doi:10.1111/evo.12687

Commentary: When does understanding phenotypic evolution require identification of the underlying genes?

Mark D. Rausher[1,2] and Lynda F. Delph[3]

[1]*Department of Biology, Duke University, Box 90338, Durham, North Carolina 27708*

[2]*E-mail: mrausher@duke.edu*

[3]*Department of Biology, 1001 East Third Street, Indiana University, Bloomington, Indiana, 47405*

Received May 8, 2015
Accepted May 10, 2015

Adaptive evolution is fundamentally a genetic process. Over the past three decades, characterizing the genes underlying adaptive phenotypic change has revealed many important aspects of evolutionary change. At the same time, natural selection is often fundamentally an ecological process that can often be studied without identifying the genes underlying the variation on which it acts. This duality has given rise to disagreement about whether, and under what circumstances, it is necessary to identify specific genes associated with phenotypic change. This issue is of practical concern, especially for researchers who study nonmodel organisms, because of the often enormous cost and labor required to "go for the genes." We here consider a number of situations and questions commonly addressed by researchers. Our conclusion is that although gene identification can be crucial for answering some questions, there are others for which definitive answers can be obtained without finding underlying genes. It should thus not be assumed that considerations of "empirical completeness" dictate that gene identification is always desirable.

KEY WORDS: **Adaptation, evolutionary processes, gene identification, Mendelian traits, phenotype, quantitative traits.**

Recently, it has been argued that there has been too much emphasis on identifying genes, and even specific mutations, associated with variation in phenotypic traits (Rockman 2012; Travisano and Shaw 2012). In many situations, it is argued, knowledge of the underlying genes may provide little additional insight regarding evolutionary processes contributing to phenotypic evolution. To the extent this is true, the time, effort, and expense required to identify underlying genes may not be justified. On the other hand, we have heard from numerous evolutionary biologists that "completeness" of an evolutionary explanation demands identification of the genes contributing to trait variation (e.g., Lee et al. 2014). It is not clear to us, however, what justifies this argument. In our minds, unless knowledge of underlying genes contributes substantial conceptual insights that could not be gained without this knowledge, the identity of the genes contributes little to scientific explanation or understanding. What is largely missing from discussion of this disagreement is an evaluation of the extent to which, and the circumstances under which, identification of genes underlying variable phenotypic traits may or may not contribute to additional understanding of evolutionary phenomena.

Our goal here is to address this issue. In particular, we offer our view of when "going for the genes" is or is not likely to be scientifically worthwhile. We believe that this discussion is warranted because although a focus on specific genes has yielded tremendous insight into evolutionary processes, we perceive that this success sometimes inappropriately affects attitudes toward, and decisions about, the types of research that is funded and the types of data that is desirable for understanding evolutionary processes. We also worry that a focus on finding "the gene" will reinforce certain ways of thinking about the underlying genetic control of phenotypes to the exclusion of others, and that it may require a degree of mechanistic detail that goes well past the interests of many evolutionary biologists. Our offerings are not intended to be exhaustive. Rather, we provide a discussion of what we believe to be some of the most important situations that evolutionary biologists are likely to encounter. In this vein,

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INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

we believe that it will be especially useful for evolutionary biologists beginning new projects to consider from the outset whether it is likely to be worthwhile to invest in gene and/or mutation identification. This will of course depend upon the evolutionary questions that are the focus of their projects—which is our main point.

Before examining specific situations, however, we wish to comment on one of the reasons given previously for devoting less attention to gene identification: genes detected through quantitative trait locus (QTL) or genome-wide association studies (GWAS) are not representative of variation in general (Rockman 2012). In particular, it is argued that genes identified through these methods are likely to be biased toward variants with large effects on the phenotype, but that variation in most traits is caused primarily by many underlying loci, each with small effect on the phenotype. We agree that there is substantial evidence suggesting that these claims may be true for variation within populations. But it is less clear that they are true for phenotypic differences between populations or species (Orr and Coyne 1992). In plants, many morphological differences between closely related species are a result of differences in one or two genes (Gottlieb 1984). Additionally, quite a few studies have detected QTLs with major effect in between-population or between-species crosses, often explaining individually substantially more than 25% of the phenotypic difference between species (e.g., Bradshaw et al. 1998; Weinig et al. 2003; Goodwillie et al. 2006; Salomé et al. 2011; Slotte et al. 2012; Wessinger et al. 2014; Dittmar et al. 2014). Although the estimated effect sizes of QTLs often tend to be biased upward (Beavis 1994) and QTLs of very small effect can fail to be detected, it is clear that QTLs of major effect are common. Moreover, these QTLs often affect characters we would think a priori are quantitative traits. However, these studies have not usually attempted to follow-up with investigations designed to identify substitutions with small effect. Thus, we have very little evidence about whether the genetic architecture of character divergence is similar to that of genetic variation within populations. In particular, at this point we cannot reject the possibility that moderate- to large-effect QTLs are actually the "stuff" of divergence.

Finally, we wish to emphasize that in many cases, evolutionary biologists are primarily interested in why *phenotypes* evolve. They wish to know whether phenotypic change is adaptive (i.e., caused by natural selection sensu lato, including individual selection, sexual selection, kin selection, and/or group selection), *why* a particular phenotype is adaptive (i.e., what constellation of environmental factors cause a particular phenotype to be favored by selection), and what nonselective constraints (e.g., pleiotropy, correlations with other traits) may prevent "optimal" phenotypes from evolving. We contend that addressing this set of questions can in many cases constitute a complete explanation of the reasons for evolutionary change (or lack thereof), one that requires only that differences in the characters of interest are at least in part caused by underlying genetic differences. The specific genes involved may be of interest for other reasons, but not necessarily for understanding adaptation.

We believe that is nowhere better illustrated than by the Grants' classic work on beak size evolution in *Geospiza fortis*, one of Darwin's finches, on the Galapagos island of Daphne Major (Grant and Grant 1993). They demonstrated that beak size is heritable, and that beak size changed after severe droughts when the population suffered unusually high mortality. They demonstrated the action of selection by showing that birds with larger beaks had a higher probability of survival. Their ecological analysis indicated the drought drastically reduced the number of seeds available, and increased the size of those seeds that remained. All of the evidence together indicated that survivors were those that could eat the larger, available seeds, that this favored individuals with larger beaks, and that differential survival of these individuals resulted in an evolutionary response to selection that increased average beak size in the next generation. This evidence satisfies the three criteria needed to demonstrate the occurrence of evolutionary change by natural selection: (1) trait variation, (2) trait heritability, and (3) a correlation between the trait and fitness (Lewontin 1970). Moreover, it identifies the "agent" of natural selection. This does not mean that nothing of evolutionary interest can be revealed by concentrating on genes involved—indeed, a recent phylogenomic analysis has shown that the ALX1 haplotype in *G. fortis*, which is associated with change in beak shape, was probably introduced into this species by hybridization and contributed to the adaptive change in beak shape (Lamichhaney et al. 2015). This study tells us the source of some of the variation involved in the adaptive change, but says little about why that change was adaptive.

Situations in Which Gene Identification May Contribute Little Conceptually

In this section, we identify situations in which complete or nearly complete explanations of evolutionary processes *may* be obtained without resort to identifying the genes responsible for trait differences. We do *not* assert that this will always be possible in these situations. For example, if for a particular organism fitness cannot be assessed reliably in the field for different genotypes or nearly isogenic lines (NILs), it may not be possible to directly detect selection acting on a trait. Determining whether trait divergence is adaptive may then require detecting signatures of selection acting on genes causing that divergence (see below). Instead, we are asserting that in many cases, with many organisms, recourse to gene identification *may not be necessary* to answer specific questions that can be answered more easily in other ways.

EXPLAINING EVOLUTIONARY CHANGE AND DIVERGENCE IN QUANTITATIVE TRAITS

Two fundamental questions are relevant to understanding the evolution of all quantitative traits: (1) is it adaptive, that is, caused by natural selection, or neutral, caused by genetic drift; and (2) if adaptive, why is it adaptive, that is, what are the environmental conditions or selective agents that generate the relationship between the trait and fitness? A number of approaches short of gene identification can often be used to answer both questions for particular quantitative traits or suites of traits. One approach is to directly quantify selection acting on traits. Lande and Arnold's (1983) demonstration that this could be done by the straightforward method of quantifying the relationship between the value of the trait and fitness stimulated literally hundreds of such investigations.

An elaboration of this approach complements studies in natural field sites with genomic analysis—it links genotype and phenotype in natural settings, allowing an investigation of the genetic architecture of adaptive divergence in relevant natural environments (Anderson et al. 2013). The approach is as follows: reciprocally transplant individuals that are the product of controlled crosses into divergent natural field sites, genotype them to create a genome-wide linkage map, phenotype the individuals for one or more fitness-related traits, and conduct a QTL analysis to identify chromosomal regions contributing to phenotypic differentiation and/or fitness (e.g., Verhoeven et al. 2004, 2008; Gardner and Latta 2006; Lowry et al. 2009). Although specific genes are not identified with this approach, in his paper entitled "Believe it or not, QTLs are accurate!" Price (2006) makes the point that the QTL approach is a truly comprehensive way to identify genomic regions coding for *multigenic* traits that contribute to adaptive variation (see also Martin and Orgogozo 2013). In other words, the QTL approach is a powerful one, although large mapping populations are required to minimize bias associated with estimates of QTL properties.

This approach is available for organisms that are amenable to field experiments in which fitness can be measured. Although this includes many plants, it cannot be used for many, if not most, animals. However, other approaches are available that do not require direct fitness measurement. One such approach is to compare divergence in the trait with divergence in presumed neutral markers, the Qst-Fst approach (Lande 1992; Spitze 1993; Whitlock 1999, 2008; LeCorre and Kremer 2003). This approach has provided evidence of divergence as a result of selection on a variety of different quantitative traits (e.g., Spitze 1993; Steinger et al. 2002; Palo et al. 2003; Streisfeld and Kohn 2005; Duncan and Rausher 2013), including in traits for which only one sex is under selection (Yu et al. 2011).

Finally, a third approach is to examine the direction of substitution of trait QTL alleles. In particular, if divergence between populations or species has been driven by selection, most of the QTLs are expected to be in the direction of the difference in species means: if species 1 has a higher mean, then most QTL alleles in species 1 should increase the value of the trait. By contrast, if genetic drift is responsible for divergence, only half of the QTLs are expected to show this pattern (Orr 1998; Rieseberg et al. 2002).

These types of analysis can detect whether selection contributes to trait evolution, but they cannot usually reveal why selection occurs. For this, experiments that manipulate specific environmental factors to determine whether the pattern of selection is affected, and whether this effect is mediated through a particular trait, are required (e.g., Wade and Kalisz 1990; Dudley 1996; Mauricio and Rausher 1997). Typically, these types of experiments do not require knowledge of the underlying genes.

DETECTING TRADE-OFFS

Evolutionary biologists are often interested in the extent to which local adaptation involves alleles with opposing effects in different environments (trade-offs) versus alleles that are beneficial in one environment and neutral in others (Rausher 1984; Futuyma and Moreno 1988; Via and Hawthorne 2002; Hall et al. 2010). A combined field/QTL approach can allow one to distinguish between these two scenarios without identifying specific genes that may be involved in the trade-off. This approach involves first identifying QTLs that affect divergence in a trait. Each QTL has an allele characteristically found in one environment and another allele characteristically associated with a second environment. The fitness of genotypes homozygous for each allele and with the genetic background randomized is then quantified in the two environments. Under the trade-off scenario, native alleles are always favored relative to nonnative alleles. In other words, alleles have a positive effect in the environment in which they are normally found, but a negative effects in the other environment (e.g., Li et al. 2003). Alternatively, with conditional neutrality alleles are either beneficial in their native environment but neutral in the other, or are neutral in their native environment but detrimental in the other environment (e.g., Verhoeven et al. 2004; Lowry et al. 2009).

EXPLAINING EVOLUTIONARY CHANGE AND DIVERGENCE IN MENDELIAN TRAITS

There are a number of circumstances in which identifying the underlying genes is not necessary for understanding of the evolution of Mendelian traits.

Maintenance of polymorphism

When segregating phenotypes conform to a Mendelian model, it is possible to manipulate allele frequencies and quantify genotype fitness without knowledge of the specific gene involved. In

principle, by doing so one can determine both whether balancing selection acts on the locus, as well as the form of balancing selection (heterozygote advantage, frequency-dependent selection, spatially or temporally heterogeneous selection, balance between selective forces acting at different life stages, etc. (see reviews in Mitchell-Olds et al. 2007 and Delph and Kelly 2014). In practice, evolutionary biologists have achieved this for a variety of traits (e.g., Halkka et al. 1975; Hori 1993; Sandoval 1994; Sinervo and Lively 1996; Subramaniam and Rausher 2000; Gigord et al. 2001; Bright and Rausher 2008; Ercit and Gwynne 2015). Moreover, through experimental manipulation of either phenotype or environment, it is possible both to identify the agent(s) of selection and confirm the phenotypic target of selection. Again, this has been achieved repeatedly by evolutionary biologists. Knowing the pattern of selection acting on a Mendelian locus as well as the environmental factors that generate that pattern of selection provides a comprehensive evolutionary explanation of the maintenance of a polymorphism, to which identification of the specific gene involved, let alone the particular single nucleotide polymorphism (SNP) or indel, adds little or nothing.

This field-based approach is likely to be successful only when allelic effects on fitness are substantial. When they are not, field experiments may not be sufficiently sensitive to detect fitness differences among genotypes. In this situation, identification of the gene involved allows examination of patterns of nucleotide variation that may reveal signatures of balancing selection (e.g., Kreitman and Hudson 1991; Roux et al. 2012). In addition, because signatures of balancing selection integrate the pattern of selection over long periods of time, they may capture the effects of episodic balancing selection that might not be detected in field experiments that examine selection for only one or a few generations. On the other hand, if balancing selection has arisen recently (e.g., Fishman and Saunders 2008), there may not have been time for divergence between alleles to build up to result in a recognizable signal of balancing selection. If so, measurement of fitness effects in the field may be the only way to detect it. On balance, because field experiments will usually be less expensive and produce results sooner than first identifying the gene involved, it will often be appropriate to attempt to detect balancing selection using such experiments first. If an unequivocal result is obtained, there is no need to proceed to identify the gene *to explain why the polymorphism persists.*

Evolutionary divergence

In similar fashion, it is in theory possible through a combination of gene-frequency and experimental manipulations to provide a complete evolutionary explanation of why a Mendelian trait has diverged between two or more populations or species. Such an approach typically involves standard reciprocal-transplant experiments using isogenic lines, which are created by introgressing each allele into the opposite genetic background (e.g., Bradshaw and Schemske 2003; Hall et al. 2010; Mojica et al. 2012). Contemporaneous experiments that manipulate environmental factors can be used to identify agents of selection, as noted above.

One potential limitation of this approach is that it assumes that the environments present today are the same ones that existed when the traits diverged. If divergence is recent, this may be a reasonable assumption; it is less so if divergence is not recent. Another potential limitation of this approach is that introgression of a particular gene will drag with it alleles of linked loci that have diverged between populations. Fitness effects of variants of the focal trait will then be confounded with fitness effects of the linked loci. During introgression, the size of the linked segment will decrease each generation. However, the length of this segment decreases as $1/n$, where n is the number of generations of introgression (Naveira and Barbadilla 1992), which means that even after 10 generations (most isogenic lines used in this type of experiment are introgressed for four to six generations), on average a segment of 5 centi-Morgans on each side of the focal locus will be present. Such a segment will typically contain hundreds of genes whose effects could be confounded with that of the focal locus. One possible way around this difficulty is (1) to determine whether the agents of selection directly target the focal trait that has been introgressed (Campbell 2009; Hopkins and Rausher 2012); and (2) by experimental manipulation of the selective agents, estimate the magnitude of selection imposed by those agents (e.g., Mauricio and Rausher 1997; Rutter and Rausher 2004). If the magnitude of selection imposed by the agents is comparable to the magnitude of selection measured on the trait, it is unlikely that linked genes are responsible for the observed fitness differences.

Currently, we have little information on how much of a problem this is likely to be. Ironically, techniques such as marker-assisted selection (Lande and Thompson 1990) and recombinant progeny testing (Keightley and Christians 2004) may be required to narrow down the size of the introgressed segment to essentially that of the focal gene itself to confidently eliminate any effects of linked genes. At this point, however, the gene will have essentially been identified.

An alternative approach to detecting whether divergent selection has acted on differences among populations is to compare patterns of allele frequency differentiation between the focal trait and neutral markers using a Qst-Fst approach, as mentioned above. Coupled with ecological analysis of the causes of selection, this approach can provide a complete picture of the processes that are responsible for divergence without needing to identify the genes underlying the trait of interest.

The invisible fraction

It could be argued that there is a purely practical advantage to identifying the gene associated with Mendelian phenotypic variation: it allows one to identify an individual's genotype for the trait early in that individual's lifetime. This identification in turn allows genotypic mortality (a fitness component) to be determined for traits that appear late in an organism's lifetime (e.g., reproductive traits), the so-called "invisible fraction" (Grafen 1988; Bennington and McGraw 1995; Mojica and Kelly 2010). However, in most cases this can be accomplished by identifying a closely linked marker locus and scoring this locus as a surrogate for the causal locus. As long as reciprocal crosses are used to generate associations between the markers and the trait in different sets of individuals, confounding effects of the marker itself and loci linked to the marker can be accounted for statistically. The often substantial extra effort needed to identify the gene underlying the polymorphism would thus be unnecessary.

Situations in Which Gene Identification Is Justified

Although we argue above that there are likely many situations in which gene identification is not expected to contribute substantially to understanding the evolution of phenotypes, there are clearly others in which this is not true. In this section, we briefly discuss some such situations.

ANY STUDY OF MOLECULAR EVOLUTION

The field of molecular evolution asks questions about processes responsible for the evolution of individual genes and their protein products. Typical issues include determining whether substitutions were caused by natural selection or genetic drift (McDonald and Kreitman 1991), whether sequence variation is actively maintained by selection (Kreitman and Hudson 1991), whether order of substitution is constrained by epistatic interactions (Weinreich et al. 2005), how frequently adaptation requires prior permissive substitutions (Ortlund et al. 2007; Bloom et al. 2010), and whether certain fates of duplicate genes are more common than others (Lynch and Force 2000; Duarte et al. 2006). It almost goes without saying that because of its focus on individual genes and gene families, prior gene identification is by definition required.

DRIFT VERSUS SELECTION

We argued above that whether populations have diverged for a trait due to selection or drift can often be determined by field experiments that measure selection on traits in different environments. For many organisms, however, this will not be possible because field experiments are not feasible. Moreover, it is likely that in many situations, selection may operate only intermittently, making its direct detection difficult. In these situations, identifying the gene(s) involved in trait divergence may allow the detection of various signatures of past selection.

This approach is not without limitations of its own. For example, one signature of past selection is a marked decrease in genetic variation in the region surrounding the target of selection due to a recent selective sweep (Nielsen 2005). It must be remembered, although, that if the selective sweep that caused the evolution of the interacting loci is older than approximately N generations, where N is population size, then the signature of the sweep likely will have disappeared (Przeworsky 2002). Another indication of past selection is a dN/dS ratio greater than 1. However, if only a few adaptive substitutions have occurred, this approach may not have sufficient power to detect them. This may be especially problematical for highly polygenic traits, in which substantial divergence in the mean of a trait may be accompanied by only minor changes in gene frequencies at numerous loci, with no fixations occurring (Berg and Coop 2014).

We also argued above that in many instances, divergent selection on a Mendelian trait leading to local adaptation can be demonstrated by comparing, along transects or among populations, gene frequencies for that trait and neutral markers. Gene identification is not necessary for this because gene frequencies can be determined by test-crossing randomly sampled individuals. However, this approach is generally not available for detecting balancing selection; although test crosses can reveal an excess of heterozygotes, other mechanisms besides balancing selection (e.g., disassortative mating, the Wahlund effect, directional selection) can also produce this result. And although there are experimental means of detecting balancing selection through fitness measurements (Bright and Rausher 2008) or perturbation experiments (Eckert et al. 1996; Subramaniam and Rausher 2000; Gigord et al. 2001; Levitan and Etges 2009), results may often not be conclusive for at least two reasons: (1) the magnitude of balancing selection may be too weak to detect with even large field samples; and (2) balancing selection may act only intermittently and thus may not be detected in short-term field studies. In these situations, identifying the gene may be a necessity. Doing so could allow detection of a signature of increased variation between regions surrounding the selected site, indicative of balancing selection (Kreitman and Hudson 1991).

Identification of specific genes underlying trait variation may thus potentially provide opportunities for distinguishing between selection and drift as causes of that variation in cases where other approaches are not available. Of course, demonstrating selection in this way usually provides no information about the causes of selection, or, in the case balancing selection, what form it takes (e.g., frequency-dependent selection, heterozygote superiority, opposing selection in haploid and diploid phases of the life cycle).

ANALYSIS OF PARALLEL EVOLUTION

A commonly asked question in evolutionary biology is whether instances of adaptive parallel phenotypic evolution are underlain by parallel genetic evolution, that is, changes in the same genes or at the same nucleotides (Arendt and Reznick 2008; Stern and Orgogozo 2008; Kopp 2009; Conte et al. 2012). In theory, parallel genetic evolution can be ruled out without identifying the specific genes contributing to the novel phenotype. In particular, a combination of QTL mapping and synteny analysis of QTLs in lineages that have independently evolved the same phenotype can show that the causal genes from one lineage are not the same as those in another lineage (Pascoal et al. 2014). By contrast, demonstrating that the same gene is involved in different lineages is more difficult. When lineages are not too divergent, complementation tests can demonstrate the involvement of the same genes (e.g., Cresko et al. 2004), although they usually cannot determine whether the same substitutions are involved. The latter may be crucial if one is trying to understand whether parallel evolution is a result of fixation of the same original mutation in different populations through gene flow, or to fixation of independent mutations (e.g., Colosimo et al. 2005).

In many organisms, complementation tests may not be possible because of an inability to cross distant lineages. In these cases, one possibility might be to take QTL co-localization as an indication of similar genetic change underlying parallel phenotypic change. However, because numerous genes typically underlie a QTL, simply demonstrating QTL overlap in a syntenic region is not sufficient to conclude the same gene is involved. Instead, the gene involved in each lineage must be determined either by fine mapping or some functional test. Moreover, distinguishing between the possible causes of parallel genetic evolution—differential mutation rates or differential pleiotropy among mutations in different genes that can produce the same novel adaptive phenotype (Steisfeld and Rausher 2011)—requires comparing mutation rates and magnitudes of deleterious pleiotropy in those genes, which is not possible without knowing the genes involved.

A related issue is that there are a number of situations in which one might want to distinguish between whether adaptive traits shared by different populations or species represent independent or shared genetic origins (e.g., Wu et al. 2013). A trait shared by one species and only some populations of a sister species may have arisen through (1) independent mutations in the two species; (2) independent lineage sorting of an ancestral polymorphism; (3) introgression (including horizontal transfer) from the first species into only some populations of the second; (4) derivation of the first species from one or more populations of the second species with the same trait; or (5) common origin in the two species but loss of the trait from some populations of one of the species. Because each of these possibilities has different implications for the phylogenetic relationships among alleles of the causal gene(s), and because these alternatives are independent of the overall genetic similarities of the populations and species involved, only analysis of the relatedness of the trait gene(s) can resolve this issue. An example is provided by the repeated fixation (presumably adaptive) of the same alleles for armor plate reduction in sticklebacks in North American lake populations (parallel genetic evolution), but fixation of a different allele in Japanese populations (nonparallel genetic evolution; Colosimo et al. 2005). Clearly being able to make this distinction requires identification of the gene(s) involved.

UNDERSTANDING ASYMMETRIES IN EVOLUTIONARY TRANSITION RATES

Many traits exhibit strong asymmetries when mapped onto phylogenies: Rates of transition in one direction are much greater than rates of change in the reverse direction (reviewed in Goldberg and Igic 2008). A number of alternative explanations can be offered for such asymmetry:

(1) Transitions in one direction tend to involve gene or pathway degeneration, caused by the accumulation of loss-of-function mutations, making genetic reversal difficult. This is the common explanation for Dollo's law, which states that complex characters, once lost, cannot be regained (Bull and Charnov 1985; Marshall et al. 1994). One example is shifts in flower color. In some taxa, loss of floral pigmentation or shifts from blue to red pigments occurs at higher frequencies than the reverse transitions (Rausher 2008). The forward transition is often accomplished by functionally inactivating a single gene (Rausher 2008), which then undergoes functional degeneration through the neutral accumulation of redundant inactivating mutations (Wessinger and Rausher 2015). Such degeneration likely makes reverse transitions highly improbable and contributes to the asymmetry in transition rates.

(2) Multiple adaptive substitutions in a particular gene render adaptive reversal impossible because of epistatic interactions among the substitutions. For example, Bridgham et al. (2009) demonstrated that the evolution of novel function in the vertebrate glucocorticoid receptor involved several substitutions that destabilized portions of the protein structure necessary to perform the ancestral function. Reversing these substitutions alone does not improve ancestral function. But unless these substitutions were reversed first, reversing the substitutions that actually changed substrate specificity was maladaptive. Genetic reversal is thus very unlikely because it would require simultaneous substitutions at multiple sites, many of which are nonadaptive.

(3) Transitions may occur less frequently in one direction because there are fewer opportunities. Thomson and Wilson (2008), for example, argue that transitions from hummingbird to bee pollination in *Penstemon* may occur less frequently than the

reverse transition because habitats with hummingbirds are less common than habitats with bees.

Distinguishing among these competing hypotheses requires evaluating hypotheses (1) and (2), which clearly requires identifying the genes involved in the evolutionary transitions.

EVALUATING THE CAUSE OF TRAIT–TRAIT AND TRAIT–FITNESS CORRELATIONS

Evolutionary biologists often want to know whether genetic correlations are caused by linkage disequilibrium or pleiotropy. There are three approaches to addressing this issue that do not involve knowledge of specific genes. One is determining whether fitness/character correlations can be broken, either by generating numerous inbred lines to see if the effects can be separated (Jinks et al. 1985) or by crossing populations for a number of generations to see if the correlation between two traits declines (Conner 2002). A second is artificially selecting directly on the correlation, rather than the means of two traits, to see whether selecting to reduce the correlation itself results in its decline (Delph et al. 2011). A third is determining if QTLs affect single or multiple characters (see David et al. 2013 for a review; Via and Hawthorne 2002). However, these approaches may be problematic if there is tight linkage between loci separately affecting correlated traits. In this situation, fine-mapping QTLs, although usually requiring substantial effort, can demonstrate that multiple loci are involved, even if neither locus is specifically identified (e.g., Wright et al. 2013). When candidate genes are available, one may employ transgenic manipulations (e.g., ectopic expression, gene knockout, CRISPR) that could distinguish between pleiotropy and linkage disequilibrium (i.e., does manipulation of one gene alter both characters in the direction of the observed correlation?; e.g., Mills et al. 2014). However, if there are many loci associated with a particular QTL, testing all candidates may be problematical. Moreover, it is always difficult, and usually impossible, to obtain permits for testing the effects of genetically modified organisms in a natural setting, which is required if fitness effects are to be realistically evaluated. Because of these limitations, fine mapping may be a more straightforward approach to determine whether different genes are involved. Moreover, it has the added advantage that, if ultimately taken to the level of individual genes because the apparent pleiotropy persists at larger scales, a gene that contributes to the trait correlation will be identified.

COSTS OF ADAPTATION

An issue related to point 5 is whether traits variable within populations have fitness costs associated with them. For example, this has been a central question in understanding the evolution of resistance and tolerance (Tiffin and Rausher 1999; Tian et al. 2003) and of ecological specialization (Futuyma and Moreno 1988). For quantitative characters underlain by several to many variable loci, addressing this issue can usually be accomplished successfully simply by using the appropriate breeding design and testing for a negative correlation between fitness and the trait in the appropriate environment (e.g., for resistance, in the absence of pathogens, herbivores, pesticides, etc.). However, if the trait is Mendelian or oligogenic, this approach may not be adequate. If the trait is newly arisen and has undergone a partial sweep, then the locus may be in linkage disequilibrium with other closely linked loci that affect fitness. Such linkage disequilibrium could generate the appearance of a cost, or even absence of a cost. As in section Evaluating the Cause of Trait–Trait and Trait–Fitness Correlations above, identifying the gene and using transgenic approaches to change or modify it to alternate alleles would control for linked loci and directly assess whether a cost is associated with the focal trait.

SELECTION-COMPONENT ANALYSIS IN UNDISTURBED NATURAL POPULATIONS

Although not widely used today, selection-component analysis (Christiansen and Frydenberg 1973) permits the estimation and decomposition of overall selection on a Mendelian trait into life-cycle components in unmanipulated natural populations. It has the advantage of avoiding artifacts introduced by experimental manipulation. It is performed by quantifying allele frequencies in samples collected from successive life-cycle stages (including gametes, if possible, such as in broadcast spawning organisms). Such an analysis clearly requires knowledge of the identity of the locus involved because the trait will not normally be expressed in all life stages. Although in principle one could determine allele frequencies at each life stage by progeny testing, such an approach will usually be prohibitively laborious compared to simple allele-scoring by techniques such as PCR and CAPS (cleaved amplified polymorphic sequence) or SNCP (single nucleotide conformational polymorphism) analysis.

Concluding Thoughts

Evolution is a genetic phenomenon. It occurs when the genetic composition of a population changes. Not surprisingly, then, the analysis of variation in particular genes has revealed much about how evolution operates. As we have outlined above, some important questions about evolutionary processes can be answered only by identifying genes involved in evolutionary change. Tremendous progress has been made in understanding, for example, the roles of selection versus drift, evolutionary constraints, the importance of regulatory rewiring and gene duplication to the generation of novelty, and other important evolutionary issues by studying particular genes. It is no coincidence that this progress has come largely over the last three decades, which are coincident with the development of gene and genome sequencing technology.

At the same time, evolution is an ecological phenomenon. The essence of adaptive change can only be grasped by uncovering the interactions between a population and its environment that generate natural selection. Moreover, the form of that selection has tremendous consequences for understanding the preservation of genetic variation within and among populations and species. Unfortunately, the prodigious effort that has gone into documenting patterns of variation in genes and genomes, which has clearly shown that natural selection is responsible for a great deal of genetic change, cannot in most cases tell us *why* that genetic change has occurred. For this, we must understand the ecological processes responsible for natural selection on traits and genes. As we have attempted to document above, describing these processes are legitimate ends in themselves, and may constitute a complete explanation of evolutionary change without recourse to identifying the particular genes involved.

In view of these considerations, the question of whether to "go for the genes" is answered by the evolutionary phenomenon that one is investigating. One should not assume either that gene identification is always necessary, or that it will not illuminate the phenomenon under study. The decision needs to be made on a case-by-case basis, based on whether knowing the gene sequences involved will significantly enhance understanding of the phenomenon being studied. Many evolutionary biologists have been asked at one time or another why they have not tried to determine what genes underlie the evolutionary issues they examine. In our view, a legitimate answer is that doing so would not significantly enhance our understanding of those issues. Given the vast range of issues knowledge of the relevant genes can address, however, in providing that answer, there are situations in which one must be able to articulate why discovering the genes is indeed irrelevant.

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Associate Editor: R. Shaw
Handling Editor: R. Shaw