis.

Topology of Pleiotropy Variation Suggests Evolution by Compensatory Changes

As already indicated, mapping studies not only reveal the particular factors involved in variation but also reveal the topology of the GP map. Thus, they invite conjecture about the origins of a particular topology. Similarly, interactions underlying variation in pleiotropy also indicate the possible mechanisms by which it evolves. It has been suggested that variation in pleiotropy involves interactions between globally acting pleiotropic factors and local, trait-specific modifiers that generate specific regional effects (Pavličev et al. 2011b). This topology is congruent with the topology of gene interaction networks that involve widely connected hubs interacting with less-connected local nodes. It is also congruent with gene expression patterns in development: Globally acting genes are crucial in the development of many local traits, although morphology is determined by the interactions of these genes with local contexts (as in body segmentation or limb divergence; Duboc & Logan 2011, Hughes & Kaufman 2002, Mann et al. 2009, Minguillon et al. 2009, Ohde et al. 2013, Ruvinsky & Gibson-Brown 2000).

The origin of such interaction topology may involve the recruitment of factors to local functions, as modifiers of the variation in more globally acting genes. The role of these factors may be to relieve pleiotropic constraint. As pleiotropic genes evolve as a result of selection on some of their functions, local modifications alleviate undesirable side effects of global changes on other functions. Such modifications either buffer or entirely change the uniform function of

global genes. As a result, local modifications generate new trait-specific variational potential in spite of persistent pleiotropic effects, and hence, the interactions represent the actual specific core of the traits. Such compensatory evolutionary dynamics have been suggested multiple times in evolutionary biology (Camps et al. 2007, DePristo et al. 2005, Glass 1957, Johnson & Porter 2007, Lenski 1988, Mayr 1970, Pavličev & Wagner 2012, Tulchinsky et al. 2014, Wright 1968).

On the basis of evidence for compensatory evolution, and the insight that the need for compensation may arise due to pleiotropy, we have developed a selection-pleiotropy-compensation (SPC) model in which a pleiotropic mutation and a mutation alleviating some of its deleterious side effects coevolve as a result of their interdependency (Pavličev & Wagner 2012). We speculate that variation detected in pleiotropy reflects variation in the effectiveness with which locally acting factors alleviate deleterious global genetic effects.

The various consequences of the SPC model have been discussed previously (Pavličev & Wagner 2012). Here, we discuss the SPC model in terms of organismal traits versus overall fitness. Compensatory mutations may not be compensatory for every trait or with respect to fitness. Pleiotropic mutations affect multiple contributors to overall fitness simultaneously, and their effects are not necessarily all concordant. For individual traits, mutations may be advantageous, neutral, or even deleterious, although the net effect on overall fitness may be advantageous (Bullaughey 2013). As a consequence, the same interdependent mutations may manifest in different patterns for different traits: a neutral, so-called permissive mutation, which renders the following mutation adaptive for one trait (Bloom et al. 2010, Bridgham et al. 2009), for example, may manifest as a locally disadvantageous mutation followed by a compensatory mutation from the perspective of another trait. Reconstructing the order and effect of changes relevant for selection would require knowing the selection target. Although we may be able to recognize selection signatures in DNA sequences, it is not straightforward to determine which trait was actually under selection.

Finally, mutations that modify each other's effects with respect to multiple traits not only change the variational relationship between traits but can also be a source of recurring variation in pleiotropy at a later time. This point was explored by Rajon & Masel (2013). Compensatory mutations that alleviate the effects of pleiotropic mutations by locally suppressing/neutralizing them may in the long term provide a source of variation. This is the case if the suppression of pleiotropic effects is perturbed or lost at a later time, revealing the ancestral pleiotropic effect (e.g., Pellmyr & Krenn 2002).

APPLICATIONS

In the previous sections, we discussed patterns and mechanisms of variation in pleiotropy as well as its evolution. Here, we turn to specific scenarios in which the consideration of varying pleiotropy is useful. The topics discussed—sexual dimorphism, the divergence of serial homologs, and the evolution of allometry—all reflect the diversification of organismal parts in the context of shared underlying variation. Additional phenomena also probably follow this scheme, with possibilities ranging from the evolutionary diversification of cell types to the evolution of asymmetries to species divergence arising from divergent G × E effects.

Evolution of Sexually Dimorphic Traits: Decoupling the Variation Between and Within the Sexes

Male and female phenotypes differ substantially in many species. All individuals in a sexual population inherit autosomal loci from both their male and female parents. Even so, the expression of many traits is either limited to one sex or is quite different between the two sexes. Moreover, sexual dimorphism is not limited to the means and variances of dimorphic traits alone but can

also involve their covariances with other traits. As sexually dimorphic traits normally covary with monomorphic traits, covariance patterns can also differ between the two sexes (Lande 1980). It is plausible that the different functions of sexually dimorphic traits in the two sexes mean that their integration with other parts of the body differs. Sexual dimorphism is thus an excellent example of the coexistence of sex- and genotype-specific pleiotropic effects in a population.

Genetically, males and females only differ at SDL, whereas they share autosomal variation. In the absence of linkage disequilibrium with SDL, two general ways to account for sexual dimorphism exist: Either the SDL directly affect trait means, or they provide the genetic background against which alleles at other loci affect phenotypic traits. The latter phenomenon is a signature of G × G interaction (epistasis) and is consistent with the evidence for sex-specific locus effects in association mapping studies (Lawson et al. 2011) and sex-specific effects in developmental genetic studies (Kopp 2011).

Genetic variation in sexual dimorphism within a population is detected by finding a significant gene-by-sex interaction. The presence of such an interaction is evidence for differences between the sexes in the phenotypic expression of a gene or genome. When there is a significant gene-by-sex interaction, we must consider the phenotypic effects of genes separately in the two sexes, treating the dimorphic trait in the two sexes as essentially different but potentially correlated. Instead of sharing a single genetic variance value for the trait, different genetic variance values exist between the two sexes (Willmore et al. 2009). Similarly, genetic covariance values may differ between the sexes.

Traits may be exposed to conflicting selection pressures in males and females; as both sexes are parts of the same interbreeding population, genetic variation in the same trait in the two sexes is interdependent. This is often referred to as intralocus conflict (Bonduriansky & Chenoweth 2009). We expect two sources of constraint to act on sexually dimorphic traits: (*a*) Genetic variation of the trait in question is shared with that of the corresponding trait in the opposite sex, and (*b*) the genetic variation of dimorphic traits is shared with that of other traits in each of the sexes (Gosden et al. 2012). Even if the mechanistic details of sexually dimorphic traits are more complex than described here, sexual dimorphism offers a clear-cut system for studying how genetic interaction modifies pleiotropic effects to release sex-specific variation from within- and between-sex constraints, as only two alternative genetic backgrounds exist.

To understand how epistasis affects the evolvability of a sexually dimorphic trait, we can compare the genetic constraint due to within-sex covariance between male and female traits. Lande (1980) developed a model in which the two sexes are considered subpopulations with their own genetic covariance matrices; the within-sex covariance matrices can be combined to create a composite population-wide genetic covariance matrix. This model formally separates all male and female traits, enabling different selection pressures to be incorporated into the model simultaneously while maintaining the constraints due to covariance between and within the sexes. The total response in each sex is then determined by combining (*a*) the direct effects via selection within the sex and (*b*) the indirect effects due to selection on the opposite sex; this approach allows a convenient parsing of effects when studying sexual dimorphism (Gosden et al. 2012, Steven et al. 2007).

Using Lande's model, the effect of variances and covariances on the evolvability of traits can be measured by estimating the conditional evolvability of sexually dimorphic traits (Jensen et al. 2003, Parker & Garant 2004). Conditional evolvability describes the ability of a trait to respond to directional selection if other traits sharing a portion of its genetic basis are under stabilizing selection (Hansen et al. 2003). A more general measure of conditional evolvability can be calculated by averaging across possible directions of selection.

Thus, the effect of genetic background on average conditional evolvability is simply the difference between the average conditional evolvability of a female and male trait. This effect may be

mediated by the difference in variance of the sexually dimorphic trait (e.g., a female trait displaying less variance than a male trait; Leutenegger & Cheverud 1982). Alternatively, the effect may involve changes in covariances with other traits.

Moreover, the specific effect of epistasis on the constraint due to different covariances with other traits can be determined by contrasting the effect of genetic background (i.e., sex) on a trait's total variance with its effect on the trait's conditional evolvability. The ratio between conditional and total variance (i.e., autonomy; Hansen & Houle 2008) is a meaningful estimate of the relative power of genetic constraint and can be compared across loci and organisms. The difference in a trait's autonomy between the sexes can be interpreted as the effect of interaction between the genes that trait shares with other traits, and SDL, hence the effect of interaction on genetic constraint.

Thus, by combining existing approaches to sexual dimorphism (Gosden et al. 2012, Lande 1980, Wyman et al. 2013) with those for studying the effects of covariance on trait evolvability (Hansen et al. 2003), it is straightforward to capture the overall effect of epistatic loci—whether SDL or others—on genetic constraint. This enables us to think not only about the effects of interaction on the variance of single traits but also about effects of genetic background (here sex) on genetic constraint.

Evolution of Allometry

Huxley (1932) demonstrated that if two parts, x and y, are determined by a common underlying growth factor, their size relationship will be of the form $y = ax^b$, or $\log(y) = \log(a) + b \times \log(x)$. The slope of the regression line, b, is the ratio of the parts' specific growth rates, and a is the intercept. As one of the traits of interest is often body size, this relationship describes how the relative size of a given part changes as body size changes. When the two traits change at different rates (allometric growth), the exponent describing this growth rate relationship differs from one, whereas in isometric growth, the parts change at the same rate ($b = 1$). The remarkable constancy of allometric relationships in populations of individuals of the same age (static allometry) and across taxa (evolutionary allometry) and the inertia of these relationships in the face of artificial selection suggest that allometry reflects evolutionary constraint (reviewed in Voje & Hansen 2013). It is noteworthy that the same static allometric slope can be generated by different combinations of growth trajectories. Thus, static allometric slopes may be attained by coordination between trait-specific growth rates, even if the actual rates change, as occurs in *Drosophila* wing discs (Parker & Shingleton 2011).

How does the evolution of allometry relate to the evolution of pleiotropy? Consider that the allometric coefficient, b, is determined by the ratio of specific growth rates and that growth of any one trait is determined by genetic contributions specific to the trait and by those shared between traits. Let us consider the shared contribution to be that of a pleiotropic gene. Variation in pleiotropy means that allele substitutions change the relative effects on the two growth rates. As a consequence, a greater or lesser part of the traits' growth rates is shared, changing their allometry. If such deviation affects the trait-specific growth rates in a manner that changes their ratio, an alternative allele at a pleiotropic locus would have an effect on the allometric slope. Indeed, genetic variation in allometric relationships has been detected, and the evolution of allometry is rare but documented (e.g., Tobler & Nijhout 2010, Wilson 2013).

Evolution of Trait Individuation

Decoupling of trait variation has been referred to in examples throughout the article, so only a brief account will be given here. Perhaps the most prominent example of the evolution of

pleiotropy is the individuation of repeated (serially homologous) traits. Most organisms manifest some form of metameric body plan (*Bauplan*), in which, to some extent, the same developmental genetic machinery is repeatedly involved in the development of single elements. Vertebrae, vertebrate limbs, and digits are examples of such elements. Phenotypic divergence of these repeated elements from one another, as in the arms and legs of bipedal primates or the long middle finger of the Aye-aye, requires that the elements' genetic bases have diverged. When serially repeated traits share much of their developmental systems, the question arises: How is the variation of the individual elements decoupled? The usefulness of viewing traits as interactive units of shared and specific factors is perhaps the most apparent in this context. However, divergent, repeated traits are just the most extreme case of decoupling because they share so much of their developmental basis. This issue is also important in considerations of homology.

CONCLUSION

Models of evolutionary phenotypic change are as important for capturing the essential ingredients of evolutionary processes as they are for identifying deviations from existing models and opening up new paths of inquiry. The history of evolutionary quantitative genetics offers an example of a succession of models and deviations. Predicting the response of a single trait to selection on heritable variation (e.g., with the simple breeder's equation) revealed the importance of deviations in response due to constraint and correlated responses (pleiotropy) affecting multiple characters. The multivariate breeder's equation addresses these deviations (Hazel & Lush 1942, Lande 1979). This model offers a prediction for one generation of selection, and the long-term deviations have become as interesting as the predictions. Long-term deviations are in part a result of changing gene effects across changing genetic contexts. The dependency of univariate gene effects on genetic context and how interaction effects combine across the genome (Hansen & Wagner 2001) are currently of great interest (Hansen 2013). It seems self-evident that context dependency and its cumulative effects concern multivariate effects as well, and at present this idea contributes much excitement in the field (Jones et al. 2014, Watson et al. 2014).

We think that the next big challenge in evolutionary biology is the integration of well-studied variational concepts with mechanistic detail, in a systems-oriented approach (Dean & Thornton 2007). To bridge the variational and mechanistic fields, it may be necessary to view traits as a result of interactions among multiple genes and their products rather than as the summation of invariant gene effects. Some of these interacting genes are shared with other traits, whether these traits are within one individual (standard pleiotropy), within groups of socially interacting partners, within the two sexes (sexual dimorphism), or expressed in different environments. Across all of these contexts, traits can be compared in terms of the genes they share, emphasizing gene-centric pleiotropy, or in terms of the interesting ways that their genes interact across different contexts. If we decide to focus on the interactions constituting the core of a trait (Wagner 2014), rather than on effects inherent to the shared genes, it follows that the evolution of trait means, variances, and covariances, and perhaps even a trait's origin (Pavličev & Widder 2015), are all consequences of evolving interactions and do not require that we consider distinct processes. In this approach, pleiotropy and constraint are aspects of the GP structure that guide, but do not limit, evolutionary change.

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