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Levels of genetic polymorphism: marker loci versus quantitative traits

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Species are the units used to measure ecological diversity and alleles are the units of genetic diversity. Genetic variation within and among species has been documented most extensively using allozyme electrophoresis. This reveals wide differences in genetic variability within, and genetic distances among, species, demonstrating that species are not equivalent units of diversity. The extent to which the pattern observed for allozymes can be used to infer patterns of genetic variation in quantitative traits depends on the forces generating and maintaining variability. Allozyme variation is probably not strictly neutral but, nevertheless, heterozygosity is expected to be influenced by population size and genetic distance will be affected by time since divergence. The same is true for quantitative traits influenced by many genes and under weak stabilizing selection. However, the limited data available suggest that allozyme variability is a poor predictor of genetic variation in quantitative traits within populations. It is a better predictor of general phenotypic divergence and of postzygotic isolation between populations or species, but is only weakly correlated with prezygotic isolation. Studies of grasshopper and planthopper mating signal variation and assortative mating illustrate how these characters evolve independently of general genetic and morphological variation. The role of such traits in prezygotic isolation, and hence speciation, means that they will contribute significantly to the diversity of levels of genetic variation within and among species.

Keywords: speciation; allozymes; reproductive isolation; geographic variation; *Chorthippus*; *Nilaparvata*

1. UNITS OF BIOLOGICAL DIVERSITY

Ecologists measure biological diversity in terms of species. There are many different ways of counting (Magurran 1988), but this is invariably the underlying unit. The still unresolved search for a universal species concept (Claridge *et al*. 1997) should, therefore, be placed at the centre of ecological debates about biodiversity. However, practising ecologists usually cannot devote the time needed to tackle this thorny issue: they necessarily make pragmatic use of the species defined by systematists. When measuring the alpha-diversity within a habitat this is likely to cause few difficulties except with a minority of species, which present problems either of asexual reproduction or of hybridization. Clones within asexual 'species' may have different ecological roles (Vrijenhoek 1994) and so may be more appropriate units of diversity where they can be distinguished. Hybridization may be sufficiently common to cause severe problems with species concepts based on reproductive isolation or on diagnosability, depending on one's perspective; for example, hybridization in a local flora has been interpreted very differently by Mayr (1992) and by others (e.g. Gornall 1997). It may well blur distinctions between ecological units.

Measuring beta- and gamma-diversity (Whittaker 1960) introduces another intractable problem with species concepts: how to deal with divergent allopatric populations. Species concepts based on reproductive isolation really have no satisfactory answer. Alternatives based on diagnosability may offer a practical solution that is satisfactory for the systematist, but it is doubtful whether diagnosability can be used as a guide to ecological distinctiveness. An approach arising from conservation biology is to recognize 'evolutionarily significant units' as entities that are sufficiently distinct in non-trivial characters to be worthy of independent conservation efforts (Vogler & DeSalle 1994). This amounts to a species concept in its own right, which aims at the common goal of recognizing lineages with independent evolutionary potential (Mayden 1997) but which, in common with its competitors, fails to provide an objective yardstick to measure 'distinctiveness'.

While species are the units of ecological diversity, alleles are the units of genetic diversity. One could argue that they are the fundamental units of biological diversity, as individuals of different species simply harbour more distinct alleles than individuals of the same species. Given that biodiversity cannot be measured in practice by counting alleles, one must consider how genetic variation is partitioned within and between species. Species diversity can then be used as a surrogate for total diversity, but only if the species is defined in a way that partitions genetic variation consistently. Therefore, one must consider how much species differ genetically, how much genetic variation there is within species and how consistent these two measures are across taxa. If these parameters vary widely, species are not equivalent units of biodiversity and must be weighted accordingly.

Weighting of ecological measures of species diversity on the basis of their genetic distance has recently been considered by several authors (May 1990; Humphries *et al*. 1995).

This, too, has been mainly in the context of conservation, with the aim being to maximize the character diversity that is maintained in a limited area or with limited resources. In general, the result of weighting is to give more emphasis to more distinct taxa. Comparable weighting for the diversity within species has also been considered in the conservation context. Part of the motivation for defining 'evolutionarily significant units' is the recognition that some species, as currently delineated, contain several genetically distinct populations, whereas others do not. At the level of genetic variation within populations, two distinct positions can be taken: either that genetically homogeneous populations are the most endangered and require the greatest intervention, or that genetically variable populations have the greatest biodiversity value, and the greatest evolutionary potential, and so should be most valued. Either way, reliable measures of genetic diversity within populations are needed. Marker loci are widely used for this purpose, but it is uncertain how well they reflect the genetic variability of quantitative traits.

In this paper, we first consider the patterns of genetic divergence between species and genetic variation within species, as revealed by allozyme electrophoresis. We then ask how well these measures reflect divergence or variability of quantitative traits. This leads to a particular consideration of divergence in traits that contribute to prezygotic reproductive isolation because of the importance of these traits in the origin, and thus the nature, of species, and because they appear to show even less dependence on general genetic divergence than other trait groups.

2. ALLOZYME DATABASES

By far the largest coherent body of data on genetic variation within and between species is provided by allozymes. DNA sequence-based methods may soon take over this position but have not done so yet, partly because of the variety of approaches possible. Ultimately, direct sequencing must provide the most powerful description of genetic variation because it can eliminate the inferential steps needed with indirect methods like allozymes or even restriction fragment length polymorphisms (RFLPs) and can, potentially, avoid bias in the representation of different types of loci (although biases are certainly present in the currently available data). Sequences of coding regions have the added advantage of internal comparisons between silent-site—presumably largely neutral—variation and non-silent variation, which is, at least potentially, under selection. Although allozyme data will remain the largest comparative resource for some time, comparisons with DNA sequence data can already help in their interpretation.

Not only is a large amount of allozyme data available in the literature, but much of it has been accumulated in databases. For example, Thorpe (1982) gathered data on Nei's genetic identity from 106 studies (typically involving several species each), while Nevo and co-workers (Nevo *et al*. 1984) and Ward, Skibinski and Woodwark (Ward *et al*. 1992; Skibinski *et al*. 1993) assembled heterozygosity estimates for 1111 species and 3728 populations of more than 1500 species, respectively. The latter database includes genetic distance information as well as heterozygosities, and data are recorded locus by locus. This allows comparisons to be made across proteins as well as across populations, and allows taxonomic comparisons to be corrected for variation in the sample of proteins studied.

The patterns revealed by these large surveys are striking. First, and reassuringly, genetic identity (a measure of the sharing of alleles and similarity of allele frequencies) is strongly related to the taxonomic hierarchy (Thorpe 1982) (figure 1). Nei genetic identities among populations within species are nearly always greater than $I=0.8$, and 80% of values are greater than $I=0.95$; identities between congeneric species typically range from $I=0.35$ to 0.85. While identity values between species in different genera and between congeneric species overlap broadly, there is remarkably little overlap between identities within and among species. This is true despite the fact that Thorpe's survey included taxa ranging from seaweeds to mammals (with an admitted concentration on North American vertebrates). Only one major group, the birds, was omitted from the figure because 'their speciation processes seem to differ fundamentally from those of most other organisms' (Thorpe 1982, p. 150). Identities among bird species tended to fall in the range of within-species comparisons from other taxa.

If allozyme differences accumulate in a clock-like fashion, then Thorpe's data imply, for many taxa, that speciation typically occurs after a consistent time interval, most probably because it is associated with a general accumulation of genetic differences. Given that, in practice, most species are defined on the basis of morphological distinctiveness, this may simply mean that morphological differences accumulate in a roughly clock-like manner along with allozyme differences. In birds, for whatever reason, morphological divergence is accelerated. This is not a very satisfying hypothesis because it does not explain the distinct gap in the distribution of identity values around $I=0.8$ (figure 1). The worry is that this gap results from Thorpe's deliberate omission of 'studies where there is taxonomic doubt (on grounds other than electrophoresis)' (Thorpe 1982, p. 150).

A protein-by-protein analysis (Skibinski *et al*. 1993) also shows a marked contrast between intra- and interspecific genetic identities. Across seven proteins that are represented in a large majority of allozyme surveys, intraspecific identities all average more than $I=0.96$ in vertebrates and $I=0.91$ in invertebrates, and mean identities between species are all below $I=0.85$ for vertebrates and $I=0.72$ for invertebrates.

Average expected heterozygosity (H, a measure of allelic diversity within a population or species) varies across species from zero to about $H=0.3$ in vertebrates or $H=0.6$ in invertebrates (Ward *et al*. 1992). There is some variation in typical levels of variation across taxonomic groups from $H=0.06$ in birds to $H=0.16$ in molluscs, but the variation is, in Gillespie's opinion 'not large enough to dispel the impression that there is a remarkable uniformity across taxa' (Gillespie 1991, p. 45). Much effort has gone into explaining allozyme heterozygosity, too much to be reviewed here. Gillespie argues (p. 51) that more progress can be made by explaining why some proteins are consistently more polymorphic than others, than by trying to predict average heterozygosities. The same is true across

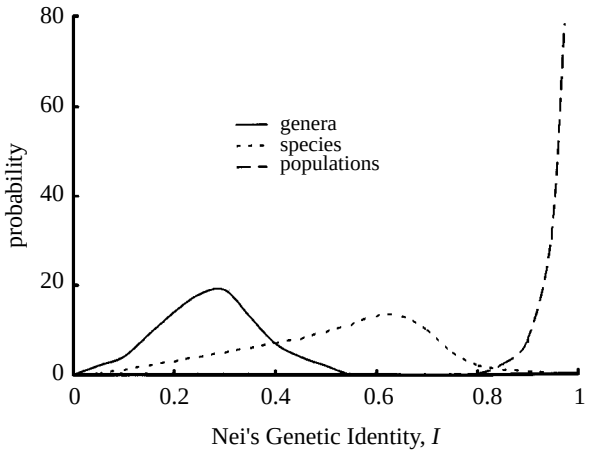

Figure 1. Distributions of Nei's genetic identity (I) values between species in different genera, between congeneric species, and between populations within species. Redrawn from data in Thorpe (1982).

species. Provided that the heterozygosity measures are comparable (and they can be made so; Ward *et al*. 1992), it is probably more important to understand why some species are more heterozygous than others than it is to explain why the overall average heterozygosity is about 0.14 across all invertebrates sampled. Variation in effective population size is expected to be important but is apparently not the whole answer (see below).

A related question, but one that has been considered less, is the extent to which variation among species or populations in allozyme heterozygosity provides an index of variation in the morphological, behavioural or life history characters that are generally of more interest to the ecologist or conservation biologist. There is often an assumption that the two are positively correlated, based on the expectation that both are influenced by effective population size, but the data available to support this assumption are few (Mallet 1996). Lande (1988) predicts that the effects of population size will be rather different between neutral markers and quantitative traits, mainly because the higher mutational input to characters influenced by many loci enables them to maintain significant genetic variability at low population sizes and recover variability rapidly after population bottlenecks.

3. HETEROZYGOSITY AND QUANTITATIVE GENETIC VARIATION

There is a significant positive relationship between population size and allozyme heterozygosity within several plant species with endangered populations (ranging in size from $1–10^5$ individuals) (Young *et al*. 1996). This presumably results from loss of variation during contraction of the smaller populations. The question is whether there is a comparable reduction in quantitative variation in such populations, and, if so, whether this impairs their ability to adapt to changing environmental conditions (Storfer 1996). This is not only a pressing conservation question but also a general question related to divergence between species and the process of speciation. The extent to which allozyme heterozygosity can be used as a guide to the genetic variability of quantitative traits depends on the way each of these varies with population characteristics, primarily effective population size.

For allozymes, the impact of population size depends on the vexed question of neutral versus selective explanations for heterozygosity. Essentially, there are three classes of model: (i) strictly neutral drift; (ii) slow removal of mildly deleterious alleles; and (iii) maintenance by balancing selection. Under each of these models heterozygosity is expected to be strongly related to effective population size, but the predicted levels of heterozygosity may be quite different (Nei & Graur 1984). The data gathered by Nei & Graur on heterozygosity and population size for 72 species do show a highly significant positive correlation between them, although the range of heterozygosity values is much less than is predicted from the range in population sizes. Gillespie (1991) points out that the correlation is mainly due to *Drosophila* species at the high end (when most other data are from mammals) and carnivores at the low end. Within higher taxa, the major effect is probably the low heterozygosity in a few species that have been through very tight bottlenecks (such as the northern elephant seal; Hoelzel *et al*. 1993), with only a weak relationship otherwise.

Nei & Graur (1984) considered that the neutral model provided the best fit to the data in their survey. Skibinski *et al*. (1993) also concluded that the majority of allozyme variation could be explained under the neutral model. Their earlier analysis (Ward *et al*. 1992) showed substantial systematic variation in heterozygosity across proteins. For example, heterozygosity declines with increase in the number of subunits in a protein and increases with subunit molecular weight. The mean heterozygosity for a protein in vertebrates is strongly correlated with its mean heterozygosity in invertebrates. These patterns suggest selective constraints. However, they observed (Skibinski *et al*. 1993) the positive relationship between heterozygosity and genetic distance, within and between either populations or species, expected under the neutral theory, indicating that the variation among proteins is mostly due to variation in the neutral mutation rate.

On the other hand, various strands of evidence against the strictly neutral hypothesis have been gathered by Gillespie (1991). Until recently, only two general types of evidence were available: pattern tests and single locus tests. Pattern tests, such as those employed by Nei & Graur (1984) or Skibinski *et al*. (1993), suffer from overlap between the predictions of the three classes of model. Single locus tests have shown convincingly that some allozyme loci are under selection. For example, Watt (1994; Watt *et al*. 1996) has provided extensive evidence for differences in molecular function among phosphoglucose isomerase allozymes in *Colias* butterflies, and has related these to organismal performance and fitness. But single locus studies cannot be extended to allozymes in general and a few selectively maintained polymorphisms are consistent with the neutral model as a general explanation. Indeed, Watt himself has argued (1995) that a general 'selectionist–neutralist' debate is fruitless: some variants are selected, others are neutral.

DNA sequence data now provide an additional approach to the issue on the basis that silent-site polymorphism is likely to be strictly neutral and, therefore,

can be used as a baseline for comparison (Kreitman & Akashi 1995). Silent-site variation is greatest in the region of the *Drosophila Adh* gene close to the codon responsible for the ubiquitous electrophoretic polymorphism for 'fast' and 'slow' alleles, supporting the interpretation of balancing selection on this locus (Hudson *et al*. 1987). More generally, if allozyme and silent-site polymorphisms are both determined by drift, they will be influenced by population size in the same way. However, there are significant departures from this pattern (Kreitman & Akashi 1995). Allozyme heterozygosity in *Drosophila melanogaster* is similar to, or greater than, heterozygosity in *D. simulans*, but *simulans* has much more silent-site variation than does *melanogaster* (Aquadro 1991), and *D. melanogaster* has similar allozyme variability to man but much greater silent-site variation (Li & Sadler 1991). If the silent-site variation reflects differences in effective population size, then the relative uniformity of allozyme heterozygosity across these three species, and generally, indicates pervasive balancing selection. Comparisons between heterozygosity and genetic distance show that amino-acid replacement substitutions are more common than expected, relative to silent-site substitutions, between species, when compared with their proportions within species (McDonald & Kreitman 1991). This suggests divergence driven by selection and disagrees with the conclusion from large-scale allozyme comparisons of heterozygosity and genetic distance, unless one concludes that the *Adh* locus in *Drosophila* is one of the exceptional examples of selection acknowledged by Skibinski *et al*. (1993).

The current balance of evidence thus favours a selective explanation for allozyme polymorphism rather than a strictly neutral explanation. Probably there is a mixture of balancing selection and selection against mildly deleterious alleles. If one assumes that the majority of quantitative traits in natural populations are under weak stabilizing selection and are influenced by several to many loci (Lande 1976; Barton & Turelli 1989), then there is a broad similarity between the forces influencing both trait groups. Loci influencing quantitative traits under stabilizing selection are also typically under weak balancing selection or have mildly deleterious alleles. Indeed, allozyme loci and quantitative trait loci are not necessarily mutually exclusive categories. The relationship between allozyme heterozygosity and population size should be reflected in a comparable relationship for heritable variation in quantitative traits (Houle 1989). The correlation may be similarly dominated by low values in populations with low effective population sizes as a result of recent bottlenecks, with Lande's (1988) proviso about the more rapid recovery of variability in quantitative traits because of the large numbers of loci involved. This relationship is not expected to extend to those quantitative traits that are under persistent directional selection. Such traits are normally expected to have low heritability, regardless of population size. Heritable variation is a ubiquitous feature of natural populations and shows the expected relationship with intensity of selection, albeit with much scatter (Roff & Mousseau 1987).

However, secondary sexual characters are a major class of traits with a history of directional selection and it has recently been argued that the operation of sexual selection on such characters can favour modifiers that enhance their additive genetic variation. A survey of data in the literature has supported this prediction (Pomiankowski & Møller 1995), although some doubt its theoretical basis (Rowe & Houle 1996). The process outlined by Pomiankowski & Møller is not necessarily specific to secondary sexual traits under female choice, it could apply to other traits that have been under directional selection. The expectation, then, is that genetic variation in quantitative traits with a history of weak stabilizing selection will be correlated with allozyme heterozygosity, through their mutual dependence on effective population size, but the relationship will be weaker, or absent, for traits under directional selection. Houle (1989) found no good empirical support for the predicted correlation of genetic variance in quantitative traits with population size, although if total phenotypic variation could be used as an indicator of genetic variation (assuming environmental variance to be relatively constant) a few studies showed trends in the right direction.

We are not aware of a test of these predictions, despite the large number of heterozygosity and heritability measures available in the literature for a comparative study. In such a test, it is clearly preferable to use the coefficient of additive genetic variation (CV_A), rather than the heritability, as the measure of genetic variability as it is not influenced by the level of non-genetic (or non-additive) variation (Houle 1992). As Pomiankowksi & Møller (1995) have accumulated data on CV_A for sets of characters currently under directional and stabilizing sexual or non-sexual components of selection, we have added data on heterozygosity to their comparisons. Note that most characters are presumably under net stabilizing selection, with any directional sexual selection balanced by other components of natural selection (Rowe & Houle 1996). However, exaggerated secondary sexual traits that are currently under directional sexual selection are likely to have a recent history of net directional selection. Despite the large number of allozyme studies in the literature, heterozygosity estimates are available for only 16 of the 32 species included in their table 2. For this small data set, the correlation between CV_A and heterozygosity is close to zero for characters under directional sexual selection ($r=-0.04$, $n=11$) and negative for characters under stabilizing selection ($r=-0.36$, $n=9$; $r=-0.21$ for the combined data). It is, of course, possible that a larger survey would reveal a significant positive relationship in one or both classes, but there is little support here for the use of allozyme data to infer the general evolutionary potential of a population or species. If there really is a negative correlation, which could, perhaps, result from weak selection in expanding populations versus strong selection in large or contracting populations, then allozyme data would be seriously misleading.

Another way in which allozymes have been suggested to reflect an important general feature of genetic variability is through an association between individual heterozygosity and fitness. Although numerous studies have reported correlations between allozyme heterozygosity and either growth rate or fluctuating asymmetry, the reasons for these relationships are unclear. It may be that allozyme loci are frequently in linkage disequilibrium with chromosomal regions containing deleterious recessive alleles, or it could be that individual allozyme loci have

large direct effects on fitness (Gillespie 1991). Either way, these associations typically only explain a very small proportion of variance in the fitness measure (Britten 1996) so that allozyme heterozygosity remains a poor surrogate for other classes of genetic variation.

4. GENETIC DISTANCE AND GENETIC DIFFERENCE

Thorpe's (1982) survey of genetic distances between species showed a broad general agreement with the taxonomic hierarchy. Phenotypic divergence also undoubtedly increases as taxonomic relatedness decreases, although this pattern has rarely been documented systematically. The study of three plant families by Gilmartin (1980) demonstrates the point, which many take for granted, that the rate of increase in phenetic distance with taxonomic distance varies markedly among higher taxa. Probably the same points could be made for ecological similarity. Allozyme and morphological variation are partitioned in broadly similar ways within and among populations but with differences in detail. For example, in *Phlox drummondii* (Schwaegerle *et al*. 1986), 94% of allozyme variation is within populations compared with 73% of heritable variation in quantitative traits. The underlying common variables are, of course, time of divergence and population size. But they are reflected imperfectly in each of these types of variation and so the correlations between them may be weak.

Speciation is more than simply accumulation of phenetic, or even genetic, differences. It involves the acquisition of evolutionary independence through the development of barriers to gene exchange. Genetic isolation then feeds back to preserve and promote differentiation. Thus, the evolution of characters contributing to reproductive isolation is of particular importance to the generation and maintenance of biological diversity. So does reproductive isolation accumulate with general genetic divergence as indicated by allozyme data? The large number of studies of species within the genus *Drosophila*, both of genetic distance and of reproductive isolation, has allowed Coyne & Orr (1989, 1997) to address this question, with striking results.

First, postzygotic isolation—failure to produce viable offspring in interspecific crosses or production of sterile offspring—does show a very strong positive correlation with genetic distance between species. There is an inflexion point in the relationship at 50% isolation because male sterility and inviability evolves more rapidly with increasing genetic distance than does female inviability and sterility, in accordance with Haldane's rule. Nevertheless, although there is a considerable scatter of points, the relationship is strong (Kendall's rank correlation, $\tau = 0.57$), stronger than most of the associations discussed above. This suggests that genetic differences at loci responsible for isolation accumulate in much the same clock-like fashion as allozyme differences, dominated by drift or under weak selection. The loci involved are known to be numerous and to produce sterility or inviability through epistatic interactions (Wu & Palopoli 1994), as predicted by Dobzhansky (1937) and Muller (1942). However, little else can be said about them because their only phenotype is sterility or inviability in crosses and this is clearly not the effect which caused their divergence. At least in *Drosophila*, postzygotic isolation is a side effect of slow general genetic divergence, which may be caused by drift or by gradual adaptive evolution involving many small substitutions.

Coyne & Orr's analyses (1989, 1997) demonstrate a very different pattern for premating isolation due to assortative mating. In many of the species pairs for which they gathered data, strong premating isolation occurs despite low genetic distance ($D < 0.5$) and weak postzygotic isolation. This pattern is especially true for sympatric species pairs, which show significantly higher premating isolation than do allopatric species pairs at low genetic distances. As a result, although there is a general increase in premating isolation with allozyme genetic distance, the relationship is much weaker than for postzygotic isolation (overall $\tau = 0.27$). The implication is that, at least in sympatry, premating isolation evolves under strong selection. This selection may be direct selection for isolation as a result of producing dysfunctional hybrid offspring (reinforcement), but could also be an indirect effect of selection on mating signals and responses for other reasons. For example, sympatric species are more likely to differ in habitat than closely related allopatric species and selection may favour mating behaviour that is adapted to these habitats.

Although there is no comparable large-scale comparative study, there is evidence that mating signals do not diverge by steady accumulation of small differences. The recent study of the acoustic signals of males of the *Drosophila willistoni* group by Ritchie & Gleason (1995) is a case in point. Despite low levels of morphological divergence, the species have very distinctive songs. *D. equinoxialis* has the most divergent song structure but is not the most distantly related on the basis of allozymes (data quoted in Coyne & Orr 1989), whereas the distantly related ($D = 1.27$) *D. paulistorum* and *D. tropicalis* have rather similar songs, perhaps reflecting their allopatry. Using sequence data to measure genetic distance, Ritchie & Gleason (1998) find no significant correlation with song divergence (Mantel test, $r = -0.27$). Although genetic distance is a good predictor of postzygotic isolation ($r = 0.68$, $p < 0.001$), it is a relatively poor predictor of prezygotic isolation ($r = 0.44$, $p = 0.035$).

A comparative study of mating signal evolution would ideally consider both signals and preferences in a group of species with known phylogenetic relationships. To some extent this type of study has been carried out in the recent tests of the 'sensory exploitation' hypothesis in *Physalaemus* toads (Ryan & Rand 1993), water mites (Proctor 1992) and swordtail fish (Basolo 1996), but these studies have concentrated on the origins of preferences and traits rather than quantitative changes. Measurements of carrier frequency spectra in the songs of a group of 20 related grasshopper species have recently been compared with sensitivity spectra of the receptor organs in the same species (Meyer & Elsner 1996). There is a strong correlation across species between the position of the low-frequency peak in the male song and the maximum sensitivity of the 'a-cells' that are responsible for registering the tympanal response to such frequencies ($r = 0.75$, $p < 0.001$). Unfortunately, grass-hopper phylogeny is not well known and the current classification is probably not a reliable reflection of true relationships (D. R. Ragge, personal

communication), so that phylogenetic interpretation of this correlation is not possible. However, it is striking that the pair *Chorthippus parallelus/montanus* and the trio *C. biguttulus/brunneus/mollis,* which are undoubtedly closely related, are divergent in this characteristic of song and correspondingly in preference. It appears that song and preference characteristics coevolve but can be modified rapidly and reversibly, a pattern that may be expected from intersexual selection (West-Eberhard 1983). As with the *Drosophila willistoni* group, these grasshoppers are very similar morphologically. They also show little genetic divergence (Butlin & Hewitt 1987; Mason *et al*. 1995). Mating signal evolution, and thus prezygotic isolation, has evolved independently of general genetic divergence.

Between-species comparisons, although valuable and informative, are limited because they cannot adequately separate divergence that occurs during speciation from divergence after speciation. Therefore, it is also important to study differentiation among populations within species in relation to their genetic divergence. For example, there is strong assortative mating among some population pairs in the salamander *Desmognathus ochrophaeus*, which is related to the degree of allozyme divergence (Tilley *et al*. 1990). Both ethological isolation and genetic distance are strongly related to geographic distance, suggesting that they both reflect accumulation of genetic differentiation in allopatry, in contrast to the between-species comparisons in *Drosophila*. Isolation is asymmetric at intermediate levels of divergence in accordance with the expectation from overlapping signal and preference distributions (Arnold *et al*. 1996). Another large study, with the túngara frog, *Physalaemus pustulosus*, shows similar strong correlations between geographic separation of samples and signal trait divergence, in this case using directly measured features of acoustic signals (Ryan *et al*. 1996). Again, genetic distance is also strongly correlated with geographic distance, but geographic distance remains a better predictor of signal divergence than genetic distance. These thorough intraspecific studies are unusual, making generalizations difficult. In the next section, we describe two further projects investigating different aspects of the divergence in mating signal systems.

5. GRASSHOPPERS AND PLANTHOPPERS

(a) Chorthippus parallelus

A hybrid zone in the Pyrenees between subspecies of the meadow grasshopper, *Chorthippus parallelus*, has attracted much attention to its evolutionary history (Butlin 1997). Traits such as morphology, chromosomal markers and mating signals form clines through the zone. Most of these clines are coincident, presumably because the zone is the product of secondary contact between divergent races separated during the last glaciation. However, non-coincident clines in a chromosomal marker (Ferris *et al*. 1993) and cuticular hydrocarbons (Neems & Butlin 1994) confirm that traits can vary independently. Outside the zone, comparison of three populations within northern Europe indicates that morphological and signal trait differences exist between parapatric populations, which also show some assortative mating (Dagley *et al*. 1994). Why the races on either side of the Pyrenees or the populations examined by Dagley *et al*. should have diverged is unknown. However, new molecular data from non-coding nuclear DNA has provided a detailed picture of the biogeographic history of populations throughout Europe (Cooper & Hewitt 1993; Cooper *et al*. 1995). This reveals that the species' northwards expansion after the last glaciation halted in the Iberian peninsula at the Pyrenees and in Italy at the Alps. Current northern European individuals are descendants from the Balkan refuge, which have spread all the way westwards across Europe (figure 2). This complex pattern shows little correlation between geographical distance and genetic divergence, but reveals populations with differing and known evolutionary histories. With this background knowledge, we set out to investigate how genetic divergence relates to prezygotic isolation and divergence in phenotypic characters.

We collected individuals from seven sites, shown in figure 2, and allowed them to lay eggs. After a winter diapause at 4 °C, we reared their offspring in the laboratory (Kelly-Stebbins & Hewitt (1972) with minor modifications and *Dactylis glomerata* provided as a food plant). All experiments and analyses were carried out on these laboratory-reared individuals.

To investigate levels of premating isolation, we conducted assortative mating experiments in which two male–female pairs from different populations were placed in the same $40 \times 30 \times 20$ cm arena, maintained at a minimum of 25 °C by an overhead 60 W bulb, and provided with fresh grass. Individuals of the same sex were matched for age to the same day when 4 days old, and as closely as possible if older (mean age difference = 6% of mean age). Only virgin females were used. The grasshoppers were continually observed for 3 h, or until a mating occurred, at which point the experiment was stopped. A minimum of 25 crosses were carried out between each pairwise combination of the seven populations (537 matings in total) allowing us to calculate the χ^2 index of isolation, an appropriate measure for this type of experiment as it takes into account differences in vigour between populations (Gilbert & Starmer 1985). The isolation matrix is given in table 1. The statistical significance of individual pairwise isolation indices is provided as a guide to the pattern of variation only. It is the overall pattern that is important and which is related, below, to genetic distances between populations, and to morphological and mating signal variation. It is clear from this table that assortative mating does occur, although no population is completely isolated. The Greek population has the highest levels of premating isolation, with evidence for assortment in all crosses. Similarly, in crosses with the English population there is a clear tendency for assortment to occur. Other crosses, such as those between Spain and northern France or Germany show no tendency to assort, indicating that the pattern of assortative mating is quite unlike that we would expect from the pattern of genetic divergence (Cooper *et al*. 1995), which predicts that the Spanish population would be most isolated. This lack of correlation is confirmed by a Mantel matrix correlation test comparing our isolation matrix and corresponding genetic distance data from Cooper *et al*.'s study (first column, table 2).

To compare patterns of phenotypic and genetic divergence we measured three suites of characters: morphology, cuticular composition and male calling

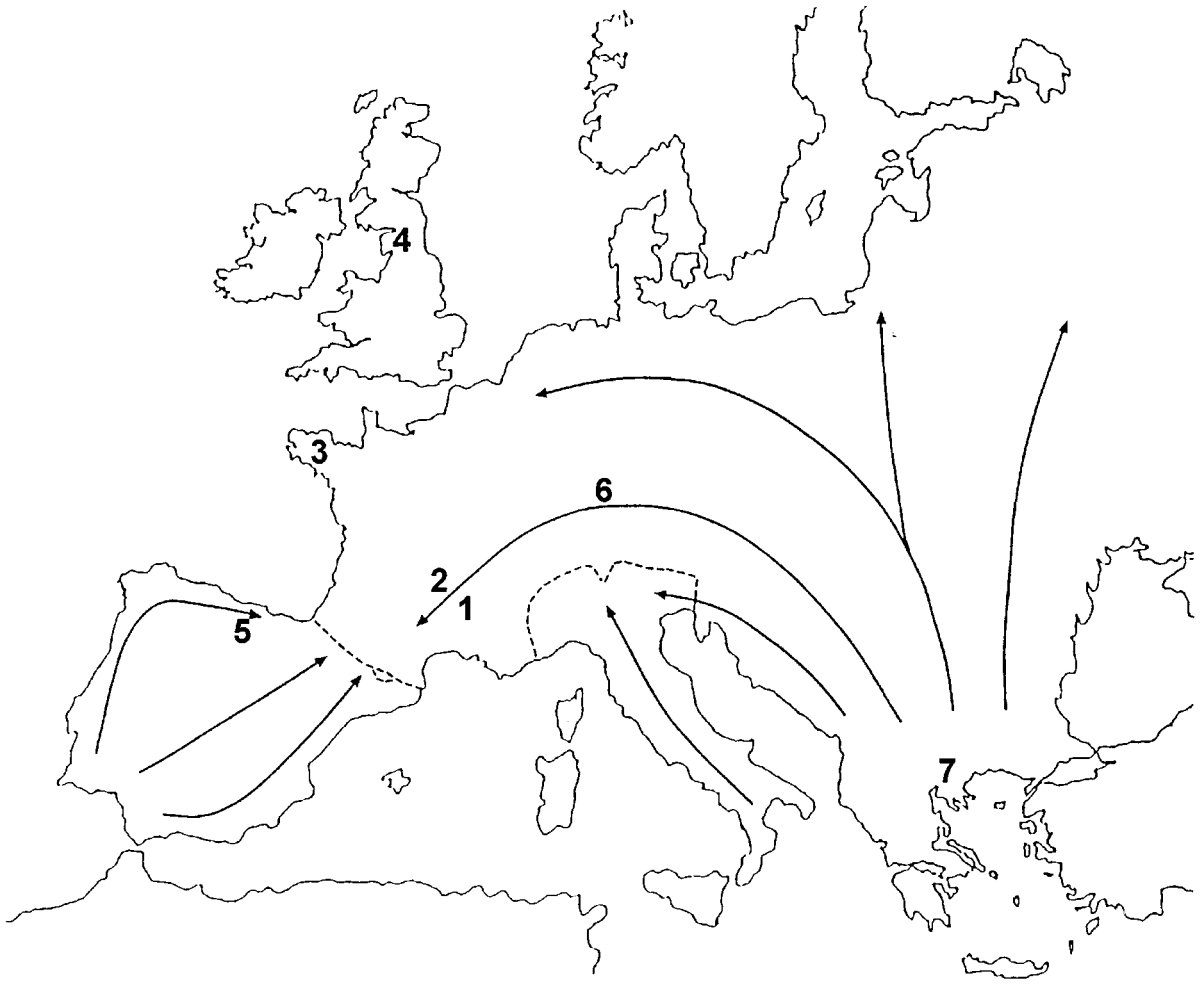

Figure 2. Colonization routes for *Chorthippus parallelus* inferred from sequence data for an anonymous single-copy nuclear DNA marker by Cooper *et al*. (1995). Glacial refugia are believed to have been in areas of deciduous woodland in southern Spain, Italy and the Balkans. Arrows indicate inferred expansion routes. Dashed lines are current areas of secondary contact. Numbers indicate the localities of populations used in the present study (full details will be given in Tregenza *et al*. (1998)).

Table 1. χ^2 *isolation indices between pairs of European populations of the meadow grasshopper*

(Locations of populations are given in figure 2.)

population	1	2	3	4	5	6
1. S. France						
2. S. France	0.021					
3. N. France	−0.122	0.379				
4. England	0.529*	0.012	0.486*			
5. Spain	0.082	0.473*	−0.205	0.272		
6. Germany	0.077	0.205	0.237	0.414*	−0.045	
7. N. Greece	0.406	0.576*	0.527*	0.250	0.583*	0.602*

*Indicates that the individual isolation index is significantly different from 0 (i.e. departs from random mating) at $p < 0.05$. Positive indices indicate assortative mating.

song. The latter two characters are likely to play important roles in the species' mating system, with cuticular composition determining contact pheromone blend (Neems & Butlin 1995; Butlin 1997) and song implicated in mate attraction and discrimination (Butlin & Hewitt 1985; Ritchie 1990). Morphological characters measured were the lengths of the two halves of the pronotum (the prozona and metazona), the length and width at its widest point of the hind femur, and, in males, the number of stridulatory pegs on the inside of the hind femur and the length of the row of pegs. A total of 597 females and 489 males were measured. Cuticular hydrocarbons were analysed from 510 females and 486 males, following the chemical and statistical procedures described in Neems & Butlin (1994). Calling songs (Butlin & Hewitt 1985) were recorded from 243 males at a mean temperature of

Table 2. *Results from Mantel matrix correlations comparing morphological and mating signal traits with genetic distance and assortative mating*

(*r*, the Mantel correlation; *p*, randomization probability test for *r*, based on 20 000 iterations, one-tailed as we have no alternative hypothesis that predicts negative correlations; 'size' is the length of the pronotum; 'other' is all morphological data after adjustment for variation explained by regression on pronotum length.)

			male morphology		female morphology		cuticle		
		mating	size	other	size	other	male	female	song
genetic	*r*	−0.20	−0.02	0.83	0.04	0.07	−0.13	−0.08	0.40
distance	*p*	n.s.	n.s	0.02	0.13	0.08	n.s.	n.s.	0.13
assortative	*r*		0.55	−0.09	0.56	0.64	0.49	0.47	−0.35
mating	*p*		0.05	n.s	0.04	0.01	0.04	0.06	n.s

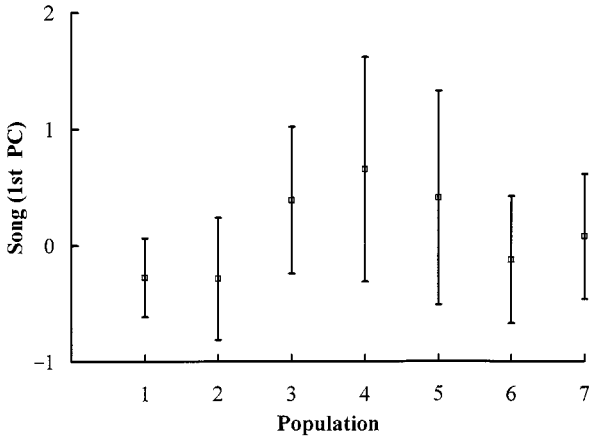

Figure 3. Comparison of male songs among the populations indicated in figure 2. Values plotted are means and 95% confidence intervals.

29.3 °C±0.17 s.e. Temperature corrections were estimated from linear regression of the entire data set, which provided an equally good fit to non-linear regressions. Four temporal song characters were measured as in Dagley *et al.* (1994).

Again, we compared the patterns of divergence of these characters with the pattern of genetic divergence, and with the pattern of assortative mating using Mantel matrix correlation tests (table 2). For purposes of comparison, trait data were converted to population difference matrices using procedures in the *Genstat 5* statistical package. Additionally, because overall size dominated morphological differences between populations, we calculated size corrections by regression of the other measurements on pronotum length, and used both pronotum length and residual morphological differences in the analysis.

Although the power of these statistical tests with a 7×7 matrix is low, there is a correlation between genetic divergence and male morphological (but not size) differences, whereas female morphology and the signal traits show little sign of any link. Comparison of phenotype and the pattern of assortative mating also show links: both morphology and likely cuticular pheromones are associated with assortative mating. Our data indicate that at least some aspects of morphological divergence are associated with genetic divergence, but that signal traits have changed independently, and have apparently influenced prezygotic isolation along the way.

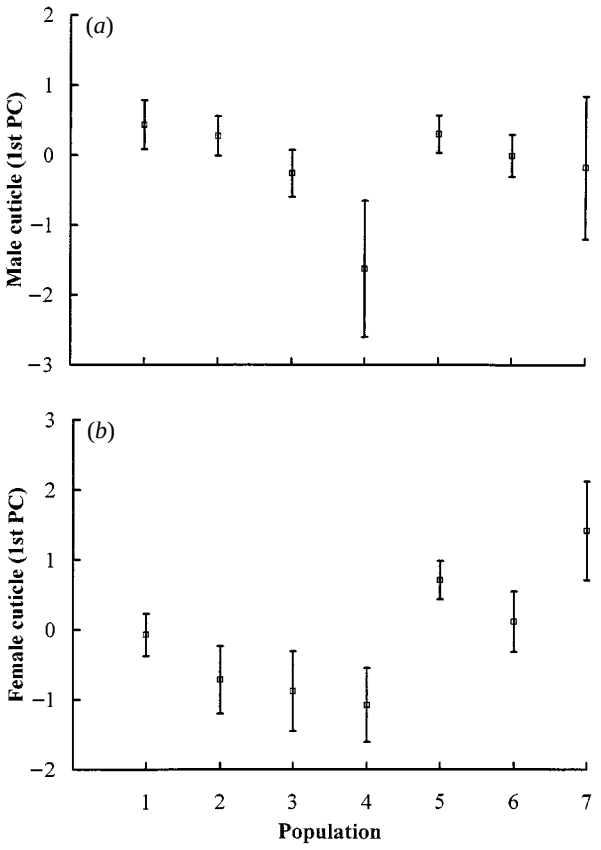

Figure 4. Comparisons of (*a*) male and (*b*) female cuticular composition among the populations indicated in figure 2. Values plotted are means and 95% confidence intervals.

Examination of the pattern of divergence of male song (figure 3), reveals very little detected variation between populations, which goes some way to explaining the lack of correlation between assortative mating and song. By contrast, cuticular composition shows considerable variation, with English males (figure 4*a*) clearly differing from other populations, and substantial differences between females from several populations (figure 4*b*). It is clear that the pattern of inter-population variation differs between the sexes, but they are nevertheless correlated (Mantel matrix correlation, $r=0.72$, $p=0.04$). Grasshoppers from the Greek population are unusually large and so size-assortative mating, which is common in insects, may explain part of the isolation of this population.

Signal trait divergence in *C. parallelus* clearly cannot be explained simply as a by-product of genetic divergence. Indeed, even the pattern of morphological variation shows a less than perfect fit to the genetic map. However, our knowledge of the evolutionary histories of the different populations suggests a number of alternative explanations for observed patterns of phenotypic divergence. Those populations in Spain and the Balkans are refugial, and unlikely to have been in contact for the last half million years (Hewitt 1996). In contrast, those in England, France and Germany are the result of post-glacial expansion and hence share more recent ancestors. The populations also differ in their likelihood of having passed through numerical bottlenecks. The sessile nature of this flightless species makes it highly probable that to reach Britain before the opening of the English Channel, northern European populations must have gone through repeated long-distance dispersal and founder events (Hewitt 1993). We can formally test these explanations by comparing prediction matrices based on the various hypotheses with the patterns we observe; this will be the subject of future work (Tregenza *et al*. 1998). However, it is evident that the greatest differences in morphology, signal traits and prezygotic isolation are between the Greek and British populations and the rest of Europe. Because these populations derive from the same refuge, this suggests that long-term isolation is not the most important engine of divergence in this species.

(b) Nilaparvata lugens

The second study organism is the brown planthopper, *Nilaparvata lugens*, which is a major pest of rice in India, south-east Asia and northern Australia. This species uses substrate-transmitted vibrations for its mating signals. Males produce a 2 s burst of rapidly damped pulses with a pulse repetition frequency (PRF) of 50–120 Hz, by shaking the plant stem, and females respond with long continued pulse trains of much lower PRF (Claridge 1985). Throughout its range, the rice-feeding race of *N. lugens* is accompanied by another host-race feeding on the weed grass *Leersia hexandra*, which often grows in close association with cultivated rice (Claridge *et al*. 1985*a*). These races are indistinguishable morphologically and can produce viable and fertile hybrid offspring in the laboratory. Sequence data from the first internal transcribed spacer region of the ribosomal DNA suggest that host shifts (probably from *Leersia* to rice) occurred very recently and independently in Asia and Australia, with the older separation between populations on the two continents being 0.5–1.0 Ma old (Jones 1994; Jones *et al*. 1996).

Despite their genetic and morphological similarity, the races of *N. lugens* show marked differences in both male and female songs (Claridge *et al*. 1985*a*) and there is also substantial variation among populations (Claridge *et al*. 1985*b*, 1988). There is evidence that this variation in PRF influences mate choice (Claridge *et al*. 1985*b*, 1988). In brown planthopper, we have tackled three questions relevant to explaining the apparently rapid evolutionary changes in PRF: (i) are the changes mirrored by changes in female response?; (ii) is female response sufficiently discriminating to impose strong selection on PRF?; and (iii) what is the genetic basis of the changes in PRF?

The first two questions can be approached using playback experiments, as female planthoppers respond readily to synthesized male signals. For three populations (rice feeding from India and Australia, *Leersia* feeding from Indonesia) with mean PRFs of 80, 95 and 100 Hz, the mean female preferences were for signals with PRFs of 83, 90 and 97 Hz, respectively (Butlin 1996). Thus there is the expected correlation between signal and preference, but preferences are less divergent than signals, suggesting that they have not led the evolution of the signal differences. Had signal divergence been due to intersexual selection, one would have expected preferences to be more different than signals. It is unlikely that signals have diverged to become adapted to the different signalling environments of their host plants, because the PRF of *Leersia*-feeding males is higher than that of rice-feeding males in Asia, but lower in Australia (Claridge *et al*. 1988).

Females are less likely to respond to signals that are further away from the mean for males of their population (Butlin 1993; Trickett 1995). Thus, mate choice is expected to impose stabilizing selection on signals and impede divergence. However, in the Indian rice population, the female response distribution is broad compared with variation among males and this is because all females have broad response windows rather than because females vary in their peak response (Butlin 1993; Trickett 1995). In this population, the overall peak response is for a slightly higher PRF than the male signal mean, implying directional selection for increased PRF, and male mating success is, indeed, greater for individuals with higher PRF (Trickett 1995). Nevertheless, selection is expected to be weak because female response windows (range of signals eliciting 50% response) are broad relative to the among individual variation in signal PRF (>4 times the signal standard deviation; Butlin 1993).

A quantitative genetic analysis of male and female signal PRF differences between the Indian rice- and either Australian rice- or Indonesian *Leersia*-feeding populations of brown planthopper provided estimates for the numbers of loci contributing that ranged from 1–5 (1.5–16 after correction for the biases in the estimation method) (Butlin 1996). There was marked dominance of the 'rice' alleles for low male signal PRF in the Indian rice by Indonesian *Leersia* cross. This pattern—a few loci of large effect with directional dominance—is normally associated with divergence under selection (Templeton 1981). However, in this case, the maximum effect of a single locus is likely to be in the order of 5–10 pulses per second. A change of this magnitude produces only a slight reduction in female response in our playback experiments (Trickett 1995) and is likely to be only weakly selected. Thus the overall conclusion at present is that rapid signal divergence in *N. lugens* is most likely to be the result of genetic drift. This may be aided by the population structure of brown planthopper, which, especially on cultivated rice, involves repeated colonizations by small numbers of individuals followed by rapid population growth, although this does not explain conservatism in other characters. Similar conclusions might be reached for rapid song divergence in the *Laupala* crickets of Hawaii (Shaw 1996). It may be possible for signal characters to drift because of the simultaneous drift in female preferences, as suggested by Lande's (1981) model of the Fisher runaway process; unlike characters under stabilizing selection due to

adaptation to the external environment, signal/response systems generate their own optima. This process may also have operated in the salamanders discussed above, but more slowly.

6. CONCLUSIONS

Species are not equivalent units of biological diversity. They vary widely in their genetic variability, both within and among populations, and in their genetic similarity to other species. By far the largest database available at present to describe the patterns of genetic variation within and among species is the massive accumulation of studies using allozymes. Unfortunately, allozyme heterozygosity within species and allozyme genetic distances between species are generally only a poor guide to other types of genetic variation.

The evidence at present suggests that allozyme variation is not strictly neutral: some polymorphisms are maintained by strong selection and many more are influenced by weak selection. Nevertheless, heterozygosity within populations is correlated with effective population size, and genetic distance between populations or species does accumulate in roughly clock-like fashion. General phenetic distance and postzygotic reproductive isolation also appear to increase steadily with time of independent evolution and thus correlate with allozyme-based genetic distance. This is probably because both trait groups are influenced by many genes that are individually under weak selection at most, like allozymes. If speciation were primarily a result of these traits, genetic distance between species and variation within species might be more consistent. But, speciation is actually dominated by the evolution of prezygotic isolation. The characters involved in prezygotic isolation frequently experience rather different patterns of selection: signal traits may be under strong directional selection, or under stabilizing selection but with a shifting optimum dependent primarily on the preferences of the other sex. They may evolve rapidly and in unpredictable directions, and can result in rapid speciation at low levels of general genetic, including allozyme, divergence. This is especially true in sympatry; in allopatry prezygotic isolation may reflect general divergence more closely as in the salamander or túngara frog examples or in *Drosophila*, but this does not seem to be true of brown planthoppers or meadow grasshoppers. Perhaps this discrepancy is a result of the shorter timescales involved in the planthopper and grasshopper examples.

Within species, signal characters under directional selection are expected to show different patterns of variation within populations from other traits, and genetic variation in signal traits is not expected to be correlated with allozyme heterozygosity even if there is such a correlation for genetic variation in other traits. Populations may vary markedly in signal traits and resulting assortative mating, without relation to patterns of genetic relationship among populations.

From the point of view of biodiversity, the outcome of speciation depends on the ecological differentiation of the resulting species. If speciation is driven mainly by prezygotic isolation, then it is likely to be decoupled from ecological differentiation. Many species pairs will initially be allopatric or parapatric and contribute to beta, rather than alpha, diversity. In contrast, ecological separation may drive the origin of prezygotic isolation in some cases (Schluter 1996). How ecological differentiation relates to allozyme divergence is an unanswered question, but it is likely that any connection will be weak. Understanding biodiversity, and preserving it, requires a knowledge of genetic variability in these critical character sets—mating signal systems and ecologically relevant traits—within and between populations and species. It is not enough either to count species, or to measure variation in marker loci, be they allozymes or the latest sequence based markers.

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