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The evolutionary genetics of speciation

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The last decade has brought renewed interest in the genetics of speciation, yielding a number of new models and empirical results. Defining speciation as 'the origin of reproductive isolation between two taxa', we review recent theoretical studies and relevant data, emphasizing the regular patterns seen among genetic analyses. Finally, we point out some important and tractable questions about speciation that have been neglected.

Keywords: hybrid inviability; hybrid sterility; reproductive isolation; sexual isolation; species

1. INTRODUCTION

When we last reviewed the evolution of reproductive isolation (Coyne & Orr 1989*a*), we complained that workers on speciation were considered poor cousins in the family of evolutionists, mired in endless and untestable speculations about a process that no one could witness. Since then, the study of speciation has grown increasingly respectable, recruiting ever more experimentalists and theorists. A number of new phenomena have been uncovered and new theories offered to explain them. Here we summarize recent progress on the genetics of speciation, highlighting areas where important and tractable questions remain unanswered.

2. WHAT ARE SPECIES?

Any discussion of the genetics of speciation must begin with the observation that species are real entities in nature, not subjective human divisions of what is really a continuum among organisms. We have previously summarized the evidence for this view and counter-arguments by dissenters (Coyne 1994). The strongest evidence for the reality of species is the existence of distinct groups living in sympatry (separated by genetic and phenotypic gaps) that are recognized consistently by independent observers. To a geneticist, these disjunct groups suggest a species concept based on gene flow. As Dobzhansky (1935, p. 281) noted: 'Any discussion of these problems should have as its logical starting point a consideration of the fact that no discrete groups of organisms differing in more than a single gene can maintain their identity unless they are prevented from interbreeding with other groups. Hence, the existence of discrete groups of any size constitutes evidence that some mechanisms prevent their interbreeding, and thus isolate them.' This conclusion inspired Dobzhansky (1935) and Mayr's (1942) biological species concept (BSC), which considered species to be groups of populations reproductively isolated from other such groups by 'isolating mechanisms', genetically based traits that prevent gene exchange. The list of such mechanisms is familiar to all evolutionists, and includes those acting before fertilization ('prezygotic' mechanisms, such as mate discrimination and gametic incompatibility), and after fertilization ('postzygotic' mechanisms, including hybrid inviability and sterility).

Like most evolutionists, we adopt the BSC as the most useful species concept, and our discussion of the genetics of speciation will accordingly be limited to the genetics of reproductive isolation. We recognize that this view of speciation is not universal: systematists in particular often reject the BSC in favour of concepts involving diagnostic characters (Cracraft 1989; Baum & Shaw 1995; Zink & McKitrick 1995). We have argued against these concepts elsewhere (Coyne *et al.* 1988; Coyne 1992*a*, 1993*a*, 1994) and will not repeat our contentions here. We note only that the recent burst of work on speciation reflects almost entirely the efforts of those adhering to the BSC. In fact, every recent study on the 'genetics of speciation' is an analysis of reproductive isolation.

3. WHY ARE THERE SPECIES?

One of the most important but neglected questions about speciation is why organisms fall into many discrete groups instead of constituting a few extremely variable 'types'. The answer to the question of why species exist may not be the same as the answer to 'how do species arise?' The only coherent discussion of this problem is that of Maynard Smith & Szathmáry (1995, pp. 163–167) who give three possible reasons for the existence of discrete species: (i) species might represent stable, discontinuous states of matter; (ii) species might be adapted to discontinuous ecological niches; and (iii) reproductive isolation (which can arise only in sexual taxa) might create gaps between taxa by allowing them to evolve independently.

The second and third hypotheses seem most plausible. There are several ways to distinguish between them. One is to determine if asexually reproducing groups form sympatric taxa just as distinct as do sexually reproducing groups. Although such comparisons are hampered by the

rarity of large asexual groups, bacteria seem reasonable candidates. Although little work has been published, two studies (Roberts & Cohan 1995; Roberts *et al*. 1996) show that forms of *Bacillus subtilis* from the American desert fall into discrete clusters in sympatry. Moreover, coalescence models (Cohan 1998) show that a combination of new mutations conferring ecological difference, periodic selection and limited gene flow can produce distinct taxa of bacteria living sympatrically.

The only empirical work on clustering in asexual eukaryotes is that of Holman (1987), who, studying successive revisions of taxonomic monographs, determined that the nomenclature of bdelloid rotifers (not known to have a sexual phase) was more stable than that of sexually reproducing relatives. From this he concluded that because they are recognized more consistently, asexual rotifers are actually more distinct than their sexual counterparts. While intriguing, this result is hardly conclusive, and we badly need similar studies based not on nomenclature but on genetic and phenotypic cluster analyses. We hasten to add that although studies of clustering in asexuals are worthwhile, they must not be overinterpreted. Such studies might show that ecological specialization can produce discrete asexual forms, but it does not follow that such specialization explains clustering in sexuals:the maintenance of discrete forms by natural selection in sexuals is far more difficult and could be far rarer.

We suggest two other approaches not discussed by Maynard Smith & Szathmáry (1995). First, if distinct niches alone (and not reproductive isolation) can explain the existence of species, then sympatric speciation should be common. The whole premise that geographic isolation is essential for speciation rests on Dobzhansky's (1935) and Mayr's (1942) idea that the swamping effect of gene flow prevents the evolution of reproductive isolation. But if reproductive isolation is not important in explaining the existence of species, adaptation to distinct niches could occur in sympatry, and phylogenetic analyses should often reveal that the most recently evolved pairs of species are sympatric. Although there is some evidence for sympatric speciation based on niche use in fish (e.g. Schliewen *et al*. 1994), we know of no other studies featuring similarly rigorous phylogenetic analyses. Perturbation experiments could also address this problem. For example, sexual isolation between sympatric species could be overcome by hybridization in the laboratory, and the resulting (fertile) hybrids reintroduced into their original habitat. If the 'ecological-niche' explanation is correct, the hybrids should revert to the parental types (or to similar, but distinct, types). But if reproductive isolation helps maintain species distinctness, the hybrids should either revert to a single parental type or remain a hybrid swarm. Such a study would, however, probably require an unrealistic amount of time.

Of course, the existence of species in sexual taxa could well depend on both distinct adaptive peaks and reproductive isolation. But it is hard to believe that ecological niches alone can explain distinct species, if for no other reason than such species, lacking reproductive isolating mechanisms, would hybridize. If they were then to remain distinct, hybrids would have to suffer a fitness disadvantage due to their inability to find a suitable niche, and this disadvantage is a form of postzygotic isolation.

4. STUDYING REPRODUCTIVE ISOLATION

(a) *What is novel about speciation?*

Some of our colleagues have suggested that speciation is not a distinct field of study because—as a by-product of conventional evolutionary forces like selection and drift—the origin of species is simply an epiphenomenon of normal population-genetic processes. But even if speciation is an epiphenomenon, it does not follow that the mathematics or genetics of speciation can be inferred from traditional models of evolution in single lineages. Under the BSC, the origin of species involves reproductive isolation, a character that is unique because it requires the joint consideration of two species, and usually an interaction between the genomes of two species (Coyne 1994). The distinctive feature of the genetics of speciation is, therefore, epistasis. This is necessarily true for all forms of postzygotic isolation, in which an allele that yields a normal phenotype in its own species causes hybrid inviability or sterility on the genetic background of another (see below). Epistasis also occurs in many forms of prezygotic isolation. Sexual isolation, for example, usually requires the coevolution of male traits and female preferences, so that the fitness of a male trait depends on whether the choosing female is conspecific or heterospecific.

These complex interactions between the genomes of two species guarantee that the mathematics of speciation will differ from that describing evolutionary change within species, and suggest that speciation may show emergent properties not seen in traditional models. Indeed, such properties have already been identified for postzygotic isolation (e.g. see discussion of the 'snowball effect' below).

Two motives usually underlie genetic analyses of speciation. First, just as with quantitative-trait-locus (QTL) analyses of 'ordinary' characters, we would like to understand the genetic basis of cladogenesis. That is, we would like to know the number of genes involved in reproductive isolation, the distribution of their phenotypic effects and their location in the genome. Second, we expect genetic analyses of reproductive isolation to shed light on the process of speciation, as different evolutionary processes should leave different genetic signatures. The observation of more genes causing hybrid male than hybrid female sterility (see below) has suggested, for example, that these critical substitutions were driven by sexual selection (Wu & Davis 1993; True *et al*. 1996).

(b) *What traits should we study?*

Because speciation is complete when reproductive isolation stops gene flow in sympatry, the 'genetics of speciation' properly involves the study of only those isolating mechanisms evolving up to that moment. The further evolution of reproductive isolation, although interesting, is irrelevant to speciation. This point is widely recognized but understandably often ignored in practice. If speciation is allopatric, and several isolating mechanisms evolve simultaneously, it is hard to know which will be important in preventing gene flow when the taxa become sympatric. *Drosophila simulans* and *D. mauritiana*, for example, are allopatric, and in the laboratory show sexual isolation, sterility of F_1 hybrid males, and inviability of both male and female backcross hybrids. We have no idea which of these factors would be most important in

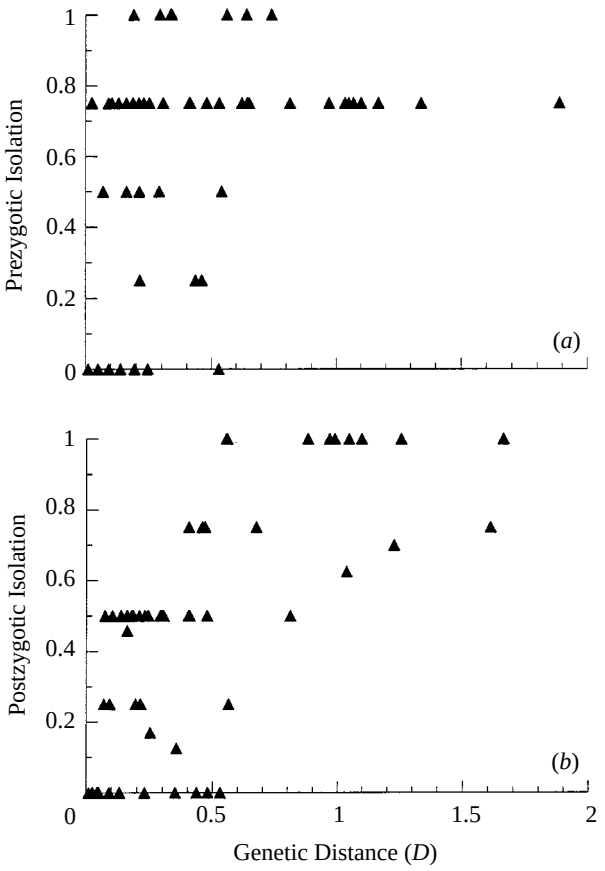

Figure 1. Strength of reproductive isolation in *Drosophila* plotted against Nei's (1972) electrophoretic genetic distance (an index of divergence time). Each point represents the average among pairs within a species group, so points are evolutionarily independent. (*a*) Prezygotic (sexual) isolation; (*b*) postzygotic isolation. See Coyne & Orr (1989*b*; 1997) for further details.

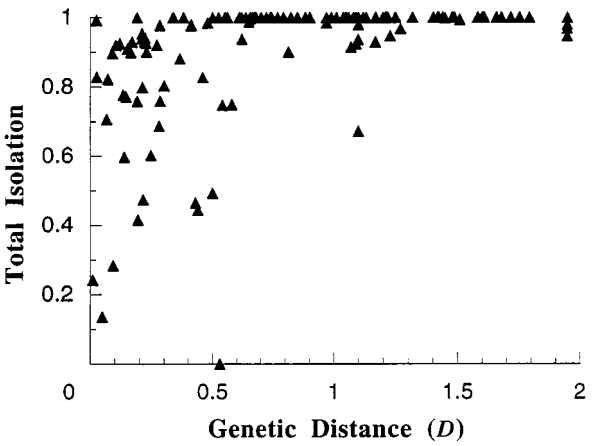

Figure 2. Total reproductive isolation in *Drosophila* (combining both pre- and postzygotic isolation) plotted against Nei's (1972) genetic distance. Each point represents a single pair of species. See Coyne & Orr (1989*b*, 1997) for further details.

preventing gene exchange in sympatry, or if other unstudied factors—such as ecological differences—would play a role.

It seems likely, in fact, that several isolating mechanisms evolve simultaneously in allopatry and act together to both prevent gene flow in sympatry and allow coexistence. (Although reproductive isolation is sufficient for speciation, different species must coexist in sympatry in order to be seen.) There are two reasons why multiple isolating mechanisms seem likely. In theory, no single isolating mechanism except for distinct ecological niches or some types of temporal divergence can at the same time completely prevent gene flow and allow coexistence in sympatry. Two species solely isolated by hybrid sterility, for example, cannot coexist: one will become extinct through excessive hybridization or ecological competition. Species subject only to sexual isolation are ecologically unstable because they occupy identical niches. Second, direct observation often shows that complete reproductive isolation in nature often involves several isolating mechanisms. Schluter (1997), for example, describes several species pairs having incomplete prezygotic isolation. When hybrids are formed, however, they are ecologically unsuited for the parental habitats, and do not thrive.

We know little about the temporal order in which reproductive isolating mechanisms appear. The only study comparing the rates of evolution of different forms of reproductive isolation is Coyne & Orr's (1989*b*, 1997) analysis of pre- versus postzygotic isolation in *Drosophila*. In these studies, prezygotic isolation (mate discrimination) between species is on average a stronger barrier to gene flow than is postzygotic isolation (figure 1). This disparity, however, is due entirely to much faster evolution of sexual isolation in sympatric than allopatric species pairs, suggesting—as we discuss below—the possibility of direct selection for sexual isolation in sympatry. Among allopatric taxa, pre- and postzygotic isolation arise at similar rates. When both pre- and postzygotic forms of reproductive isolation are considered simultaneously, the total strength of reproductive isolation increases quickly with time (figure 2). We are unaware of any analogous data on the rate at which total reproductive isolation increases in other taxa. It would certainly be worth obtaining such information, as it would allow one to see if the 'speciation clock' ticks at the same rate in different groups.

In other taxa, the data are far more impressionistic. In many groups of plants, such as orchids and the genus *Mimulus*, temporal or pollinator isolation sometimes evolve faster than postzygotic isolation, because related species produce fertile offspring when forcibly crossed in the greenhouse but fail to hybridize in nature (Grant 1981). Students of bird evolution have noted that prezygotic isolation often seems to evolve well before hybrid sterility and inviability (Prager & Wilson 1975; Grant & Grant 1996).

But these conclusions must be seen as preliminary. We require additional and more systematic studies in which different forms of reproductive isolation are assessed among pairs of species that diverged at about the same time. Such work is especially practicable in plants, as ecological differences can be studied in the greenhouse, pollinator isolation can be studied *in situ*, and postzygotic isolation can be studied through forced crossing.

(c) *A summary of genetic studies*

Because there are relatively few studies of the genetics of speciation, we have summarized them all in table 1. We

Table 1. *Summary of existing genetic analyses of reproductive isolation between closely related species*

(The reference to each study includes a summary of the results, emphasizing any interesting features. The text gives our criteria for including studies in this table.)

species pair	trait	number of genes	reference (see Appendix 1)
Drosophila heteroneura–D. silvestris	head shape	9	1
Drosophila melanogaster–D. simulans	hybrid inviability	⩾9	2
	female pheromones	⩾5	3
Drosophila mauritiana–D. simulans	hybrid male sterility	⩾15	4
	hybrid female sterility	⩾4	5
	hybrid inviability	⩾5	5
	male sexual isolation	⩾2	6
	female sexual isolation	⩾3	6
	genital morphology	⩾9	7
	shortened copulation	⩾3	8
Drosophila mauritiana–D. sechellia	female pheromones	⩾6	9
Drosophila simulans–D. sechellia	hybrid male sterility	⩾6	10
	hybrid inviability	⩾2	11
	female sexual isolation	⩾2	12
Drosophila mojavensis–D. arizonae	hybrid male sterility	⩾3	13
	male sexual isolation	⩾2	14
	female sexual isolation	⩾2	14
Drosophila pseudoobscura–D. persimilis	hybrid male sterility	⩾9	15
	hybrid female sterility	⩾3	15
	sexual isolation	⩾3	16
Drosophila pseudoobscura U.S.A.–Bogota	hybrid male sterility	⩾5	17
Drosophila buzatti–D. koepferae	hybrid male inviability	⩾4	18
	hybrid male sterility	⩾7	19
Drosophila subobscura–D. madeirensis	hybrid male sterility	⩾6	20
Drosophila virilis–D. littoralis	hybrid female viability	⩾5	21
Drosophila virilis–D. lummei	male courtship song	⩾4	22
	hybrid male sterility	⩾6	23
Drosophila hydei–D. neohydei	hybrid male sterility	⩾5	24
	hybrid female sterility	⩾2	24
	hybrid inviability	⩾4	25
Drosophila montana–D. texana	hybrid female inviability	⩾2	26
Drosophila virilis–D. texana	hybrid male sterility	⩾3	27
Drosophila auraria–D. biauraria	male courtship song	⩾2	28
Ostrina nubialis, Z and E races	female pheromones	1	29
	male perception of pheromones	2	29
Laupala paranigra–L. kohalensis	song pulse rate	⩾8	30
Spodoptera latifascia–S. descoinsi	pheromone blend	1	31
Xiphophorus helleri–X. maculatus	hybrid inviability	2	32
Mimulus lewisii–M. cardinalis	8 floral traits	(see note)	33
Mimulus guttatus–M. micranthus	bud growth rate	8	34
	duration of bud development	10	34
Mimulus, four taxa	(see note)	(see note)	35
Mimulus guttatus pops.	hybrid inviability	2 (system 1)	36
		⩾2 (system 2)	36
Mimulus guttatus–M. cupriphilus	flower size	3–7	37
Helianthus annuus–H. petiolarus	pollen viability	⩾14	38

used two criteria for including a study in this table. First, the character studied must be known to cause reproductive isolation between species in either nature or the laboratory, or be plausibly involved in such isolation. Second, the genetic analysis must have been fairly rigorous, using one of three methods: (i) classical-genetic analyses, in which species differing in molecular or visible mutant markers are crossed, and the segregation of reproductive isolation with the markers examined. Here we included only those studies in which markers were distributed among all major chromosomes; (ii) simple Mendelian analyses in which segregation ratios in backcrosses or F_2s indicated that an isolating mechanism was due to changes at a single locus; or (iii) biometric analyses, in which measurement of character means and variances in backcrosses or F_2s yielded a rough estimate of gene number. Table 1 also gives the actual or minimum number of genes involved for each isolating mechanism. The notes in Appendix 1 give more detail about the type of genetic analysis, whether the species pair was sympatric or allopatric, and a brief summary and critique of the results.

We must add several caveats. First, despite our attempts to comprehensively comb the literature, we have surely missed some studies. Second, the quality of the analyses is

uneven: some classical-genetic studies involved detailed mapping experiments with many markers, whereas others relied on only one marker per chromosome. Third, most studies have underestimated the number of genes causing a single reproductive isolating mechanism. This may reflect a limited number of markers, failure to test all possible interactions between chromosomes, or the use of biometric approaches, which nearly always underestimate true gene number. Finally, data are given for single isolating mechanisms, but several mechanisms may often operate together to impede gene flow in nature.

The most striking feature of table 1 is the imbalance of both species and isolating mechanisms. Roughly 75% of all the studies involve *Drosophila*, with only seven other pairs of taxa, mostly plants from the genus *Mimulus*. Moreover, nearly two-thirds of the *Drosophila* work is on hybrid sterility and inviability. There is no published genetic study of ecological isolation (but see below). Obviously we must extend such studies to other groups and other forms of reproductive isolation.

For convenience, we discuss pre- and postzygotic isolation separately.

5. PREZYGOTIC ISOLATION

Although there are many forms of prezygotic isolation, including differences in ecology, behaviour, time of reproduction, gametic incompatibility and (in plants) differences in pollinators, only one form—sexual isolation—has been the subject of much theory and experiment.

(a) *Sexual isolation*

Many evolutionists have noted that closely-related animal species, particularly those involved in adaptive radiations, seem to differ most obviously in sexually dimorphic traits. This impression has been confirmed by two phylogenetic studies of birds. Barraclough *et al.* (1995) found a positive correlation between the speciosity of groups and their degree of sexual dimorphism, suggesting a link between sexual selection and speciation. (It is not likely that species are simply recognized more easily in strongly dimorphic groups, for the authors note that females of such species can also be distinguished easily.) Mitra *et al.* (1996) found that taxa with promiscuous mating systems contain more species than their non-promiscuous sister taxa. Promiscuously mating species are, of course, more likely to experience strong sexual selection.

The connection between sexual selection and sexual isolation may seem obvious. After all, it is a tenet of neo-Darwinism that reproductive isolation is a by-product of evolutionary change occurring within populations. Two populations undergoing sexual selection may readily diverge in both male traits and female preferences, and the natural outcome of this would be sexual isolation between the populations. This idea is much easier to grasp than, say, the notion that adaptation among isolated populations would cause sterility or inviability of their hybrids; under sexual selection, the pleiotropic effect of the diverging genes (sexual isolation) is obviously connected to their primary effect (exaggeration of male traits or female preferences).

Nonetheless, consideration of the role of sexual selection in speciation was remarkably late in coming. The earliest paper even mentioning such a possibility appears to be that of Haskins & Haskins (1949), who posited that sexual selection in guppies might lead to female recognition of only conspecific males as mates, which would serve as an isolating mechanism among sympatric species. This line of thought then vanished from the literature until Nei (1976) made a model of sexual selection in which one gene controlled the male trait and another the female preference. Exploring the conditions that could lead to the joint evolution of these traits, Nei noted that such a process could cause reproductive isolation. More recently, Ringo (1977) proposed that sexual selection might explain both the adaptive radiation and the strong sexual dimorphism of Hawaiian *Drosophila*.

The most extensive theoretical work on this problem is that of Lande (1981, 1982). In 1981, he showed that sexual isolation could be the by-product of sexual selection if random genetic drift in small populations triggered the 'runaway' process suggested by Fisher (1930). Later workers (e.g. Kirkpatrick 1996) have shown, however, that such instability is unlikely if natural selection acts on female preference. Lande's 1982 model, an explicit quantitative-genetic treatment of clinal speciation via sexual selection, is more realistic. Here he showed that adaptive geographic differentiation in male traits could be amplified by the evolution of female preferences, resulting in reproductively isolated populations along a cline. Data that may support this model come from the guppy *Poecilia reticulata*, in which local populations have differentiated so that females prefer to mate with local rather than foreign males (Endler & Houde 1995).

There is only one theoretical analysis of sympatric speciation resulting from sexual selection. Turner & Burrows (1995) constructed a genetic model of a male trait affected by four loci and a female preference for that trait affected by a single locus with two alleles. Under some conditions, this model produced sympatric taxa showing complete sexual isolation; but these results may be highly dependent on the assumptions. (One such assumption was that the preference locus showed complete dominance, and more realistic assumptions of intermediate dominance and additional loci would almost certainly reduce the probability of speciation.)

The only theoretical study of allopatric speciation resulting from sexual isolation is that of Iwasa & Pomiankowski (1995). Their quantitative-genetic model of sexual selection on a male trait and on female preference assumes a fitness cost to increased female preference. This cost results in a cyclical fluctuation of both trait and preference. Different populations undergoing the same selection regime could then fall out of synchrony, causing sexual isolation. Kirkpatrick & Barton (1995), however, note that this model neglects the possibility of stabilizing selection acting on the male trait around its optimum value for survival, and that such selection (even if very weak) could eliminate the cycling. Moreover, related species do not usually show different stages of elaboration or diminution of the same male-limited trait, but instead the exaggeration of completely different traits. (Some species of bowerbirds, for example, have different forms of elaborate male plumage, while others lack such

plumage but have males who build elaborate bowers; Gilliard 1956.) A useful extension of Iwasa & Pomiankowski's (1995) model might incorporate either novel environments or genetic drift that could launch populations on different trajectories of sexual selection. Schluter & Price (1993) also suggest that, if the secondary sexual traits of males reflect their fitness or physiological condition, differences among habitats that change the female's ability to detect different traits could lead to sexual isolation among populations.

Sexual isolation is often asymmetric, i.e. species or populations show strong isolation in only one direction of the hybridization. This is a common phenomenon in *Drosophila* (Watanabe & Kawanishi 1979; Kaneshiro 1980), is also seen in salamanders (Arnold *et al.* 1996), and may be ubiquitous in other animals. Kaneshiro (1980) offered an explanation of this pattern based on biogeography, but newer data contradict his theory (Cobb *et al.* 1989; David *et al.* 1974). Arnold *et al.* (1996) proposed that mating asymmetry is a transitory phenomenon that decays rapidly as populations diverge, but such asymmetry is often seen among even distantly related species of *Drosophila* (Coyne & Orr 1989*b*). (Of course asymmetry must eventually disappear, because sexual isolation will, with time, become complete in both directions.) There are likely to be other explanations for mating asymmetry, such as the combination of open-ended female preferences and sexual selection operating in only one of two populations. If asymmetry does prove ubiquitous, it may provide important clues about how sexual selection causes speciation.

Unfortunately, there are too few data about the genetics of sexual isolation to confirm or motivate any theory. The few studies listed in table 1 show only that sexual isolation may sometimes have a simple basis, as in races of *Ostrina nubialis*, where complete sexual isolation is apparently based on changes at only three loci (one each for differences in female pheromones, male perception and male attraction), or can be more complicated, as in *Drosophila mauritiana*/*D. sechellia*, where differences in female pheromones alone involve at least five loci. One conclusion that seems reasonable, based as it is on three independent studies (table 1), is that for a given pair of species, the genes causing sexual isolation of males differ from those causing sexual isolation in females. This is not surprising, as there is no obvious reason why genes affecting male traits should be identical to those affecting female perception. (It is possible that sexual selection could cause male and female genes to be closely linked by selecting for genetic correlations between trait and preference, but there is no theory addressing this possibility.) This lack of correlation is worth verifying, however, as its absence is assumed in many models of sexual isolation (e.g. Spencer *et al.* 1986).

Given the likely importance of sexual selection in animal speciation, more genetic analyses are clearly needed. Fortunately, the advent of QTL analysis has made such studies feasible in any pair of species that produces fertile hybrids.

(b) *Reinforcement*

One of the greatest controversies in speciation concerns reinforcement: the process whereby two allopatric populations that have evolved some postzygotic isolation in allopatry undergo selection for increased sexual isolation when they later become sympatric. Reinforcement was introduced and popularized by Dobzhansky (1937), who apparently considered it the necessary last step of speciation. Its wide popularity may have reflected its appealing assumption of a creative (and not an incidental) role for natural selection in speciation (Coyne 1994). Although theoretical studies of reinforcement appeared only recently, two analyses of *Drosophila* (Ehrman 1965; Wasserman & Koepfer 1977) supported the idea: in species pairs with overlapping ranges, sexual isolation was stronger when populations derived from areas of sympatry than from allopatry.

In the 1980s, however, several critiques eroded the popularity of reinforcement. First, Templeton (1981) pointed out that the pattern of stronger sexual isolation among sympatric than allopatric populations could be caused not by reinforcement but by 'differential fusion', in which species could persist in sympatry only if they had evolved sufficiently strong sexual isolation in allopatry. Thus, stronger isolation in sympatry might reflect not direct selection, but the hybridization and fusion of weakly isolated populations. Moreover, it became clear that some of the data offered in support of reinforcement was flawed (Butlin 1987). Finally, the first serious theoretical treatment of reinforcement (Spencer *et al.* 1986) showed that even under favourable conditions (e.g. complete sterility of hybrids), extinction of populations occurred more often than reinforcement.

Recently, however, a combination of empirical and theoretical work has resurrected the popularity of reinforcement. In an analysis of 171 pairs of *Drosophila* species, Coyne & Orr (1989*b*, 1997) found that recently diverged pairs show far more sexual isolation when sympatric than allopatric (figure 3). (An independent analysis of these data by Noor (1997*a*), making less restrictive assumptions, arrived at similar conclusions.) Although in 1989 there was no theoretical work showing that reinforcement was feasible, two such studies have appeared recently. Liou & Price (1994) and Kelly & Noor (1996) showed that reinforcement can occur frequently even if hybrids have only moderate postzygotic isolation. The important difference between these models and that of Spencer *et al.* (1986) is that the former explicitly allow for sexual selection, which greatly enhances the evolution of sexual isolation.

Newer data also support reinforcement. These include a reanalysis of the earlier literature, finding many more possible examples (Howard 1993), and two new studies of species pairs with partially overlapping ranges (Noor 1995; Saetre *et al.* 1997). The work of Saetre *et al.* (1997) on two species of European flycatchers is especially interesting, as the reduced hybridization in sympatry is caused by the divergence of male plumage occurring in that area, a difference presumably caused by sexual selection.

Although the pattern of stronger isolation in sympatry shown in figure 3 might in principle be explained by several processes, including reinforcement and differential fusion (Coyne & Orr 1989*b*), reinforcement seems most likely for several reasons. First, differential fusion posits that cases of strong sexual isolation in sympatry form a subset of the levels of isolation seen in allopatry. Although allopatric taxa might show either strong or weak isolation,

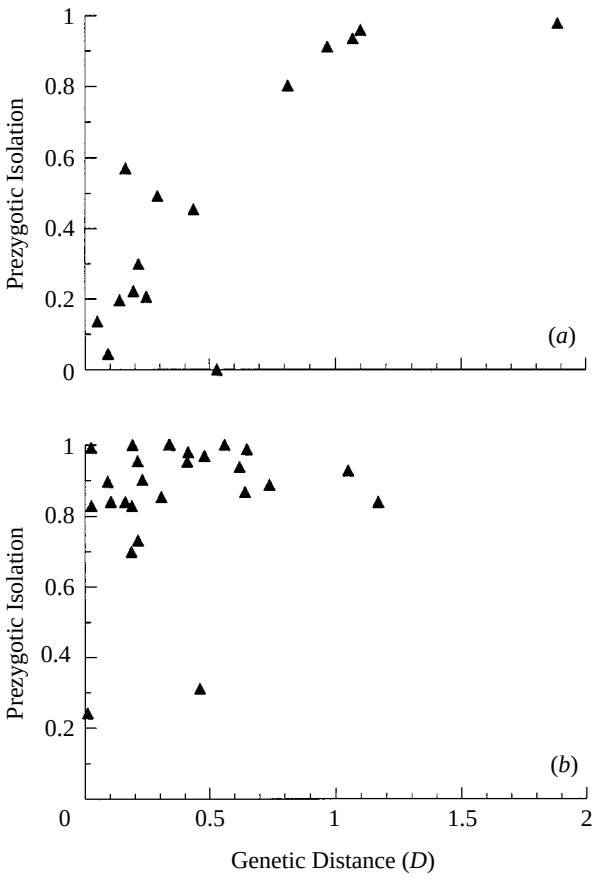

Figure 3. Prezygotic isolation in *Drosophila* plotted against Nei's (1972) electrophoretic genetic distance. Each point represents the average among pairs within a species group. (*a*) Allopatric taxa; (*b*) sympatric taxa. See Coyne & Orr (1989*b*, 1997) for further details.

the latter cases disappear by fusion on geographic contact, leaving us with a pre-existing set of strongly isolated taxa. This is not, however, the pattern seen in *Drosophila*. Figure 3 shows that no recently diverged allopatric taxa have sexual isolation as strong as that seen among sympatric taxa of the same age. Furthermore, differential fusion predicts that postzygotic as well as prezygotic isolation will be stronger in sympatry, as the probability of fusion should decrease with any form of reproductive isolation. The *Drosophila* data also fail to support this prediction: although prezygotic isolation is much stronger in sympatry than allopatry, postzygotic isolation is virtually identical in the two groups (Coyne & Orr 1997).

Finally, it should be noted that reinforcement does not necessarily require the pre-existence of hybrid sterility or inviability, but might also result when allopatric populations evolve some behavioural or ecological difference that leaves hybrids behaviourally or ecologically maladapted. Stratton & Uetz (1986), for example, observed that hybrids between two species of wolf spiders were 'behaviourally sterile', so that hybrid males were rejected by females of both species, and hybrid females refused to mate with any male. Similarly, Davies *et al.* (1997) observed that hybrid females between two species of butterflies suffer a reduced tendency to mate.

The data and theory reviewed above strongly suggest that reinforcement of sexual isolation can occur. This conclusion represents one of the most radical changes of views about speciation over the last decade. Future work must determine whether reinforcement is rare or ubiquitous across animal taxa, and whether it occurs in plants.

(c) *Ecological isolation*

Ecological isolation must play a role in maintaining biological diversity as—even if it is not a primary isolating mechanism—ecological differences are required for the sympatric coexistence of taxa. Consider, for instance, the fate of a newly arisen polyploid plant species. Although all auto- or allopolyploids are automatically postzygotically isolated from their parental species (hybridization produces mostly sterile triploid hybrids), any polyploid that does not differ ecologically from its ancestral species will be quickly driven extinct through either competition or the production of sterile hybrids with its ancestors, rendering it unavailable for study.

'Ecological isolation' actually subsumes three phenomena:

1. Individuals of different species live in the same region and may encounter each other, but confine mating and/or reproduction to different habitats so that hybrids are not formed (e.g. the sympatric, host-specific *Drosophila* that breed on different cacti; Ruiz & Heed 1988). This is a form of prezygotic reproductive isolation, and the only type of reproductive isolation that can by itself both cause complete speciation and allow persistence of species in sympatry. This form of isolation may be the most common result of sympatric speciation (Rice & Hostert 1993).
2. Species live in different subniches of the same area and rarely, if ever, come into contact (e.g. the spadefoot toads *Scaphiopus holbrooki hurteri* and *S. couchi*). Although their ranges overlap extensively, heterospecific toads almost never meet because they are restricted to different soil types (Wasserman 1957). Although this situation corresponds to Dobzhansky's (1937, p. 231) definition of ecological isolation, such species are effectively allopatric.
3. Species live in different subhabitats of the same area and sometimes come into contact with one another and mate, forming hybrids that are not well-adapted to available habitat. Such cases may be common, and have been studied extensively in stickleback fish (Schluter 1996). They constitute examples of *postzygotic* isolation that, while technically a form of hybrid inviability, depend on ecological details of the environment and not on inherent problems of development. Like any other form of postzygotic isolation, 'ecological inviability' could trigger reinforcement between sympatric species (Coyne & Orr 1989*b*). Unlike other forms of postzygotic isolation, however, ecological inviability may—depending on how population size is regulated in the different habitats—allow two species to coexist without prezygotic isolation. In addition, postzygotic isolation based on ecological divergence need not involve complementary gene interactions (epistasis) between alleles of two species. Instead, the genetics of this type of ecological isolation would presumably resemble the genetics of ordinary adaptation (about which we unfortunately know little; Orr & Coyne 1992).

Schluter (1996, 1997) makes a strong case for the importance of ecological isolation (especially type 3) in the origin and persistence of species. Unfortunately, there has been only one study, not yet published, of the genetics of ecological isolation. The island endemic *Drosophila sechellia* breeds exclusively on the normally toxic fruit of *Morinda citrifolia*. All of *D. sechellia*'s relatives, including its presumed ancestor *D. simulans*, succumb to *Morinda*'s primary toxin, octanoic acid. Although *D. sechellia* and *D. simulans* were originally allopatric, they have recently become sympatric and breed on separate hosts. Recent work (Jones 1998) shows that *D. sechellia*'s resistance to octanoic acid involves at least five mostly dominant alleles, distributed over all three of the major chromosomes, with the largest effect mapping to chromosome three.

(d) *Pollinator isolation*

Pollinator isolation probably represents a common form of reproductive isolation in plants (Grant 1981). A variant of this is the isolation of insect-pollinated plants from self-compatible species, a mechanism described in *Mimulus*. The few data at hand (table 1) show that the difference between outcrossing and inbreeding is due to several genes, but that differences in flower shape, colour or nectar reward, that attract different pollinators, may be due to one or a few major genes (Prazmo 1965; Bradshaw *et al*. 1995). This latter observation, if common, might suggest a rapid form of speciation.

(e) *Postmating, prezygotic isolation*

Biologists have begun to appreciate that sexual selection is not limited to obvious behavioural and morphological traits that act before copulation, but can include 'cryptic' characters acting between copulation and fertilization. Such selection (as well as sexually antagonistic selection; Rice 1996) can lead to female control of sperm usage, male–male sperm competition within multiply inseminated females and the mediation of such competition by the female. Selection acting between copulation and fertilization has also been invoked to explain the striking diversity of male genitalia among animal species, bizarre conformations of female reproductive tracts and postcopulation 'courtship' behaviour by males (Eberhard 1985, 1996). Moreover, the relatively rapid evolution of proteins involved in reproduction (Coulthart & Singh 1988; Metz & Palumbi 1996; Tsaur & Wu 1997) is also consistent with sexual selection.

Just as sexual selection acting on male plumage or courtship behaviour can pleiotropically produce sexual isolation, so postcopulation, prezygotic sexual selection can produce 'cryptic' sexual isolation detectable only after fertilization. Such isolation may take the form of either blocked heterospecific fertilization, such as the 'insemination reaction' of *Drosophila* (Patterson 1946) or the preferential use of conspecific sperm when a female is sequentially inseminated by heterospecific and conspecific males. This latter phenomenon has recently been described in grasshoppers (Bella *et al*. 1992), crickets (Gregory & Howard 1994), flour beetles (Wade *et al*. 1994) and *Drosophila* (Price 1997). In three species of *Drosophila*, single heterospecific inseminations produce large numbers of hybrid offspring, but females doubly inseminated by both conspecific and heterospecific males produce very few hybrids. Such reproductive isolation thus depends on competition between conspecific and heterospecific sperm, and resembles interspecific pollen competition in some plants, in which heterospecific pollen tubes grow more slowly than conspecific tubes, yielding reproductive isolation detectable only after double pollination (Rieseberg *et al*. 1995). The only genetic analysis among these studies is that of Price (1997), who showed that, among females, 'conspecific sperm precedence' was dominant, so that F_1 female hybrids between *D. simulans* and *D. mauritiana* induce the same sperm preference as do *D. simulans* females. Gametic incompatibilities may be among the earliest-evolving forms of reproductive isolation, and are worthy of more attention.

6. POSTZYGOTIC ISOLATION

Postzygotic isolation occurs when hybrids are unfit. Evolutionists have, historically, pointed to three types of genetic differences as causes of these fitness problems: species may have different chromosome arrangements, different ploidy levels, or different alleles that do not function properly when brought together in hybrids. Although each of these modes of speciation has enjoyed its advocates, it is now clear that the latter two are by far the most important.

Speciation by auto- and allopolyploidy is clearly common in plants, as about 60% of angiosperms are of polyploid origin (Masterson 1994). We will not, however, discuss polyploid speciation here as it has been thoroughly reviewed elsewhere (see, for example, Grant 1981; Ramsey & Schemske 1998). Instead, we concentrate on the question of whether postzygotic isolation in animals is based on chromosomal or genic differences. We will conclude that genes play a far more important role in hybrid sterility and inviability than do structural differences in chromosomes.

(a) *Chromosomal speciation*

The notion that chromosome rearrangements are a major cause of hybrid sterility was once very popular (White 1969, 1978), and still enjoys a few adherents (e.g. King 1993). The idea of chromosomal speciation, however, suffers from both theoretical and empirical difficulties. The theoretical problems have been discussed elsewhere (Lande 1979; Walsh 1982; Barton & Charlesworth 1984), but the empirical problems have not been widely recognized.

The first such difficulty is that many species producing sterile hybrids are homosequential, that is, they do not differ in chromosome arrangement. White (1969, p. 77), the greatest champion of chromosomal speciation, argued that such cases 'represent only a small fraction of the total number of species complexes that have been extensively studied;'. But this argument is potentially misleading: many of the taxa showing fixed chromosomal differences are old, and may have accumulated many—if not all—of their chromosomal differences after the actual speciation event. Resolving this issue requires systematic comparisons of the extent of chromosomal divergence with both the age of taxa (as determined by molecular data) and the strength of postzygotic isolation. In addition, although

sterile hybrids often suffer meiotic pairing problems, this sterility is often limited to the heterogametic sex (see below). This is not expected if sterility results from the disruption of chromosome pairing, which in most cases should afflict both males and females. Indeed, in some species, such as *Drosophila*, intraspecific chromosomal rearrangements typically sterilize females only (male *Drosophila* have no recombination), but *hybrid* sterility is far more severe in males (Ashburner 1989).

Third, even in species hybrids whose chromosomes fail to pair during meiosis, we do not know whether this failure is caused by differences in chromosome arrangement or differences in genes. As Dobzhansky (1937) emphasized, both rearrangements and gene mutations can disrupt meiosis within species and so, presumably, within species hybrids. The best attempt to disentangle these causes remains the first: *Drosophila pseudoobscura* and *D. persimilis*, which differ by at least four inversions, produce sterile hybrid males (Dobzhansky 1937). Meiotic pairing in hybrids is abnormal and univalents are common. Dobzhansky (1933) showed that islands of tetraploid spermatocytes are often found in hybrid testes. All chromosomes in these 4*N* cells have a homologous pairing partner, and hence should show improved pairing if pairing problems in hybrid cells reflect structural differences between chromosomes. However, univalents are just as common in the hybrid tetraploid as in diploid spermatocytes. The hybrid meiotic problems must therefore have a genetic and not a chromosomal basis. This test has apparently not been repeated in any other hybridization.

A further problem with chromosomal speciation is that it depends critically on the semisterility of hybrids who are heterozygous for chromosome rearrangements. It is not widely appreciated, however, that heterozygous rearrangements theoretically expected to be deleterious (e.g. fusions and pericentric inversions) in reality often enjoy normal fitness, probably because segregation is regular or recombination is prevented (see discussion in Coyne *et al.* (1997)). Any putative case of chromosomal speciation requires proof that different rearrangements actually cause semisterility in heterozygotes, and almost no studies have met this standard.

Finally, direct genetic analyses over the last decade have shown conclusively that postzygotic isolation in animals is typically caused by genes, not by large chromosome rearrangements. Many of these genes have been well mapped, and several have been genetically characterized or even cloned (Wittbrodt *et al.* 1989; Orr 1992; Perez *et al.* 1993). Our main task, therefore, is to understand how the evolution of genic differences can produce hybrid sterility and inviability.

(b) *The Dobzhansky–Muller model*

Understanding the evolution of postzygotic isolation is difficult, because the phenotypes we are hoping to explain—the inviability and sterility of hybrids—seem maladaptive. The difficulty is best seen by considering the simplest possible model for the evolution of postzygotic isolation: change at a single gene. One species has genotype *AA* and the other *aa*, and *Aa* hybrids are completely sterile. Regardless of whether the common ancestor was *AA* or *aa*, fixation of the alternative allele cannot occur because the first mutant individual has genotype *Aa* and so is sterile. Using the metaphor of adaptive landscapes, it is hard to see how two related species can come to reside on different adaptive peaks unless one lineage passed through an adaptive valley.

This problem was finally solved by Bateson (1909), Dobzhansky (1937) and Muller (1942), who noted that, if postzygotic isolation is based on incompatibilities between two or more genes, hybrid sterility and inviability can evolve unimpeded by natural selection. If, for example, the ancestral species had genotype *aabb*, a new mutation at one locus (allele *A*) could be fixed by selection or drift in one isolated population because the *Aabb* and *AAbb* genotypes are perfectly fit. Similarly, a new allele (*B*) at the other locus could be fixed in a different population since *aaBb* and *aaBB* genotypes are also fit. But it is entirely possible that when the *AAbb* and *aaBB* populations come into contact, the resulting *AaBb* hybrids could be sterile or inviable. The *A* and *B* alleles have never been 'tested' together within a genome, and so may not function properly in hybrids.

Alleles showing this pattern of epistasis are called 'complementary genes'. Such genes need not, of course, have drastic effects on hybrid fitness; any particular incompatibility might lower hybrid fitness by only a small amount. It should also be noted that the Dobzhansky–Muller model is agnostic about the evolutionary causes of substitutions that ultimately produce hybrid sterility or inviability; purely adaptive or purely neutral evolution within populations can give rise to complementary genes and thus to postzygotic isolation.

The Dobzhansky–Muller model is the basis for almost all modern work in the genetics of postzygotic isolation. There is now overwhelming evidence that hybrid sterility and inviability do indeed result from such between-locus incompatibilities (reviewed in Orr 1997). Curiously, there have been few theoretical studies of the Dobzhansky–Muller model. Recent analyses, however, predict that the evolution of postzygotic isolation should show several regularities.

(c) *Patterns in the genetics of postzygotic isolation*

Long before any formal studies of the Dobzhansky–Muller model, Muller (1942) predicted that the alleles causing postzygotic isolation must act asymmetrically. To see this, consider the two-locus scenario sketched above. Although the *A* and *B* alleles might be incompatible in hybrids, their allelomorphs *a* and *b* must be compatible. This is because the *aabb* genotype must represent an ancestral state in the evolution of the two species.

There is now good evidence that genic incompatibilities do in fact act asymmetrically. The best data come from Wu & Beckenbach's (1983) study of male sterility in *Drosophila pseudoobscura–D. persimilis* hybrids. When an X-linked region from one species caused sterility upon introgression into the other species' genome, they found that the reciprocal introgression had no such effect. This observation has now been confirmed in other *Drosophila* hybridizations (e.g. Orr & Coyne 1989).

The second pattern expected under the Dobzhansky–Muller model was pointed out more recently. If hybrid sterility and inviability are caused by the accumulation of complementary genes, the severity of postzygotic isolation, as well as the number of genes involved, should 'snowball'

much faster than linearly with time (Orr 1995; see, also, Menotti-Raymond *et al.* 1997). This follows from the fact that any new substitution in one species is potentially incompatible with the alleles at every locus that has previously diverged in the other species. (In our discussion above, the *B* substitution is potentially incompatible with the previous *A* substitution in the other species.) Later substitutions are therefore more likely to cause hybrid incompatibilities than earlier ones. Consequently, the cumulative number of hybrid incompatibilities increases much faster than linearly with the number of substitutions, *K*. If all incompatibilities involve pairs of loci, the expected number of Dobzhansky–Muller incompatibilities increases as K^2, or (assuming a rough molecular clock) as the square of the time since species diverged (Orr 1995). If incompatibilities sometimes involve interactions between more than two loci (see below), the number of hybrid incompatibilities will rise even faster. This snowballing effect requires that we interpret genetic studies of postzygotic isolation with caution. Because the genetics of hybrid sterility and inviability will quickly grow complicated as species diverge, it is easy to overestimate the number of genes required to cause strong reproductive isolation (see Orr (1995) and below).

The limited data we possess are consistent with a snowballing effect, although they do not prove it (see Orr (1995) for a discussion). The simplest prediction of the snowballing hypothesis is that the number of mapped genes causing hybrid sterility or inviability should increase quickly with molecular genetic distance between species. But given the enormous difficulties inherent in accurately mapping and counting 'speciation genes', it may be some time before such direct contrasts are possible.

Third, while we have assumed that hybrid incompatibilities involve pairs of genes, analysis of the Dobzhansky–Muller model shows that more complex hybrid incompatibilities, involving interactions among three or more of genes, should be common. The reason is not intuitively obvious, but is easily demonstrated mathematically (Cabot *et al.* 1994; Orr 1995). Certain paths to the evolution of new species are barred because they would require passing through intermediate genotypes that are sterile or inviable. It is easy to show, however, that the proportion of all imaginable paths to speciation allowed by selection increases with the complexity of hybrid incompatibilities (Orr 1995). Thus, for the same reason that two-gene speciation is 'easier' than single-gene speciation, so three-gene is easier than two-gene, and so on.

The evidence for complex incompatibilities is now overwhelming. They have been described in the *Drosophila obscura* group (Muller 1942; H. A. Orr, unpublished data), the *Drosophila virilis* group (Orr & Coyne 1989), the *Drosophila repleta* group (Carvajal *et al.* 1996), and the *Drosophila melanogaster* group (Cabot *et al.* 1994; Davis *et al.* 1994). Indeed, such interactions could prove more common than the two-locus interactions discussed at length by Bateson, Dobzhansky and Muller.

Theoretical analysis of the Dobzhansky–Muller model also has yielded counterintuitive results about the effect of population subdivision on speciation. Many evolutionists have maintained, for example, that speciation is most likely in taxa subdivided into small populations. At least for postzygotic isolation, this idea is demonstrably false. Orr & Orr (1996) showed that if the substitutions ultimately causing Dobzhansky–Muller incompatibilities are driven by natural selection—as seems likely (Christie & Macnair 1984)—the waiting time to speciation grows longer as a species of a given size is splintered into ever smaller populations. If, however, the substitutions causing hybrid problems are originally neutral, population subdivision has little effect on the time to speciation. In no case is the accumulation of hybrid incompatibilities greatly accelerated by small population size. Unfortunately, we have little empirical data bearing on this issue. Although it might seem that the effect of population size on speciation rates could be estimated by comparing the rate of evolution of postzygotic isolation on islands versus continents, this comparison is confounded by the likelihood of stronger selection in novel island habitats, which itself might drive rapid speciation.

Finally, much of the theoretical work on the evolution of postzygotic isolation has been devoted to explaining one of the most striking patterns characterizing the evolution of hybrid sterility and inviability, Haldane's rule. Because this large and confusing literature has recently been reviewed elsewhere (e.g. Wu *et al.* 1996; Orr 1997), we will not attempt a thorough discussion here. Instead, we briefly consider the data from nature and sketch the leading hypotheses offered to explain them.

(d) *Haldane's rule*

In 1922, Haldane noted that, if only one hybrid sex is sterile or inviable, it is nearly always the heterogametic (XY) sex. More recent and far more extensive reviews (Coyne 1992*c*) show that 'Haldane's rule' is obeyed in all animal groups that have been surveyed, e.g. *Drosophila*, mammals, Orthoptera, birds and Lepidoptera (the latter two groups have heterogametic females). Indeed, it is likely that Haldane's rule characterizes postzygotic isolation in all animals having chromosomal sex determination. Moreover, these surveys show that Haldane's rule is consistently obeyed. In *Drosophila*, for instance, 114 species crosses produce hybrids that are sterile in only one gender. In 112 of these, it is the males who are sterile (Coyne 1992*c*). Comparative work in *Drosophila* also shows that Haldane's rule represents an early stage in the evolution of postzygotic isolation: hybrid male sterility or inviability arises quite quickly, whereas female effects appear only much later (Coyne & Orr 1989*b*, 1997).

For obvious reasons, Haldane's rule has received a great deal of attention: it represents one of the strongest patterns in evolutionary biology, and perhaps the only pattern characterizing speciation. In addition, the rule implies that there is some fundamental similarity in the genetic events causing speciation in all animals.

Although many hypotheses have been offered to explain Haldane's rule, most have been falsified. We will not consider these failed explanations here (for reviews, see Orr 1997; Wu *et al.* 1996; Coyne *et al.* 1991; Coyne 1992*c*). Instead, we briefly review the three explanations of Haldane's rule that remain viable. There is strong evidence for two of these, and suggestive evidence for the third. In a field that has historically been rife with disagreement, a surprisingly good consensus has emerged that some combination of these hypotheses explains Haldane's rule (Orr 1997; Wu *et al.* 1996; True *et al.* 1996).

The first hypothesis, the 'dominance theory', posits that Haldane's rule reflects the recessivity of X-linked genes causing hybrid problems. This idea was first suggested by Muller (1942), and his verbal theory was later formalized by Orr (1993*b*) and Turelli & Orr (1995). The mathematical work shows that heterogametic hybrids suffer greater sterility and inviability than homogametic hybrids whenever the alleles causing hybrid incompatibilities are, on average, partially recessive $\overline{d} < \frac{1}{2}$; this parameter incorporates both the effects of dominance *per se* and any correlation between dominance and severity of hybrid effects (see Turelli & Orr 1995). The reason is straightforward. Although XY hybrids suffer the full hemizygous effect of all X-linked alleles causing hybrid problems (dominant and recessive), XX hybrids suffer twice as many X-linked incompatibilities (as they carry twice as many Xs). These two forces balance when $\overline{d} = \frac{1}{2}$. But if $\overline{d} < \frac{1}{2}$ the expression of recessives in XY hybrids outweighs the greater number of incompatibilities in XX hybrids, and Haldane's rule results. Obviously, the dominance theory can account not only for Haldane's rule, but also for the well-known large effect of the X chromosome on hybrid sterility and inviability (Wu & Davis 1993; Turelli & Orr 1995).

There is now strong evidence that dominance explains Haldane's rule for hybrid inviability. In particular, the dominance theory predicts that, in *Drosophila* hybridizations obeying Haldane's rule for inviability, hybrid females who are forced to be homozygous for their X chromosome should be as inviable as F_1 hybrid males. (Such 'unbalanced' females possess an F_1 male-like genotype in which all recessive X-linked genes are fully expressed.) In both of the species crosses in which this test has been performed, unbalanced females are, as expected, completely inviable (Orr 1993*a*; Wu & Davis 1993). Similarly, there is evidence from haplodiploid species that hybrid backcross males (who are haploid) suffer more severe inviability than their diploid sisters (Breeuwer & Werren 1995).

There is also weaker indirect evidence that the alleles causing hybrid sterility act as partial recessives; Hollocher & Wu (1996) and True *et al.* (1996) found that although most heterozygous introgressions from one *Drosophila* species into another are reasonably fertile, many homozygous introgressions are sterile. It therefore seems likely that dominance contributes to Haldane's rule for both hybrid inviability and hybrid sterility. Last, it is worth noting that the dominance theory—unlike several alternatives—should hold in all animal taxa, regardless of which sex is heterogametic (Orr & Turelli 1996).

The second hypothesis posits that Haldane's rule reflects the faster evolution of genes ultimately causing hybrid male than female sterility (Wu & Davis 1993; Wu *et al.* 1996). Wu and his colleagues offer two explanations for this 'faster male' evolution: (i) in hybrids, spermatogenesis may be disrupted far more easily than oogenesis, and (ii) sexual selection may cause genes expressed in males to evolve faster than those expressed in females. Although there is now good evidence for faster male evolution (see below), this theory cannot be the sole explanation of Haldane's rule. First, it cannot explain Haldane's rule for sterility in those taxa having heterogametic females, e.g. birds and butterflies. After all, spermatogenesis and sexual selection involve males *per se*, whereas Haldane's rule pertains to heterogametic hybrids, male or not. Second, the faster-male theory cannot account for Haldane's rule for inviability in any taxa. Because there is strong evidence that genes causing lethality are almost always expressed in both sexes (reviewed in Orr 1997), it seems unlikely that hybrid 'male lethals' can evolve faster than 'female lethals'. Finally, it is not obvious that sexual selection would inevitably lead to faster substitution of 'male' than 'female' alleles. One can easily imagine, for instance, forms of sexual selection in which each substitution affecting a male character is matched by a substitution affecting female preference for that character. If sexual selection causes faster evolution of male sterility, one may need to consider processes like male–male competition in addition to male–female coevolution.

Despite these caveats, there is now good evidence—at least in *Drosophila*—that alleles causing sterility of hybrid males accumulate much faster than those affecting females (True *et al.* 1996; Hollocher & Wu 1996). (Unfortunately, both of these studies analysed the same species pair; analogous data from other species are badly needed.) Although we cannot be sure of the mechanism involved, it certainly appears that 'faster-male' evolution plays an important role in Haldane's rule for sterility in taxa with heterogametic males.

The last hypothesis, the 'faster-X' theory, posits that Haldane's rule reflects the more rapid divergence of X-linked than autosomal loci (Charlesworth *et al.* 1987; Coyne & Orr 1989*b*). Charlesworth *et al.* (1987) showed that, if the alleles ultimately causing postzygotic isolation were originally fixed by natural selection, X-linked genes will evolve faster than autosomal genes if favourable mutations are on average partially recessive ($\overline{h} < \frac{1}{2}$). (It must be emphasized that this theory requires only that the favourable effects of mutations on their 'normal' conspecific genetic background are partially recessive; nothing is assumed about the dominance of alleles in hybrids. Conversely, the dominance theory requires only that the alleles causing hybrid problems act as partial recessives in hybrids; nothing is assumed about the dominance of these alleles on their normal conspecific genetic background.) Under various scenarios, this faster evolution of X-linked genes can indirectly give rise to Haldane's rule (Orr 1997).

There is some evidence that X-linked hybrid steriles and lethals do in fact evolve faster than their autosomal analogues. In their genome-wide survey of speciation genes in the *Drosophila simulans–D. mauritiana* hybridization, True *et al.* (1996) found a significantly higher density of hybrid male steriles on the X chromosome than on the autosomes. Hollocher & Wu (1996), however, found no such difference in a much smaller experiment. Thus, while faster-X evolution may contribute to Haldane's rule, the evidence for it is considerably weaker than that for both the dominance and faster-male theories. (The faster-X theory also suffers several other shortcomings described elsewhere; Orr 1997.) Definitive tests of the faster-X theory must await new experiments that, following True *et al.* (1996), allow direct comparison of the number of hybrid steriles and lethals on the X versus autosomes.

In summary, there is now strong evidence for both the dominance and faster-male theories of Haldane's rule. Future work must include better estimates of the

dominance of hybrid steriles, better tests of the faster-X theory, and, most important, genetic analyses of Haldane's rule in taxa having heterogametic females. Although both the dominance and faster-male theories make clear predictions about the genetics of postzygotic isolation in these groups (Orr 1997), we have virtually no direct genetic data from these critical taxa. Last, it is important to determine if Haldane's rule extends beyond animals, particularly to species of plants with heteromorphic sex chromosomes.

(e) *Hybrid rescue mutations*

No discussion of postzygotic isolation would be complete without mentioning a recent and remarkable discovery, 'hybrid rescue' mutations. These are alleles that, when introduced singly into *Drosophila* hybrids, rescue the viability or fertility of normally inviable or sterile individuals (Watanabe 1979; Takamura & Watanabe 1980; Hutter & Ashburner 1987; Hutter *et al.* 1990; Sawamura *et al.* 1993*a*,*b*,*c*; Davis *et al.* 1996). All rescue mutations studied to date involve the *D. melanogaster*–*D. simulans* hybridization. In one direction of this cross, hybrid males die as late larvae; in the other, hybrid females die as embryos. All surviving hybrids are completely sterile (Sturtevant 1920). Several mutations are known that rescue the larval inviability: *Lethal hybrid rescue* (*Lhr*) from *D. simulans* (Watanabe 1979), and *Hybrid male rescue* (*Hmr*) and *In(1)AB* from *D. melanogaster* (Hutter & Ashburner 1987; Hutter *et al.* 1990). Despite some early confusion, it now appears that these rescue mutations have no effect on hybrid *embryonic* inviability, which is instead rescued by a different set of mutations: *Zygotic hybrid rescue* (*Zhr*) from *D. melanogaster* (Sawamura *et al.* 1993*c*), and *maternal hybrid rescue* (*mhr*) from *D. simulans* (Sawamura *et al.* 1993*a*). The fact that larval and embryonic lethality are rescued by non-overlapping sets of mutations strongly suggests that these forms of isolation have different developmental bases (Sawamura *et al.* 1993*b*).

Recently, Davis *et al.* (1996) described a mutation that rescues, albeit weakly, the *fertility* of *D. melanogaster*–D. simulans hybrid females. Although little is known about this allele, its discovery suggests that it may be possible to bring all of the genetic and molecular technology available in *D. melanogaster* to bear on speciation. (This species has previously been of limited use in the genetics of speciation because it produces no fertile progeny when crossed to any other species.)

The discovery of hybrid rescue genes has several important implications. First, it suggests that postzygotic isolation may have a simple genetic basis. It seems quite unlikely that a single mutation could restore the viability of hybrids if lethality were caused many different developmental problems. But a simple developmental basis implies in turn a simple *genetic* basis; if many genes were involved, it seems unlikely that they all would affect the same developmental pathway. These inferences have been roughly confirmed by more recent work on the developmental basis of larval inviability in *D. melanogaster*–*D. simulans* group hybrids, which shows that lethal hybrids suffer a profound mitotic defect that can be reversed by introduction of hybrid rescue mutations (Orr *et al.* 1997). This suggests (but does not prove) that hybrid inviability in this case results from a single developmental defect, namely, failure to condense chromosomes during mitosis. (Such a suggestion may seem contradicted by recent introgression experiments showing that many different chromosome regions cause postzygotic isolation when introgressed from one *Drosophila* species into another (True *et al.* 1996; Hollocher & Wu 1996). But the overwhelming majority of these introgressions have discernible effects only when homozygous and, so, would not play any role in F_1 hybrid inviability or sterility (Hollocher & Wu 1996).)

The discovery of hybrid rescue genes may also provide an important short-cut to the cloning and characterization of speciation genes. It is possible that rescue mutations are alleles of the genes that normally cause hybrid inviability or sterility (Hutter & Ashburner 1987). If so, characterization of these mutations might quickly lead to the molecular isolation of speciation genes. Alternatively, rescue mutations might be second-site suppressors, i.e. mutations at some second set of loci that suppress the problems caused by a different, primary set of genes.

(f) *The role of endosymbionts in speciation*

Recent work on postzygotic isolation has pointed to a novel cause of hybrid incompatibilities, cytoplasmic incompatibility (CI) resulting from infection by cellular endosymbionts. Although CI has now been found in at least five orders of insects (Stevens & Wade 1990; Hoffmann & Turelli 1997), most work has focused on two model systems, the fly, *Drosophila simulans* (Hoffmann *et al.* 1986; Turelli & Hoffmann 1995), and the small parasitic wasp, *Nasonia* (Perrot-Minnot *et al.* 1996; Werren 1997).

In both systems, hybrid embryonic lethality results when males from infected populations or species are crossed to females from uninfected populations or species. Moreover, in both cases the infective agent is the *rickettsia*-like endosymbiont *Wolbachia* (Turelli & Hoffmann 1995; Werren 1997). Remarkably, antibiotic treatment of infected lines cures *Wolbachia* infections, allowing fully compatible crosses. Because infected-by-infected crosses are compatible—while the same crosses using genetically identical but cured females are incompatible—the presence of *Wolbachia* in females must confer immunity to the effects of fertilization by sperm from infected males. The basis of this immunity is unknown.

Because CI only occurs when 'naive' uninfected cytoplasm is fertilized by sperm from infected males, CI is typically unidirectional. Recently, however, cases have been found in both *Drosophila* (O'Neill & Karr 1990), and *Nasonia* (Breeuwer & Werren 1995; Perrot-Minnot *et al.* 1996), in which two different populations or species are infected by different varieties of *Wolbachia*. Crosses between individuals carrying these different types of *Wolbachia* are bidirectionally incompatible, so that postzygotic isolation is complete. This represents a remarkable mode of speciation, for no changes are required in the host's genome.

Although *Wolbachia* infections will surely prove common—and speciation workers should routinely test for them—there are reasons for questioning whether CI will prove an important cause of speciation. First, *Wolbachia* cannot explain Haldane's rule, which is ubiquitous among animals and characterizes (at least in *Drosophila*) an early and nearly-obligate step in the evolution of postzygotic

isolation (Coyne & Orr 1989*b*, 1997). Although CI results in dead embryos, we know of no cases in which *Wolbachia* causes lethality of one sex only. Similarly, *Wolbachia* seems unlikely to be a common cause of hybrid sterility; among the many genetic analyses of hybrid sterility, only a single case involves an endosymbiont (Somerson *et al*. 1984).

7. CONCLUSIONS

The increasing prominence of work on speciation reflects real progress: a number of important questions have been resolved by experiment and a number of patterns explained by theory. This progress, in turn, derives from several fundamental but rarely recognized changes in our approach to speciation. First, the field has grown increasingly *genetical*. As a consequence, a large body of grand but notoriously slippery questions (How important are peak shifts in speciation? Is sympatric speciation common?), have been replaced by a collection of simpler questions (Is the Dobzhansky–Muller model correct? What is the cause of Haldane's rule?). Although it would be fatuous to claim that these new questions are more important than the old, there is no doubt that they are more tractable. Second, the connection between theory and experiment has grown increasingly close. Whereas speciation once seemed riddled with amorphous and untestable verbal theories, the last decade of work has produced a body of mathematical theory yielding clear and testable predictions about the basis of reproductive isolation. Last, but most important, many of these predictions have been tested.

Despite this progress, many questions about speciation remain unanswered. Throughout this review we have tried to highlight those questions that seem to us both important and tractable. Most fall into two broad sets. The first concerns speciation in taxa that have been relatively ignored: does reinforcement occur in plants? Do pre- and postzygotic isolation evolve at about the same rate in most taxa as in *Drosophila*? Do plants with heteromorphic sex chromosomes obey Haldane's rule? Do hybrid male steriles still evolve faster than female steriles in taxa having heterogametic females? How distinct are asexual taxa in sympatry?

The second set of questions concerns the evolution of prezygotic isolation, which has received less attention than postzygotic isolation: how complex is the genetic basis of sexual isolation? How common is reinforcement? Why is sexual isolation so often asymmetric? What is the connection between adaptive radiation and sexual isolation?

It may seem that trading yesterday's grand verbal speculations for today's smaller, more technical studies risks a permanent neglect of the larger questions about speciation. We believe, however, that more focused pursuits of tractable questions will ultimately produce better answers to these bigger questions. Just as no mature theory of population genetics was possible until we understood the mechanism of inheritance, so no mature view of speciation seems possible until we understand the origins and mechanics of reproductive isolation.

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APPENDIX 1. NOTES FOR TABLE 1

1. (Templeton 1977; Val 1977.) Sympatric species, biometric analysis. Head shape difference conjectured (but not known) to be involved in sexual isolation between the two species. Templeton (1977, p. 636) concludes that shape difference 'is a polygenic trait determined by alleles with predominantly additive effects'. No confidence limits are given for either of the two estimates of gene number (nine in one study and ten in the other).
2. (Pontecorvo 1943.) Sympatric species, genetic analysis. (Both species are cosmopolitan human commensals.) Gene number is a minimum estimate and includes factors from both species. Results based on analysis of 'pseudobackcross' hybrids between marked triploid *D. melanogaster* females and irradiated *D. simulans* males.
3. (Coyne 1996*a*.) Sympatric species, genetic analysis. (Both species are cosmopolitan human commensals.) Genes affect the ratio of two female cuticular hydrocarbons that appear to be involved in sexual isolation.
4. (True *et al*. 1996; Wu *et al*. 1996.) Allopatric species, genetic analysis. True *et al*.'s (1996) analysis implicates at least 14 regions of the *D. mauritiana* genome that cause male sterility when introgressed into a *D. simulans* background. Wu *et al*. (1996) report 15 genes on the *D. simulans* X chromosome causing hybrid sterility; by extrapolating to the entire genome, they estimate at least 120 loci causing hybrid male sterility. This latter figure may, however, be an overestimate, as some of the X-linked regions studied were known in advance to have large effects on sterility. See also Coyne & Charlesworth (1986), Coyne (1989), Davis & Wu (1996).
5. (True *et al*. 1996.) Allopatric species, genetic analysis. Estimate based on homozygous introgression of *D. mauritiana* segments into *D. simulans*; we have given our minimum estimate of gene number based on the size of introgressions. Regions causing inviability invariably affect both males and females. No large effect of the X chromosome for either character. There could be many more factors affecting both female sterility and inviability, as 19 sites out of 87 cytological positions produced hybrid inviability and 12 sites caused female sterility. Density of male steriles is probably higher than that of female steriles: 65 out of the 185 regions caused male sterility. See also Davis *et al*. (1994), Hollocher & Wu (1996).
6. (Coyne 1989*a*, 1992*b*, 1996*b*.) Allopatric species, genetic analysis. Character studied was sexual isolation between *D. mauritiana* females and *D. simulans* males. The major chromosomes had different effects in the two sexes, implying that different genes are involved in female discrimination versus the male character that females discriminate against.
7. (True *et al*. 1997.) Allopatric species, genetic analysis. Character measured was the shape of the posterior lobe

of the male genitalia. The interspecific difference in this character is thought, but not proven, to be involved in shortened copulation time between *D. mauritiana* males and *D. simulans* females, which itself causes reduced progeny number in hybrid matings (see note 8 below, and Coyne 1983).

8. (Coyne 1993*b*.) Allopatric species, genetic analysis. Shortened copulation time between *D. simulans* females and *D. mauritiana* males contributes to the reduced number of progeny in interspecific crosses.
9. (Coyne & Charlesworth 1997.) Allopatric species, genetic analysis. Ratio of two female cuticular hydrocarbons that appears to affect sexual isolation between these species.
10. (Coyne & Kreitman 1986; Coyne & Charlesworth 1989; Cabot *et al.* 1994; Hollocher & Wu 1996.) Allopatric species, genetic analysis. At least three genes on the X, two on the second, and one on the third chromosome cause sterility in hybrid males.
11. (Hollocher & Wu 1996.) Allopatric species, genetic analysis. A total of two out of three large non-overlapping regions on the *D. sechellia* second chromosome (the only chromosome analysed) cause inviability when introgressed as homozygotes into *D. simulans*.
12. (Coyne 1992.) Allopatric species, genetic analysis. Only two of three major chromosomes are involved in female traits leading to reduced mating propensity of *D. sechellia* females with *D. simulans* males.
13. (Vigneault & Zouros 1986; Pantazidis *et al.* 1993.) Sympatric species, genetic analysis. Trait studied was sperm motility in hybrid males. The Y chromosome and two autosomes affect the character, whereas two other chromosomes do not. The X chromosome was not studied.
14. (Zouros 1973, 1981.) Sympatric species, genetic analysis. Different pairs of chromosomes (and hence genes) are involved in sexual isolation among males versus females (see note 6).
15. (Orr 1987, 1989*b*.) Sympatric species, genetic analysis. Large X-effect was observed in both male and female hybrid sterility. Genes causing male and female sterility are probably different given the different locations and effects. See, also, Wu & Beckenbach (1983).
16. (Noor 1997*b*.) Sympatric species, genetic analysis. In each of the two backcrosses, one X-linked and one autosomal gene affect the male traits discriminated against by heterospecific females. Because the two X-linked genes map to different locations, the minimum estimate of genetic divergence between the species is three loci. See, also, Tan (1946).
17. (Orr 1989*a*,*b*; H. A. Orr, unpublished data.) Genetic analysis between USA populations and an allopatric isolate from Bogota, Colombia. Hybrid male sterility is mostly caused by X-autosomal incompatibilities. Recent work shows that at least three genes are involved on the Bogota X chromosome and two on the USA autosomes (H. A. Orr, unpublished data). Nonetheless, large regions of genome have no discernible effect on hybrid sterility (e.g. chromosome 4, half of chromosome 2, Y chromosome, etc.).
18. (Carvajal *et al.* 1996.) Sympatric species, genetic analysis. Male inviability in backcrosses is caused by at least two factors on the X chromosome of *D. koepferae*. A total of two X-linked loci from *D. koepferae* cause male lethality on a *D. buzzatii* genetic background; this inviability can be rescued by co-introgression of two autosomal segments from *D. koepferae*. Therefore, at least four loci are involved.
19. (Naveira & Fontdevila 1986, 1991.) Sympatric species, genetic analysis. This is probably a considerable underestimate of gene number, particularly on the X, as heterospecific X chromosomal (but not autosomal) segments of any size cause complete male sterility. Marin (1996), however, argues that Naveira & Fontdevila over-estimated the number of autosomal factors causing hybrid male sterility.
20. (Khadem & Krimbas 1991.) Sympatric species, genetic analysis. Male sterility quantified as testis size. Both X-linked and autosomal factors affect testis size in hybrids; the X chromosome has the largest effect.
21. (Mitrofanov & Sidorova 1981.) Sympatric species (*D. virilis* is a cosmopolitan species associated with humans), genetic analysis. Character measured was reduced viability of offspring from backcross females.
22. (Hoikkala & Lumme 1984.) Sympatric species, genetic analysis. A total of four factors (at least one on each autosome) affect the interspecific difference in number of pulses in pulse train of male courtship song. No apparent effect of X. Character is possibly but not yet definitely known to be involved in sexual isolation between these species.
23. (Heikkinen & Lumme 1991.) Sympatric species, genetic analysis. F_1 males are weakly sterile. Backcross analysis uncovered X-2, X-4, X-5 and Y-3, Y-4, Y-5 incompatibilities. Slight female sterility was also detected in backcross.
24. (Schäfer 1978.) Sympatric species (*D. hydei* is a cosmopolitan species associated with humans), genetic analysis. F_1 hybrids are fertile, but backcross hybrids show severe sterility. Female sterility involves a 3–4 incompatibility; some hint of X–A incompatibilities were also suggested, but not proven. Male sterility involves 3–4, X–3, X–4, Y–3, Y–4, and Y–5 incompatibilities.
25. (Schäfer 1979.) Genetic analysis, see note 24 for geographic distribution. Backcross hybrid inviability involves chromosomes X, 2, 3, and 4. Chromosomes 5 and 6 play no role. Thus, whereas a modest number of genes are involved, lethality is not truly polygenic.
26. (Patterson & Stone 1952.) Sympatric species, genetic analysis. F_1 females having *D. montana* mothers are inviable. Analysis showed that inviability results from an incompatibility between a dominant X-linked factor from *D. texana* and recessive maternally acting factor(s) from *D. montana*. Whereas the *D. texana* factor appears to be a single gene (mapped near the *echinus* locus), nothing is known about the number or location of the maternally acting factor(s).
27. (Lamnissou *et al.* 1996.) Sympatric species (*D. virilis* is a cosmopolitan species associated with humans), genetic analysis. Male sterility appears only in backcrosses. Sterility is primarily caused by a Y_{tex}–5_{vir} incompatibility, although milder Y_{tex}–2–3_{vir} incompatibility also occurs. (2–3 is fused in *D. texana*; thus, 2–3 behaves as single linkage group in backcrosses.)

28. (Tomaru & Oguma 1994; Tomaru *et al.* 1995.) Sympatric species, genetic analysis. These species show strong sexual isolation in the laboratory and differ in male courtship songs. Genetic analysis showed that both major autosomes carried at least one gene affecting the interpulse interval (IPI) of the male mating songs, but the X chromosome had no effect (Tomaro & Oguma 1994). In a later study, Tomaru *et al.* (1995) tested *D. biauraria* females with conspecific males whose wings and antennae had been removed, and played synthetic courtship song during these encounters. Conspecific matings occurred more frequently and female rejection behaviours less frequently, when artificial songs contained the conspecific IPI than when they contained the longer IPI characteristic of *D. auraria*. This indicates that the song differences probably contribute to sexual isolation.
29. (Roelofs *et al.* 1987; Lofstedt *et al.* 1989.) Genetic analysis, sympatric races of European corn borers in New York State, USA. Characters studied were female pheromone blend (ratios of two long-chain acetates, whose interspecific difference is apparently caused by a single autosomal locus). The electrophysiological response to these pheromones by male antennae sensilla is caused by another, unlinked autosomal locus, and the behavioural response of male to female pheromones is caused by a single X-linked factor.
30. (Shaw 1996.) Allopatric species, biometric analysis. Trait studied was male song pulse rate of two species of Hawaiian crickets, which may be involved in sexual isolation. Reciprocal F_1 crosses indicate no disproportionate effect of the X chromosome.
31. (Monti *et al.* 1997.) Sympatric species, genetic analysis. Trait studied was ratio of two pheromones, apparently caused by a single factor. The timing of female emission of the pheromones, which differs between the species, appears to be polygenic, and the authors give no estimate of number of factors. Male perception of traits must, of course, also differ if there is to be reproductive isolation, so that, as in corn borers (see note 28), sexual isolation must be caused by changes in at least two genes.
32. (Wittbrodt *et al.* 1989.) Sympatric species, genetic analysis. Hybrid inviability between *X. helleri* (swordtail) and *X. maculatus* (platyfish) is caused by appearance of malignant melanomas, which are often fatal. These cancers are caused by the interaction between a dominant, X-linked oncogene encoding a receptor tyrosinane kinase, and an autosomal suppressor that is either missing in the swordtail or dominant in the platyfish. Some backcross hybrids inherit the oncogene without the suppressor, yielding melanomas.
33. (Bradshaw *et al.* 1995.) Sympatric species, genetic analysis. Traits studied by QTL analysis include flower colour, corolla and petal width, nectar volume and concentration, and stamen and pistil length. All of these traits affect whether a flower is pollinated by bumblebees (*M. lewisii*) or hummingbirds (*M. cardinalis*). Species are at least partly reproductively isolated by pollinator difference. For most traits, the species difference involved at least one chromosome region of large effect. The difference in carotenoids in petal lobes was governed by a single gene.
34. (Fenster *et al.* 1995.) Sympatric species, biometric analysis. Bud-growth rate and duration reflect difference in flower size between these species: *M. micranthus* is largely selfing and apparently derived from the outcrossing *M. guttatus*. It is not known whether this difference causes reproductive isolation in nature, but this seems likely given the decrease in gene flow caused by selfing. No evidence for factors of large effect. Lower bound of 95% confidence interval for gene number is 3.2 for bud growth rate and 4.6 for duration of bud development.
35. (Fenster & Ritland 1994.) Biometric analysis. A total of four taxa of controversial status, named *M. guttatus, M. nasutus,* (both outcrossing), and *M. micranthus* and *M. laciniatus* (predominantly selfing). The latter three taxa are sometimes classified as subspecies of *M. guttatus*. Traits studied included differences in flowering time, corolla length, corolla width, stamen level, pistil length, stigma–anther separation. Selfing species have shorter and narrower corollas, shorter stamens and pistils, and less stigma–anther separation than outcrossers. Flower characters are therefore associated with breeding systems. Minimum number of genes for character differences averaged across all taxa varied between 5 and 13 per trait. Standard errors are large. Our impression is that these phenotypic differences involve several to many genes, with no single locus causing most of the difference in any character between any two taxa. Within a cross, positive genetic correlations were often seen between many traits, so that genes causing these character differences are not necessarily independent.
36. (Macnair & Christie 1983; Christie & Macnair 1984, 1987.) Allopatric populations, genetic analysis. Complementary lethal loci are polymorphic (one in each population) in two North American populations of *Mimulus guttatus*. There are two separate systems of inviability. The first involves only two genes, both polymorphic for complementary lethals. The other involves one locus (possibly the gene involved in copper tolerance) that interacts with an unknown number of genes in non-tolerant population.
37. (Macnair & Cumbes 1989.) Sympatric species, biometric analysis. A total of seven flower-size characters studied, some of which (e.g. height, width, pistil length) are probably related to difference in breeding systems between these species. *M. cupriphilus* selfs much more often than does *M. guttatus*, and this difference in breeding systems may contribute to reproductive isolation. Stamen–pistil length ratio is also important in reproductive isolation, but it was not studied genetically. Each character difference was caused by between three and seven genes. High genetic correlations were observed between many characters, so that traits are not genetically independent.
38. (Rieseberg 1998.) Sympatric species, genetic analysis. Within the colinear portions of the genome of these two sunflower species, Rieseberg estimates that at least 14 chromosomal segments are responsible for inviability of hybrid pollen. Thus, approximately 40 genes are involved if one assumes a similar density of factors in the rearranged portions of the genome.

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