Reuniting Phenotype and Genotype in Biodiversity Research

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Using phenotypes to explore and describe biological diversity has become less popular than using genetics to do so. Results from the two approaches often conflict at the species level and below, the very ground floor of biodiversity. However, because in today's data sets phenotypic divergence is probably driven mostly by selection and genetic divergence by stochastic processes, we should not expect them to be tightly coupled at population-to-species evolutionary depths. For heuristic purposes it is useful to consider phenotypic and genetic data as largely unidimensional axes in an inherently multidimensional process, and this is perhaps the source of the controversies surrounding each approach. Phenotypic and genotypic data sets might give very different portrayals of evolutionary trajectories in adaptive and nonadaptive space. Integrating these data sets provides a roadmap for theoretical and empirical research. The exploration of the multidimensional relationships of the two types of differentiation in diverging populations is providing important insights both into the units of biodiversity and into the processes responsible for their generation.

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Taxonomic assessments have historically been made on the basis of organismal phenotype and, since the modern evolutionary synthesis, on an assumption of a genetic basis for the phenotypic characters used to make taxonomic determinations (Mayr 1963, 1969). The phenotype of "traditional" characters has generally been considered to be the product of selection, and phenotypic divergence among populations (or subspecies, etc.) at and below the level of species is usually considered to represent a process of selection: nonrandom, differential reproductive success among differing genotypes. Genetic variation within species, on the other hand, is largely presumed to be neutral or near neutral, at least as interpreted from the data sets most common today (Kimura 1983, Ohta 2002). Consequently, genetic divergence among populations (or subspecies, etc.) throughout most of the genome is likely to be a result of stochastic processes.

In both types of data, there are open questions regarding how much influence selection may have in genetic data sets and how much neutral processes might influence inter-population divergence in phenotypic data (this is discussed below). However, few researchers disagree with the general assessment that in the data sets systematists and taxonomists currently use, phenotypic divergence between populations is driven mostly by selection and genetic divergence mostly by stochastic factors. This heuristically useful generalization is a consequence of the facts that morphological characteristics are exposed to natural selection and a great deal of genetic variation is not (e.g., DNA mutations that do not cause protein-coding changes). There is no reason to suppose that at shallow evolutionary depths these two fundamentally different processes—divergence by selection versus divergence by stochastic factors—should ever be tightly coupled. Yet, despite these considerations, it has become rather common to attempt to use genetic measures, such as percentage divergence or reciprocal monophyly (two groups monophyletic with respect to each other), often using data from just a single locus, to come to taxonomic conclusions at the levels of species or subspecies (e.g., König et al. 1999, Hebert et al. 2004, Zink 2004; see also Hickerson et al. 2006, Meier et al. 2006, Phillimore and Owens 2006).

Genetic data have tremendous value in systematics and population genetics, largely because most of the variation in today's data sets is selectively neutral and consequently provides relatively clear reconstructions of phylogenetic relationships, relative timing of divergence, and gene flow. But neutral characters are not drivers of evolutionary divergence in the process of speciation. The genetic bases of phenotypic differences between populations are almost certainly not included in most biodiversity data sets; this is both a strength, because it helps us recover population history, and a weakness, because it may or may not reveal concordant patterns with phenotypic differences. It is thus inappropriate to consider our usually very small genomic data sets (with variation dictated largely by evolutionarily neutral, stochastic

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processes) to represent a definitive assessment of species status or subspecies validity, or to erect new taxa solely on the basis of these data. Moreover, because the two processes generating variation in phenotype and genotype should be expected to be decoupled at shallow levels of genomic divergence, strictly genetic assessments of shallow taxonomic units are likely to be looking in the wrong place: We expect time to have differential effects in the two very different regimes of stochastic versus selection-driven divergence.

For example, selection might drive the rate of phenotypic change much more rapidly than the rates of mutation and gene-lineage sorting between two diverging populations. Or, conversely, a lack of divergent selection (or canalization—selection for a particular phenotype despite underlying genetic variation) might cause phenotypic change to be slower than the accumulation of genetic divergence between populations. As a consequence, a sister pair of recently diverged but genuine species could theoretically differ very simply in one critical gene and be virtually identical in the rest of the genome; or another putative pair of species could exhibit seemingly deep neutral genetic divergence at some loci and yet interbreed freely with no hybrid disadvantage where their ranges overlap. Regardless of one's concept of species, these simple theoretical scenarios illustrate the likelihood that at shallow evolutionary depths—populations to species—our phenotypic and genotypic data sets might give very different portrayals of evolutionary trajectories in adaptive and nonadaptive space.

Genetics tools are very useful, but using genetic data without considering phenotype or traits that are under selection should be understood to provide, in effect, a unidimensional view of a multidimensional problem. Perhaps most important, recovery of a single-locus (or other simple genetic) history should not be viewed as a full representation of the process of evolutionary divergence. Selection quite likely plays a much larger role than neutral genetic phenomena in this process (Coyne and Orr 2004).

The multidimensional space of the speciation process

When we consider that the process of differentiation involves both phenotype and genotype, we can use the generalizations described above to visualize a process space and begin to better integrate data from each to understand not only the process but also its evolutionary products. Moritz (2002) used this approach to emphasize the importance of including evolutionary processes in biological conservation (figure 1), and it can also be used to improve our understanding of the speciation process itself.

A gross theoretical division of this speciation process space into coupled and uncoupled rates of divergence in genotype and phenotype is helpful when considering routes to (or modes of) speciation (figure 2). Such a simple consideration of the multidimensional aspects of differentiation offers insight into the speciation process—particularly into how it might vary among taxa or across geographic or ecological space. When, where, and in what taxa speciation is driven largely by adaptive divergence as opposed to mostly neutral divergence are focal questions in speciation research (Schluter 2000, Coyne and Orr 2004). So while we recognize that phenotype can be a misleading indicator of relationships—this is why DNA data have become so important for determining evolutionary history—it is important to revisit inclusion of the phenotype. Large-scale data sets such as species- and subspecies-level taxonomies and single-locus genetic data sets enable us to make rapid and profound progress in this area, but this integration has yet to effectively occur at the broad scale that is needed.

Subspecies and DNA barcoding: Effectively unidimensional approaches

Let us consider two areas of descriptive biodiversity research that are important but incomplete in their oversimplification of the processes of differentiation.

Subspecies and DNA barcoding are each controversial topics. Both are fraught with problems vociferously debated in the literature (e.g., Hebert et al. 2004, Moritz and Cicero 2004, Zink 2004, Hickerson et al. 2006, Phillimore and Owens 2006, Rubinoff et al. 2006). Yet each has value to many researchers. The naming of subspecies through formal, trinomial nomenclature attempts to describe the geographic partitioning of phenotypic variation below the species level. Few would deny that the study of subspecific variation has played an important historic role in organismal and evolutionary study, just as few would deny that the application of subspecies status to minor geographic variants went too far in many cases. DNA barcoding adopts a single-locus, sequence-based approach to the genetic description of biodiversity. Using mitochondrial DNA (mtDNA) sequence data (in animals) has had great value in biodiversity research in past decades, but few would deny that the DNA barcoding initiative is not a panacea or that early promises—such as the discovery of new species using these data alone—were overreaching. (I use the term "DNA barcoding" in its broad sense, not restricting it to a portion of the mtDNA *COI* gene in animals; see box 1.)

For better or worse, each of these approaches focuses largely on only one of the dimensions of differentiation illustrated in figure 1. It could be argued that a substantial portion of the controversy surrounding each is that they do not adequately represent the multidimensional space of the evolutionary processes involved in differentiation. In other words, much of the disagreement about these topics might be considered a clash of different unidimensional approaches against an inherently multidimensional problem.

Both approaches also suffer from the risks of sampling error, although this is not often discussed (but see Funk and Omland 2003, DeSalle et al. 2005). Historically, many described subspecies have been synonymized ("lumped") when increased sampling has revealed a more complete picture of individual variation and how it is partitioned (e.g., by showing a species' variation to be smoothly clinal). Similarly, we can expect that many reciprocally monophyletic mtDNA

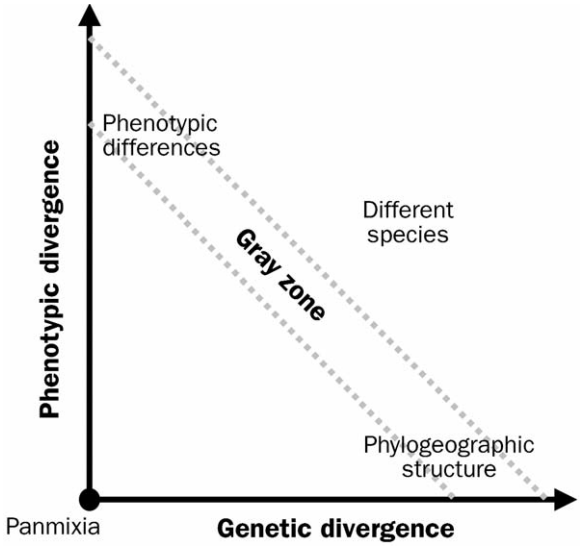

Figure 1. Speciation process space in two dimensions, considering the largely adaptive axis of phenotypic divergence and the largely neutral axis of genetic divergence (adapted from Moritz 2002, and based on traditional biodiversity data sets). Differentiating units progress from panmixia (total genetic admixture) to speciation in this space. The gray zone indicates variation among species concepts about when differentiating units become full species during this process.

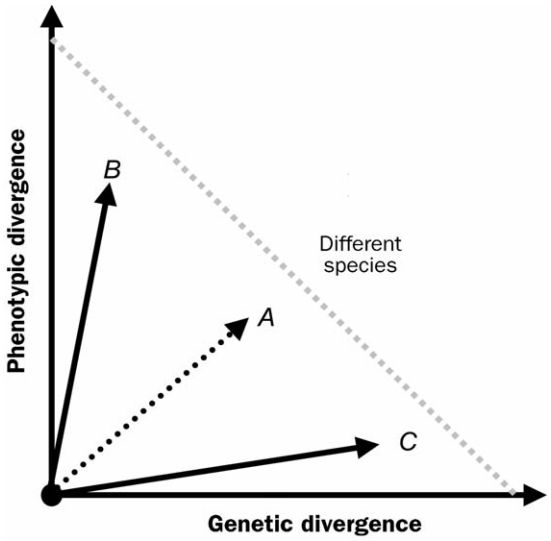

Figure 2. Different routes to speciation possible in the process space of figure 1 (with conceptual variation of "species" arising from different species concepts being collapsed). Route A (dotted line) occurs when phenotypic and genotypic divergence rates are coupled. Differentiation driven largely by adaptive divergence is depicted by route B, whereas that driven by largely neutral divergence is depicted by route C. Route A is dotted because evidence in birds (Zink 2004, Phillimore and Owens 2006) suggests that these two divergence rates are not usually coupled.

clades (or otherwise character-based gene clades; see, e.g., Rach et al. 2007) that are recovered from the small sample sizes typical of most phylogeographic studies today will no longer be monophyletic as sampling increases. This is not only a phenomenon of random branching of genealogical lineages, as recently treated by Rosenberg (2007). It is also one of spatial coverage and of gene flow between diverging populations.

Insofar as gene flow at even relatively low levels can prevent speciation (Rice and Hostert 1993, Hostert 1997), and its effects are highly nonlinear (Cabe and Alstad 1994), sampling theory becomes very important when interpreting results describing the distribution of genetic variation. For example, 10 individuals each from two populations is a good sample

Box 1. DNA barcoding.

Sequencing the base pairs of all or part of a gene from animal mitochondrial DNA (mtDNA) or plant chloroplast DNA has been a mainstay of systematics and phylogeography for decades. The goal is to reconstruct the evolutionary history of biotic lineages, and these genetic markers often prove to be more accurate in determining these histories than phenotypically based characters. Recently, the term "barcoding" has been applied to these techniques for their utility in identifying samples—diagnosing what an unknown organism is by comparing its DNA sequence to a digital library of the DNA of the world's organisms. That library is under construction using a standardized set of markers to enable global use (*www.barcoding.si.edu*). In animals, for example, the marker chosen is 648 base pairs of the cytochrome *c* oxidase subunit 1 gene (*COI*), found in the mtDNA of all animals.

Controversy appeared early in the barcoding program, however, when those promoting the use of mtDNA sequence data in this narrower sense viewed its utility as going beyond simple diagnostics, and also promoted its use for discovering new species (e.g., Hebert et al. 2004, Moritz and Cicero 2004). Because species are not readily definable using only a small fragment of DNA and simplistic rules for its interpretation, and because taxonomy—the naming and description of subspecies, species, and other taxa—has a strong basis in phenotype, this aspect of DNA barcoding has been contentious. Aspects of mtDNA itself, such as maternal inheritance and heterogeneous mutation rates, add to uncertainties in interpretation (Rubinoff et al. 2006). Nonetheless, the potential role of DNA barcoding in diagnostics remains promising because animal identification is often difficult or impossible, as when trying to identify larval stages of agricultural pests or small fragments of birds that have damaged or destroyed aircraft. However, with respect to biodiversity, many researchers feel that the presently narrowly defined barcoding effort will just provide another data set—in addition to other genetic and phenotypic data—to consider when studying and describing biodiversity and the evolutionary processes that generate it.

size for coalescent-based analyses of gene-lineage histories (Harding 1996), and such sample sizes provide excellent power over the question of gene-lineage monophyly (Rosenberg 2007); however, substantial levels of gene flow between these populations could occur without detection. For example, a sample size of 11 gives one a 95% probability of detecting all alleles (or haplotypes) in a population that occur at a frequency of only 30% or more (Gregorius 1980). This is remarkably little power over a factor that strongly influences divergence at even low levels of occurrence. Gene flow, clinal variation, reticulation (rejoining of divergent lineages), isolation by distance, and paraphyly and polyphyly (types of allelic mingling between groups) are routine phenomena in the populational processes of divergence. Thus, just as studies of divergence using phenotype have improved dramatically with increased sampling, so too will those using genetics tools.

Coupling phenotypic and genotypic data

Zink (2004), among others, provided evidence from birds that single-locus genetic divergence and phenotypic divergence below the species level (in this case, named subspecies) are not tightly coupled; it would thus seem that speciation by this coupled route (route A in figure 2) is not the predominant means by which divergence occurs in the class Aves. Differentiation driven largely by adaptation (figure 2, route B) would place diverging populations into divergence space favoring the recognition of subspecies, provided those adaptations were reflected in characteristics typical of phenotypic data sets (figure 3). Differentiation resulting largely from neutral divergence (figure 2, route C) would cause cryptic diversity (figure 4). Cases in which genetic divergence crosses a perceived threshold (e.g., monophyly or a particular percent divergence), but phenotypic divergence apparently does not, represent a growing area of research suggesting that we have much to discover—probably in unmeasured or unappreciated characteristics—about phenotypic divergence and its effects (Bickford et al. 2007).

There are good examples of bird lineages diverging along routes B and C (figure 2). For example, shallow genetic divergence with species-level phenotypic divergence (route B) occurs between lineages in *Plectrophenax* buntings (Klicka et al. 2003, Maley and Winker 2007), *Vermivora* warblers (*Vermivora chrysoptera* and *Vermivora pinus*; Vallender et al. 2007), and *Icterus* orioles (*Icterus galbula* and *Icterus albeillei*; Kondo et al. 2008). Avian examples of relatively deep genetic splits with seemingly small phenotypic divergence (route C) occur within *Schiffornis* "*turdina*" (Nyári 2007) and *Troglodytes* "*troglodytes*" (Toews and Irwin 2008). Other examples of each could be cited, but this type of biodiversity research is still in the exploratory phase.

Do these different divergence routes (figure 2) occur nonrandomly with respect to taxonomy or geography? We do not yet know. Genetic analyses of recent taxa—sister species of birds and mammals—have suggested that both speciation and extinction rates are highest at high latitudes and decline toward the tropics (Weir and Schluter 2007). This finding is controversial, not least because there is a well-understood latitudinal bias in effective taxonomic treatment, with a correspondingly imperfect understanding of species limits and sister taxa upon which to base tropical genetic sampling (Tobias et al. 2008). The effect of this bias on these analyses cannot at present be quantitatively resolved, but there is agreement that further study in basic taxonomy and systematics is necessary (Tobias et al. 2008). Integrated studies of genotype and phenotype are considered exemplary in this regard (e.g., see Isler et al. 2007).

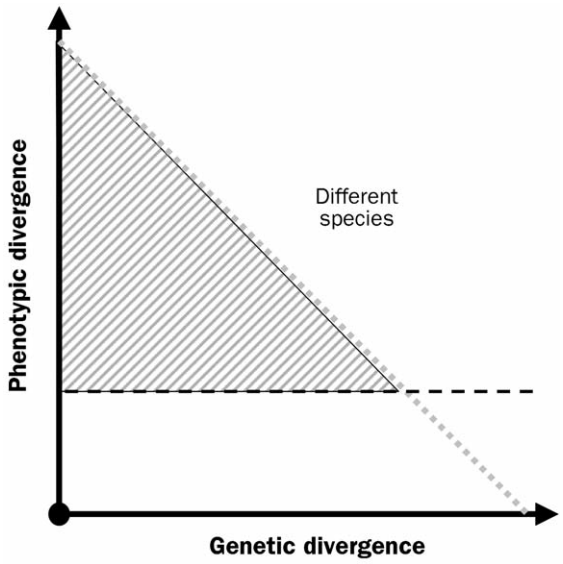

Figure 3. When phenotypic divergence reaches a threshold (horizontal dashed line), subspecies, races, or varieties may be described. Uncertainty about how well this divergence reflects species limits means that this line can cross the species boundary (i.e., many described subspecies turn out upon closer study to be full species). The hatched region can be considered the divergence space dominated by described subspecies.

Further examination of the data in Weir and Schluter (2007) in a different context shows another limitation of an exclusively genotypic approach. Graphing the distribution of genetic divergences (in the mtDNA gene cytochrome *b*) between the sister species of birds included in their study (figure 5) demonstrates that avian sister species (or putative sisters, at any rate) exhibit remarkably broad variation in genetic divergence, and there is clearly no threshold in such a continuous-but-ragged distribution at which speciation can be said to occur. Fully 21.9% of the 192 sister-species pairs in the study by Weir and Schluter (2007) had genetic divergences of less than 2.0% corrected sequence divergence (26.6% had divergences < 2.5%; corrections are made using a model that accounts for the nonlinear accumulation of evidence for mutations as the time since divergence increases). The typological concept that there is some genetic divergence threshold that delineates species limits is both philosophically and practically unwarranted (Winker et al. 2007).

If there is no lower threshold of genetic divergence indicating when speciation has occurred, is there some upper boundary beyond which genetic incompatibilities are so likely that speciation is certain? Perhaps, but empirical evidence suggests that it is not easy to put a threshold value on such a boundary; in birds, genetic incompatibilities arise at quite variable levels of divergence (Price 2008). Using data on avian hybrids, Price (2008) showed that genetic incompatibilities increase with lineage divergence such that complete hybrid infertility occurs between lineages that are on average 14% divergent in corrected mtDNA sequence data. The relationship is messy, though, and it does not tell us when environment-dependent postzygotic reproductive isolation (Rice and Hostert 1993) might occur between diverging lineages—when hybrids experience reduced fitness not from genetic incompatibilities, but rather from how their phenotypes react to their environment. Importantly, however, as Price (2008) noted, in birds "complete speciation can occur without any intrinsic loss of hybrid viability or fertility" (p. 373). Thus, prezygotic isolating mechanisms are probably more important in birds than postzygotic ones.

This would seem to suggest that selection, and not neutral divergence, is on average a more important force in avian speciation. However, at roughly 2.1% mtDNA sequence divergence per million years in birds (Weir and Schluter 2008), there is a long temporal window (millions of years) in which a lack of divergent selection (or the presence of canalization) and neutral divergence might produce cryptic diversity (contrast figure 4 with figure 5). Thus, in birds it seems that it is possible to have a long period of genetic divergence between lineages without incurring genetic incompatibilities or other hybrid disadvantages, during which phenotype (prezygotic isolating mechanisms) apparently determines, on secondary contact, the outcome of the lineages in terms of evolutionary independence. Finding these sorts of lineages (route C, figure 2) and readdressing phenotype in light of genetic evidence for cryptic diversity is an important area of current and future research (Bickford et al. 2007). Remember that it is our human perception of organisms that determines cryptic diversity, and to understand evolutionary divergence (i.e., how selection has caused species-level differences), we need to use a full definition of phenotype, one that includes all the traits of an organism except its genome (West-Eberhard 2003). We are likely to learn a lot more about phenotype and which aspects of it are evolutionarily important (i.e., contribute to speciation) in different taxa.

Because the conditions under which cryptic diversity is most likely to develop (route C, figure 2) probably require the relative homogeneity and stability found in tropical regions, and because these regions remain the least known, a lot of interesting data points must be acquired to fully understand how speciation occurs in birds and other organisms. In other words, we should expect variation in this process in geographic and taxonomic space, although it may be subtle, given early, global evidence that cryptic species are relatively evenly distributed taxonomically and geographically when corrected for species richness and research intensity (Pfenninger and Schwenck 2007). There are patterns that make geographically variable speciation processes seem likely. For example, in birds and other vertebrates there is evidence that degrees of interpopulation genetic differentiation increase with decreasing latitude (Hackett and Rosenberg 1990, Winker

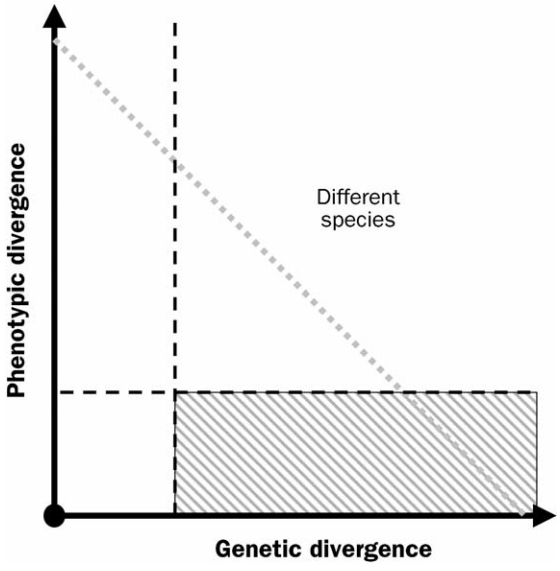

Figure 4. When genetic divergence reaches a threshold (vertical dotted line), such as percentage divergence or single-locus reciprocal monophyly, divergence space becomes further partitioned, and cryptic diversity space (hatched area) is defined. Note that the genetic threshold concept and cryptic diversity space cross the species boundary, but neither condition alone may be definitive in this multidimensional evolutionary process space.

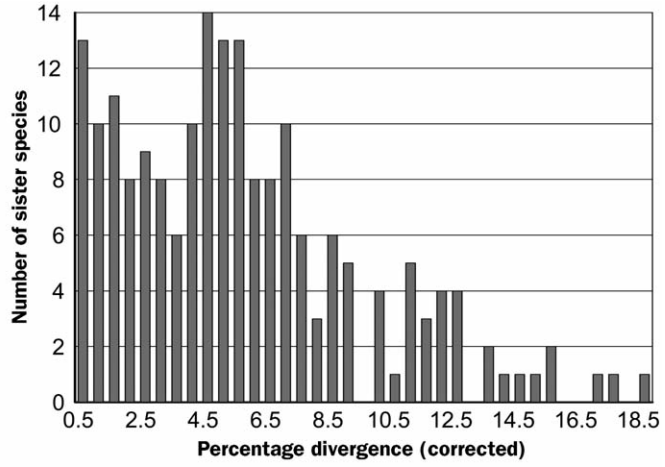

Figure 5. Frequencies of mitochondrial DNA (cytochrome* b*) genetic divergence values among 192 pairs of avian sister species (Weir and Schluter 2007). The relationship shows a decline in frequencies as divergence increases, as we expect, but the relationship is not clean, and it exhibits no obvious thresholds indicating when speciation has occurred.

et al. 2000, Chek et al. 2003, Martin and McKay 2004). Whether this might be linked to latitudinally variable speciation rates (e.g., Weir and Schluter 2007) or might in another way contribute to the well-recognized latitudinal gradient in biodiversity remains to be determined, but it is another important area of research (Mittelbach et al. 2007).

How we measure genetic and phenotypic divergence is not a trivial issue, and there are many ways to measure each. I have put no values on the conceptually based divergence-process space (figures 1–4), in part for this reason. Through the Q_{ST} method, which uses a statistic that compares among-population genetic variance in phenotypic traits with the null hypothesis of divergence among neutral genetic loci (G_{ST} or F_{ST}, measures of genetic differentiation between populations), numerous researchers have shown that divergent selection can indeed be an important driver of differentiation (see Leinonen et al. 2008). But there is a considerable degree of heterogeneity among studies, and obtaining quantitative genetic data on phenotype in wild populations is laborious. Thus, if broadscale contrasts between phenotypic and genetic divergence are to be fleshed out, we must turn to other methods. Classic methods of quantifying phenotype through morphometrics (e.g., Bookstein et al. 1985) are very useful, but they have not yet achieved the degree of among-taxon comparability that classic F_{ST} statistics have done in genetics (but see Jost 2008). Nor do we yet have a broader concept of "phenotypic metrics." Progress is being made, however, in methods enabling comparison between disparate phenotypic attributes (e.g., Verga and Gregorius 2007, Toews and Irwin 2008).

Toward an integrated future

The pendulum of genotypic versus phenotypic data in the study of biodiversity seems to have swung recently to its genotypic extreme (e.g., Hebert et al. 2004, Zink 2004). However, this has unleashed a storm of dissent around the discovery and diagnosis of the diversity of life, and approaches that integrate data from genotype and phenotype are likely to dominate future research (Paquin and Hedin 2004, DeSalle et al. 2005, Meier et al. 2006, Schlick-Steiner et al. 2007). In some respects this dichotomy is artificial, because taxonomists and systematists have generally incorporated new methods in an integrative fashion to help solve these traditional, ongoing problems (Mayr 1969, DeSalle et al. 2005, Burns et al. 2008). In addition, evolutionary biologists have been studying the relationships among genotype, phenotype, and environment for decades (e.g., van Noordwijk 1989, West-Eberhard 2003). Nonetheless, the recent debates are genuine, perhaps because new technologies are often viewed hopefully—perhaps as a silver bullet—and because, as outlined here, overemphasis is occasionally placed on effectively unidimensional approaches to the inherently multidimensional process of biological differentiation (figure 1).

Empirically derived ecogeographic rules that find widespread concordance among unrelated taxa between phenotype and climate have long been considered evidence that geographic variation in phenotype reflects population adaptations to geographically variable environments. Examples include Bergmann's and Gloger's rules in endothermic vertebrates, which describe the positive relationship between cooler, drier environments and larger body size, and the correlation between increased humidity and darker plumage or pelage, respectively (Zink and Remsen 1986, James 1991). To these traditional, morphological phenotypic characteristics can be added other attributes of phenotype that vary with climate, such as reduced basal metabolic rates among tropical birds (Wiersma et al. 2007). Evidence that subspecific-level morphological phenotypic characters can be adaptive (Zink and Remsen 1986, Mumme et al. 2006), and that such adaptations can have simple genetic bases (Hoekstra et al. 2006), assures us that continued genomic exploration will yield important information not now represented in most of our genetic data sets—adaptive geographic variation. We can anticipate an explosion in genomic-based research into adaptation and speciation (Wu and Ting 2004, Begun et al. 2007, Storz and Hoekstra 2007, Carroll 2008, Ellegren and Sheldon 2008).

However, studies of biodiversity must elucidate patterns as well as begin to explore processes, both for applied science, to inform conservation and management, and for basic science, to create effective baselines for ecological, evolutionary, behavioral, and other biological research. This, at least initially, requires broad but shallow coverage, which encourages the very oversimplifications that have historically led to effectively unidimensional approaches to the multidimensional divergence problem. Considering phenotypic and genotypic attributes in two simplified dimensions of divergence (figures 1–4) should encourage better exploration of this multidimensional process space, but unanswered questions remain here as well.

What does phenotypic or genotypic divergence mean in an evolutionary sense? When are genotypic or phenotypic divergences important (i.e., reflecting responses to selection), and when are they not? And, integrating them, when do genotypic and phenotypic divergences reflect aspects integral to the speciation process? These deceptively simple questions have no simple answers, yet they are relevant to applied and basic sciences from conservation biology and medicine to theoretical biology.

While the simplifications of axes of divergence portrayed in figures 1–4 have heuristic value, the generalities of "largely adaptive" and "largely neutral" divergence require that some care be taken, especially as data sets grow deeper. The attribution of phenotypic differences to adaptation has a history of excess, with important consequences for the philosophy of science, teleonomy, and reductionism (Gould and Lewontin 1979, Mayr 1983). Nonadaptive and nongenetic influences, such as environment and developmental plasticity, can produce phenotypic divergence between populations. How environment affects phenotype and the evolutionary significance of these effects remain central issues in evolutionary biology; theoretical and empirical advances are being made (Price et al. 2003, West-Eberhard 2003, Suzuki and Nijhout 2007).

Importantly, environmentally induced phenotypic variation and the developmental plasticity underlying it can be a target of selection, and be adaptive (James 1991, West-Eberhard 2003).

Similarly, however, we are most likely in an era of overzealousness with respect to attributing variation in our genetic data sets to neutrality. Single-locus studies are particularly susceptible to departures from neutrality, if only through possible linkage to genes or loci under selection. Even the abundant noncoding variation dominating mtDNA sequence data, the workhorse of animal biodiversity genetics, is irrevocably tied as a single linkage unit to the rest of the mitochondrial genome, which, as the genetic basis of the powerhouse of animal cells, is under strong selection. The consequences in terms of neutrality are not yet fully understood, but strict neutrality seems unlikely (Ballard et al. 2007), and nonneutrality in the nuclear genome (e.g., through linked selection; Gillespie 2000) may be "rampant" (Hahn 2008).

Issues of simplification aside, interest in and debate about what can be done in comparing values between these axes of phenotypic and genotypic divergence (figure 1) have been strong for decades (e.g., Templeton 1980, Felsenstein 1986). Interactions, associations, and what we can infer from relationships between these axes remain intriguing. Because of uncertainties in our attributions of adaptive phenotypic and neutral genetic divergences, we can expect some fuzziness in our determinations of where diverging lineages fall along both axes (figure 2); deeper data sets should help here. But the consideration of divergences in such process space (figures 2–4) unquestionably provides testable hypotheses and heuristic value in determining how evolutionary divergence occurs in nature and, in an applied sense, in what biodiversity units exist and where we should aim to be effective in their management and conservation.

Conclusions

The process of differentiation occurs in multidimensional space, and it has long been recognized that intraspecific variation among populations is the raw material from which species arise (Mayr 1963, Kimura 1983, West-Eberhard 2003). Taxonomy is a categorical tool that results in discrete "bins" along a continuum of differentiation. Unsurprisingly, this tool, which provides categorical data based on phenotype, often disagrees with genetic measurements of variation, which are largely disconnected from the phenotype used in taxonomy and provide data of a continuous rather than a discrete nature. Legions of systematists and taxonomists have demonstrated that both approaches can be misleading. For example, convergent and divergent morphological evolution can obscure lineage histories, and a single genetic locus can fail to accurately track relationships at and below the species level.

Research that integrates genotypic and phenotypic data is clearly advancing our understanding of biodiversity and its generation. Across the spectrum of such studies, we see research ranging from taxonomically narrow and data-deep approaches to taxonomically broad and data-shallow efforts. As we continue to describe and study biodiversity, integrating phenotypic and genotypic data and exploring their relationships in the multidimensional process space of diverging populations will very likely provide new insights both into the units of biodiversity and into the processes responsible for their generation. For example, increasing attention to developmental plasticity in evolutionary divergence suggests the possibility that environmentally induced phenotypic changes causing novel, adaptive phenotypes and subsequent genetic accommodation (i.e., genes as followers rather than leaders in evolutionary change) would favor route B to speciation in figure 2 (Price et al. 2003, West-Eberhard 2003). Alternatively, long-term isolation and drift might cause populations to diverge to species-level incompatibility in the absence of divergent selection; this would reflect the cryptic diversity shown by route C in figure 2. How often might such processes occur in nature, and where do they occur geographically, ecologically, and taxonomically?

We still have a long way to go to fully describe and understand the diversity of life on Earth. A growing number of examples integrating genotypic and phenotypic data sets show that, at the same time that biodiversity is anthropogenically threatened, we are making unprecedented progress in developing our knowledge of it. It is indeed an exciting time to be a biologist.

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