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The Genetics of Sexual Isolation

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For many, the term *speciation* is synonymous with the evolution of reproductive isolation between diverging taxa (Mayr 1963; Dobzhansky 1951). Even if one accepts a less demanding species definition, for example, involving levels of clustering or occupancy of adaptive peaks, the achievement of reproductive isolation is a major evolutionary event as it "fixes" genetic divergence, vastly reducing the potential for subsequent reintegration. The determinants of reproductive isolation are usually classified into premating or postmating factors, after Dobzhansky, depending on whether they come into play before or after sexual partners mate. It is increasingly apparent that isolating factors can act after mating but before fertilization (Howard et al., this volume), blurring the distinction somewhat, but the major classes of premating isolation involve ecological factors (breeding period, season, or location) and mating behaviors contributing to sexual isolation between species, while those of postmating isolation involve hybrid inviability and sterility.

Coyne and Orr (1989a) have carried out the only detailed comparison of the rate of appearance of pre- versus postmating isolation and concluded that both evolved at a similar rate in *Drosophila*, though premating isolation appeared most quickly between sympatric (partially overlapping) species. Against this, there are a number of more anecdotal studies suggesting that many animal species are isolated only by mating behaviors, possibly as a result of sexual selection generating rapid evolution (reviewed in Butlin and Ritchie 1994), though counterexamples can be found. In Dobzhansky's and others' highly influential early studies of North American *Drosophila*, postmating isolation was used in practice as the main criterion for defining species (possibly because it was easier to measure in the laboratory). This could have inflated the apparent importance of postmating factors in this group. Perhaps as a consequence, much of the early literature about the genetics of speciation concerns identifying genetic causes of hybrid dysfunction. Another considerable stimulant behind much of this research is Haldane's Rule, the pattern whereby the heterogametic sex shows most dysfunction in the F_1 of crosses between species (Coyne and Orr 1989b). This is consistent for animals with nonconventional sex chromosome systems such as birds, Lepidoptera, and Orthoptera, and usually holds for both hybrid inviability and infertility. Haldane's Rule has multiple causes (Hollocher and Wu 1996; Wu et al. 1996). In the same species pair, hybrid inviability and sterility can be caused by different genes (Orr 1993). One consistent finding in early studies of the genetics of Haldane's Rule was that epistatically acting sex-linked genes were commonly involved (Coyne and Orr 1989b). This would be expected if the X accumulates differences between developing species more quickly than autosomes (Charlesworth et al. 1987). However, recent studies suggest that sex linkage may simply appear to be involved because of differential expression due to the hemizygosity, rather than accumulation, of X-linked genes in *Drosophila* (Hollocher and Wu 1996; True et al. 1996). Thus there may be no "special effect" of the X-chromosome in postmating isolation (at least in *Drosophila*) in the sense that sex-linked genes are not inherently more likely to effect reproductive isolation, though their divergence is more likely to be seen due to their greater probability of expression. The recent renaissance of the "Dominance Theory" (Turelli and Orr 1995) may provide the most general explanation for Haldane's Rule (Davies and Pomiankowski 1995; but see Hollocher and Wu 1996).

In comparison to postmating isolation, little concerted effort has been spent on analyzing either the genetic causes of, or identifying general rules about, premating isolation. Here we ask if studies of the genetics of sexual isolation show any evidence of a disproportionate affect of sex-linked genes. We also ask to what extent is there evidence that "major genes" may influence premating isolation, that is, that single genes may play a significant role in this aspect of speciation.

Behavior, Sex Linkage, and Major Genes

Following his studies of *Drosophila* courtship song, Ewing (1969) argued that X-linked genes were more likely to be involved in behavioral differences between closely related species because favorable partial recessives would be immediately exposed to selection, allowing more rapid evolution. This clearly could apply to genes with favorable effects on any trait expressed in males (Ewing supposed that male and female genes affecting sexual isolation might show "genetic coupling"). Charlesworth et al. (1987) suggest this could favor more rapid divergence of the X-chromosome. If there is any tendency for genes to accumulate on the X-chromosome, this could encourage further differentiation of any affecting sexual isolation because linkage disequilibrium can facilitate rapid coevolution through reduced recombination between male and female components of sexual communication. The fact that many traits involved in sexual isolation are sex-limited in expression must also favor sex linkage (Rice 1984).

One possible reason why sex-linkage may be less likely is if behaviors are polygenic. It has been the position of some authorities that the conventional allopatric model of speciation (and, indeed, a neodarwinian view of evolution) requires that most adaptive changes are polygenic and as a consequence divergence is piecemeal and gradual. However, recent reviews do not strongly support this position (Orr and Coyne 1992), and it seems likely that the increasing application of Quantitative Trait Loci (QTL) marker methodologies to evolutionary studies will provide further examples of major genes for traits previously thought to be polygenic (e.g., Bradshaw et al. 1995; Mitchell-Olds 1995; Liu et al. 1996). Single genes may be particularly important in rapid differentiation, especially involving selection (and changes between "adaptive peaks"). Behavior may seem particularly likely to be under polygenic control. Behavior genetics is traditionally studied using the methods of quantitative genetics, but mutant studies are finding many examples of major genes, such as *period* (Kyriacou and Hall 1980), which influence behavioral traits. Henry (1994) has argued that bisexual courtship communication may favor the appearance of major genes because coordinated communication systems are resistant to minor disruption, and Bakker and Pomiankowski (1995) found that 8 of 36 studies of the genetics of female mating preferences (not necessarily involved in premating isolation) were suggestive of or compatible with major gene effects. Asymmetrical selection in communication systems might allow mutations of large effect to persist in either signals or receivers (see below; see also Löfstedt 1993; Butlin and Trickett, 1996). We therefore might expect the involvement of major genes in sexual isolation not to be exceptional. However, other authors have argued that coevolving sexual selection may lead to a polygenic architecture (Coyne and Orr 1989a; Coyne et al. 1994). Given the lack of a clear prediction in the background literature, it seems appropriate to consider also evidence for the involvement of major genes in premating isolation.

Scope of the Review

We have reviewed research examining the genetics of assortative mating and behaviors implicated in this—primarily male mating signals and female preferences. We have excluded host plant choice or seasonality unless these are strongly implicated in premating isolation. Methods include conventional crosses between species with examination of F_1s and sometimes the first or second segregating generations. These can identify sex linkage fairly clearly, and sometimes implicate major genes. With *Drosophila,* the use of marked chromosomes in recombinant individuals allows better identification of potential major genes. We only consider a trait polygenic if every marked chromosome contributes to interspecific differences. Of course, under these circumstances major genes could still exist, especially as many studies only involve one or two marker genes per chromosome. The distinction between polygenic and "major gene" systems is necessarily somewhat arbitrary, there will be a continuum from a few genes up to so many that separate analysis is unlikely or unfeasible. Other techniques include biometrical methods which assume polygeny, though allow identification of linkage and nonadditive effects. Mutant studies are fairly common in *D. melanogaster*, but only the *period* example provides a clear connection with interspecific differences, and the mutant recovery techniques are usually biased toward finding sex-linked genes. We have therefore excluded mutant studies. Finally, we have concentrated on interspecific differences but have freely included studies between well-differentiated forms. In some ways, intraspecific studies may be better models of changes contributing to the process of speciation, because many differences between species may have accumulated following the speciation event and therefore be incidental to the process. Polymorphisms within populations are sometimes included (e.g., where a selection experiment has been carried out to mimic interspecific differences), but we have tried not to overemphasize such studies as they may not be representative of speciation. We do not imagine that our survey is exhaustive.

Background

Detailed studies of natural populations are contradicting the once common notion that variation in assortative mating or in "species-specific" mating behaviors is low within species (Paterson 1993; Henderson and Lambert 1982). Numerous studies testify to the continuous nature of speciation, with races of species showing partial isolation, numerous hybrid zones (Hewitt 1989) and considerable variability in signals and preferences (Butlin [1995] found 69 cases of variation in signals or preferences within recognized species versus 18 cases without such variation). It is therefore not surprising that arguments persist regarding species definitions. Recently Pomiankowski and Møller (1995) have shown that traits involved in courtship may be characterized by particularly high levels of genetic variation within populations. A few studies have documented intraspecific variation in mating preferences (Bakker and Pomiankowski 1995), and there are even a handful of studies that have measured genetic covariances between trait and preference. It is therefore surprising to find how few studies really contribute detail to our review. A minority were capable of detecting major genes. Although this is a general issue in quantitative genetics (Barton and Turelli 1989), the major reason for the paucity of good data is probably the frustrating scarcity of crossable species (Wu 1996), especially those with easily analyzable behaviors.

An important question is what constitutes significant sex linkage. Any difference between reciprocal F_1s is evidence of a role of the sex chromosomes (ignoring for the moment maternal effects). Evidence of a disproportionate role of the sex chromosomes requires this to be greater than expected were genes distributed randomly throughout the karyotype. For a sex-limited male trait, the expectation under the standard quantitative genetic model (all additive effects, (+)alleles in one species, (–)alleles in another, assuming further the X-chromosome is of approximately average size) is that the difference between reciprocals as a proportion of the parental difference should be $1/(2n - 1)$. So, for example, the expected magnitude is 14% in *D. melanogaster* (where effectively $n = 3$). This prediction is complicated by unequal chromosome sizes, dosage compensation, and asymmetrical gene action. In *D. melanogaster*, sex-linked genes are doubled in expression, which results in an expectation of simply $1/n$, that is, 33% of the difference (nearer 25% accounting for chromosome size). Plainly, one would expect to find a substantial role for X-linked genes in organisms with low chromosome number even in the absence of differential accumulation of these genes. Many studies do not give the haploid number of the organisms involved.

A substantial literature exists regarding the significance of finding major gene effects, usually in the context of QTL, but also for biometrical methods. In practice most of the studies here have either looked for segregation following crosses or cosegregation with marked chromosomes. Sometimes we have reanalyzed these data using appropriate statistical models to examine the significance of major gene effects where these were not given by the authors.

Case Studies

Table 22.1 summarizes the major components of the database. Below we describe major studies and issues they raise, taking different groups of organisms in turn.

Drosophila

Understandably the most detailed studies available concern *Drosophila*. Traits examined include assortative mating, species-specific acoustic and pheromonal components of courtship, and female preferences (for specific traits or via assortative mating).

Assortative mating (albeit in the laboratory) is probably the most direct method of ascertaining premating isolation. Only six studies are available. Tan (1946) examined assortative mating between recombinant females of crosses between *D. pseudoobscura* and *D. persimilis* (from 50% to 100% *D. pseudoobscura*, effective $n = 4$) when combined with different combinations of wild-type males and females. The best-studied crosses involve recombinant females with wild-type *D. persimilis* males and *D. pseudoobscura* females. The tendency to mate "homogametically" was significantly associated with only the marker for chromosome II with a subsidiary positive interaction between the X and IV (Tan defined homogametic matings here as the tendency for the *D. persimilis* males to mate with the recombinant females—this is positive assortment for *D. persimilis* genes). When the alternative wild-type female in the trio was *D. persimilis* rather than *D. pseudoobscura*, heterogametic matings (once more, matings with the recombinant females) were also mainly associated with chromosome II, though IV played a role. "Homogametic" matings (with the wild-type *D. persimilis* female) were influenced by the origin of the X in the recombinant females. Tan (1946) interpreted this last pattern as effectively male discrimination against sex-linked *D. pseudoobscura* genes, but recent work (Noor 1996) using a more conventional design has shown a lack of species discrimination in males of these species, implying the patterns of assortment seen in Tan's rather complex experimental design is probably due to female preference for unidentified male traits. Using recombinant males in a similar experiment does not reveal an X-effect (Noor, 1997).

Zouros's (1981) study of assortative mating between *D. mojavensis* and *D. arizonae* used a more conventional design involving recombinants (F_1 backcrossed into *D. mojavensis*) of both sexes. Male behaviors were assessed by scoring mating success of recombinant males with wild-type *D. arizonae* males and females. There was a strong effect of both sex chromosomes, with only marginal effects among other chromosomes (and interactions). Female behaviors (pure *D. mojavensis* males with females from backcrosses to *D. arizonae*) also showed clear evidence of major gene effects, with only two auto-

Table 22.1. Major studies of the genetics of sexual isolation in *Drosophila*, Orthoptera, and Lepidoptera.

Organism	Traits	Sex Linkage?	Major Genes?	Authors	Comments
Drosophila pseudoobscura and *D. persimilis*	Assortative mating	Possibly	Yes	Tan 1946	Chromosome II, plus X-linked effect
D. simulans and *D. sechellia*	Assortative mating (BX females to (+)males)	No	Yes	Coyne 1992	Few markers No dominance
D. auraria	Song (ipi)	No	No	Tomaru and Oguma 1994	3 markers
D. paulistorum	Assortative mating	No	No	Ehrman 1961	Assortment weak in one pair
D. mojavensis and *D. arizonensis*	Assortative mating			Zouros 1981	
	BX females	No	Yes, 2 of 5 markers		
	BX males	Yes	Yes, 1 or 2 of 4		
D. simulans and *D. mauritiana*	Assortative mating	No	No	Coyne 1989, 1992	Dominance in F_1 marker arms additive
	Song	No	No	Pugh and Ritchie 1996	Additive
D. melanogaster	Song	No	No	Ritchie et al. 1994 Ritchie and Kyriacou 1996	Intraspecific
D. pseudoobscura and *D. persimilis*	Song "types"	Yes	—	Ewing 1969	Quantitative aspects autosomal
D. virilis and *D. littoralis*	Song traits	Yes	Yes	Hoikkala and Lumme 1987	Disproportionate role of X between *virilis* and *montana* phylads
D. virilis and *D. lummei*	Song	No	No	Hoikkala and Lumme 1984	Within *virilis* phylad
D. simulans and *D. sechellia*	7-T/7-11HD pheromones	No	Yes	Coyne et al. 1994	Maps to chromosome 3
D. melanogaster	7-T/7-P pheromone races	No	Yes	Ferveur and Jallon 1996	
	Female preference for pheromone races	No	Yes	Scott 1994	
D. melanogaster	Assortative mating	No	—	Wu et al. 1995	Intraspecific
Chorthippus parallelus and *C. erythropus*	Various song traits	Possible, also maternal effects	No	Butlin and Hewitt 1988	Intraspecific Trait importance?
Laupala paranigra and *L. kohalensis*	Pulse rate	Yes, but proportional	No; Ne = n	Shaw 1996	Fits polygenic model well, though a genetic background effect
Acheta spp.	Song	Yes	—	Bigelow 1960	
Poecolimon veluchianus subspp.	Body size (under sexual selection)	Yes	—	Reinhold 1994	Testis size Y-linked (in XO system!)
C. bigguttulus and *C. mollis*	Song	Yes	—	von Helversen and von Helversen 1975a,b	male and female songs maternal? Behaviorally uncoupled

Ephippiger ephippiger races	Song and preference	Yes	No	Ritchie 1996	Intraspecific
Teleogryllus oceanicus / *T. commodus*	Song; Intertrill interval	Yes		Bentley and Hoy 1972	Probably polygenic, but quite large differences between F_1s and n = 29
	Pulses per trill, etc.	No		Bentley and Hoy 1972	
	Preference	Yes	Yes	Hoy et al. 1977	
Gryllus spp.	Song			Bentley and Hoy 1972	Sometimes intermediate, but highly variable
Heliothis virescens / *H. subflexa*	Hairpencil glands	Yes	Yes	Teal and Oostendorp 1995	*H. virescens* males normally have hair pencil glands; *H. subflexa* males lack hair pencil glands
Ostrinia nubilalis E/Z pheromone races	Female pheromone production	No	Yes	Roelofs et al. 1987	Intraspecific
	Detection of pheromones by males	No	Possibly	Roelofs et al. 1987	
	Male behavioral contact with female—hair pencil display	No	Possibly (could be linked to the female autosomal factor for determining pheromone components)	Roelofs et al. 1987	
Colias philodice/*C. eurytheme*	Male ultraviolet wing color	Yes	Yes	Silberglied and Taylor 1978	
	Pheromone composition in males	Yes	Yes	Grula and Taylor 1980	
	Mate selection by females	Yes	Yes	Silberglied and Taylor 1978	
Choristneura fumiferana / *C. pinus*	Pheromone composition	Yes	Yes	Sanders et al. 1977	
	Female calling time	Yes	Yes	Sanders et al. 1977	
Y. ponomeuta padellus / *Y. malinellus*	Pheromone composition of females	No	—	Hendrikse 1988	
Papilio glaucus / *P. canadensis*	Oviposition preference	Yes	—	Scriber 1992	
Papilio machaon / *P. zelicaon*	Oviposition preference	Yes	—	Thompson 1988	

Sex linkage is entered as "No" if any effect detected was not disproportionate, and "—" indicates no result reported or not tested.

somes accounting for variation in insemination rates. The X had no effect, clearly showing a lack of any common genetic basis to the sexual behaviors of males and females.

Against those examples, Ehrman's (1961) study of crosses between two pairs of subspecies of *D. paulistorum* showed that every chromosome carries genes contributing to assortment, with no clear major gene effects, and Coyne's (1992) study of isolation in *D. simulans/ D. sechellia* showed every autosome contributing, apart from the X (providing a striking contrast to his examination of postmating isolation between this pair). Wu et al. (1995) obtained similar results in a study of assortative mating between the differentiated Zimbabwe and control strains of *D. melanogaster*, and Welbergen et al.'s (1992) biometrical study of male sexual isolation between *D. simulans* and *D. melanogaster* (a diallel analysis of strains of *D. melanogaster* differing in frequency of interspecific mating with *D. simulans*) showed no sex-linkage effect (and directional dominance rather than additivity). Genetic variation for premating isolation between these species is fairly easily found (e.g., Jamart et al. 1993). The Hawaiian species *D. heteroneura* and *D. silvestris* also show substantial genetic polymorphism for premating isolation, and there is some evidence for epistatic interactions (Ahearn and Templeton 1989).

Turning to traits implicated in premating isolation, the most detailed studies concern cuticular hydrocarbons and male courtship song. Cuticular hydrocarbons are known to have a strong influence on premating isolation in the melanogaster group of *Drosophila*. The 7,11-Heptacosadiene (7,11-HD)/7-tricosene (7-T) polymorphism plays an important role in male recognition of females (Cobb and Jallon 1990; Coyne and Oyama 1995). Male *D. simulans* (7-T) will not court *D. sechellia* females (who carry the other pheromone compound, 7,11-HD). If 7,11-HD is transferred from *D. sechellia* to *D. simulans* females, males will cease to court their conspecific females. Similarly, *D. simulans* males will court *D. sechellia* females (even dead ones) who have acquired 7-T. Analysis of the pheromone blend of backcross females showed that most of the variation was dependent only on the origin of chromosome III (figure 22.1). A pheromone blend polymorphism exists in *D. melanogaster* that influences female mating speed. Females of the common form (with 7-T as the main component) mate more slowly with males carrying 7-pentacosene (7-P) (found in the Tai-Y strain, of Afrotropical origin). The mating discrimination also maps to chromosome III. As Tai-Y females mate randomly, this could be a gene that blocks discrimination against 7-P (Scott 1994). The difference in production of this blend maps to chromosome II (Ferveur 1991). Recent intrachromosomal studies have suggested that both the 7-T/7-P polymorphism and 7-T/7,11-HD species difference are produced by more than one linked gene (Ferveur and Jallon 1996; Coyne 1996). Pheromonal differences between *D. persimilis* and *D. pseudoobscura* are determined by X- and second chromosome-linked genes (Noor and Coyne 1996).

The courtship song of *Drosophila* has been studied for many years, especially in *D. melanogaster*. The X-linked *period* (*per*) gene has been suggested as a possible example of a "speciation gene." It was identified by mutant studies but has been shown to influence species specific differences in courtship song (Wheeler et al. 1991) and activity cycles (Petersen et al. 1988). The song difference influences female mating speed in playback experiments (but the *period* gene itself does not determine the effect in females; Greenacre et al. 1993). However, the total contribution of *per* encoded traits to premating isolation is unclear as Ritchie and Kyriacou (1994a) found *D. melanogaster* females could not be selected to distinguish between males transformed with either the *D. melanogaster* or *D. simulans* allele. Other song traits, notably interpulse interval (IPI), are also likely to play a role. IPI in *D. melanogaster* has an unusual pattern of variability. Mean IPI varies little between and within populations, has low heritability, and shows considerable environmental variation in the laboratory (Ritchie et al. 1994; Ritchie and Kyriacou 1994b). Where variation between populations has been found it has been shown to be additive autosomal (Cowling 1980; Ritchie et al. 1994). Genetic variation can be uncovered by artificial selection experiments. Ritchie and Kyriacou (1996) managed to take the IPI of one line of *D. melanogaster* outside the range of the species within six generations. The response fitted expectations for a polygenic quantitative trait—about 25% of the variation was due to sex-linked genes, which fits expectations for *D. melanogaster* very well. Differences in IPI between *D. simulans* and *D. mauritiana* show a similar architecture (figure 22.2; see also Kyriacou and Hall 1986; Cowling and Burnet 1981).

Other *Drosophila* species have been less well studied. Hoikkala and colleagues have extensive information on the virilis group; for example, Hoikkala and Lumme (1987) carried out a diallel analysis of interspecific song differences. There is evidence of substantial nonadditive effects, directional dominance for characteristics of the pulse thought to be under strongest sexual selection and ambidirectional dominance (indicating possible stabilising selection) for pulse length. In contrast, crosses between species of the virilis subgroup suggested an additive autosomal architecture to species differences, whereas crosses between *D. virilis* and *D. littoralis* (from different subgroups) indicated strong, disproportionate, sex linkage (figure 22.3). Hoikkala and Lumme (1987) speculate that the change in song pattern associated with the X of the Montana group was a key event in the evolution of song pattern within this group, facilitating many structural differences. Ewing (1969) similarly found in crosses between *D. subobscura* and *D. persimilis* that, while quantitative differences in song were additive autosomal, qualitative differences (notably the presence or

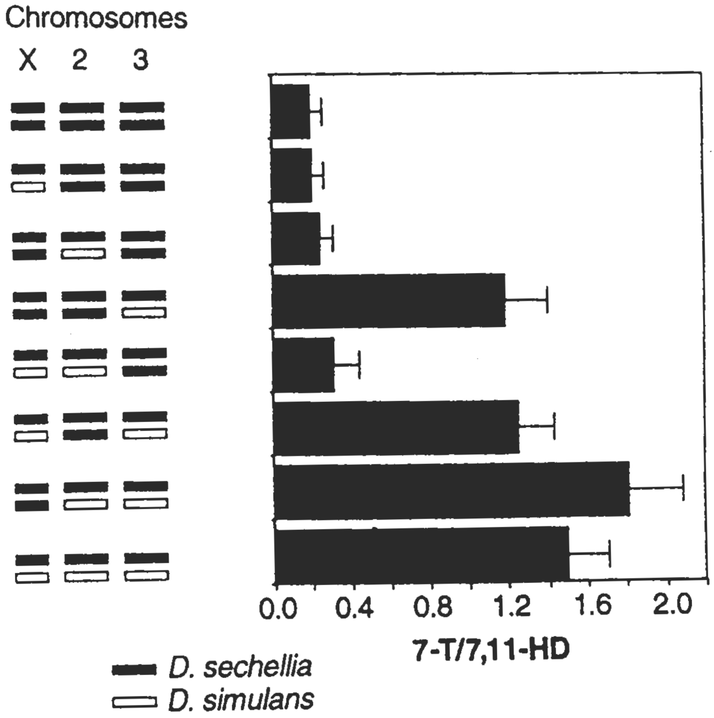

Figure 22.1. The ratio of the pheromones 7-T and 7,11-HD present in backcrosses between *D. simulans* and *D. sechellia* is almost solely determined by the origin of the third chromosome (when heterozygous, more 7,11-HD was produced than 7-T). Shading indicates the species of origin of each chromosome. Reprinted with permission from Coyne et al. (1994). Copyright 1994 American Association for the Advancement of Science.

absence of song types) were sex linked. Tomaru and Oguma (1994) found song to be polygenic in *D. auraria*.

Perhaps the technique with the greatest power to resolve the number of genes influencing a continuous trait is the use of molecular markers for quantitative trait loci. A relevant example is Liu et al.'s (1996) recent study of the shape of genitalia, potentially a character influencing sexual isolation, in crosses between *D. simulans* and *D. mauritiana*. Using 15 markers, they found that nine showed significant linkage effects with the trait and concluded this is sufficiently few to reject the classic "polygenic" model. Exactly where a boundary lies between a trait influenced by major genes and polygenic determination is not clearly defined. Fewer than 10 genes each explaining a significant proportion of the variance of the trait is probably sufficiently few to contemplate independent characterization and study of each locus, which may be as good a definition of a "major gene trait" as any.

Lepidoptera

The Lepidoptera are unusual in that sex linkage of traits that differ among species seems to be common, despite a relatively high chromosome number in this group (Sperling 1994). Prowell (this volume) discusses this phenomenon in some detail and makes some suggestions why this might occur. Here we only briefly summarize some major studies as these relate to sexual isolation.

The method of chromosomal sex determination within the Lepidoptera causes females to be the heterogametic sex (ZZ for male and ZW for female). The traits involved in assortative mating include visual (e.g., *Heliconius*), pheromonal (e.g., *Ostrinia*), and phenological (e.g., many noctuid moths) differences. Of these, the most amenable to study is pheromonal communication. An important aspect of pheromonal communication is that the sex roles are usually also reversed; hence, the highly investing females are the signalers (emitting the pheromone) and males the responders (though further mate assessment might occur once in close proximity). This sex role reversal can have important implications for the ease with which shifts in signal might occur (see discussion below).

At least four Lepidopteran species groups or strains have been clearly shown to demonstrate sex-linked differences in factors affecting premating isolation. The two pierid butterflies *Colias philodice* and *C. eurytheme* are genetically very similar with offspring from F_1, F_2, and

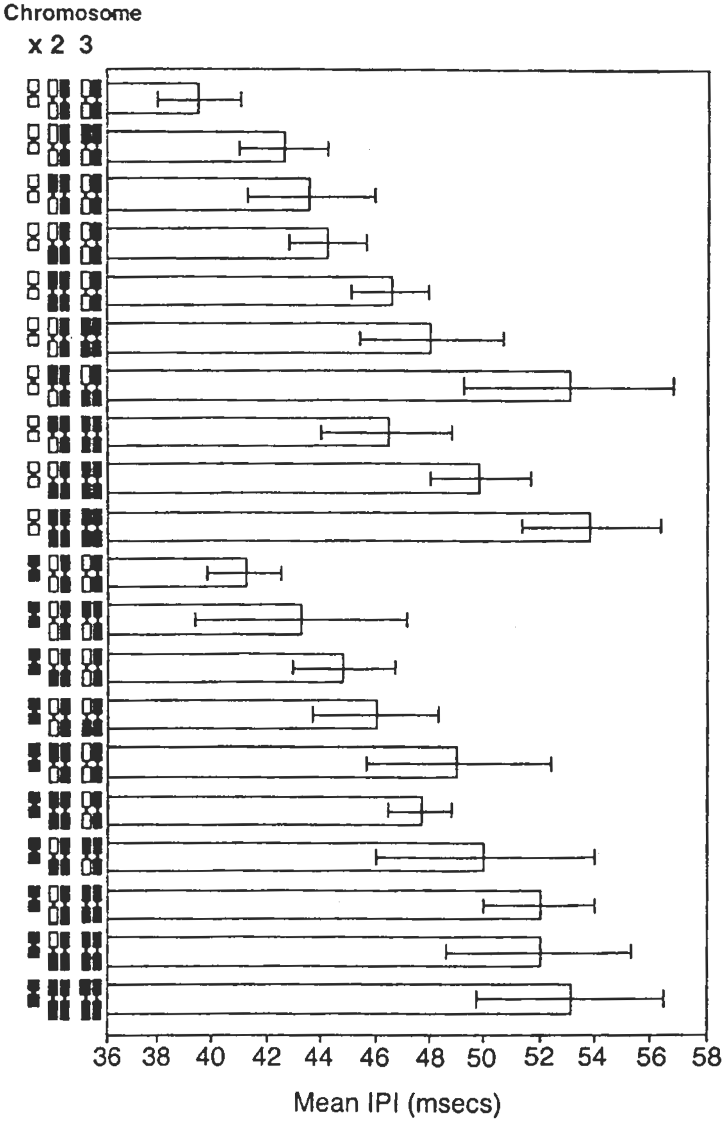

Figure 22.2. The interpulse interval (± 1 standard error) of backcross genotypes generated by crossing F_1 *D. simulans* × *D. mauritiana* females into *D. simulans*. Shading of chromosomes indicates the species of origin of each chromosome arm (open = *D. mauritiana*; solid = *D. simulans*, ignoring recombination in the F_1). Note that the genotypes are first arranged according to the origin of the X-chromosome, but no step is apparent in the IPI data. This would be expected if sex-linked genes made a major contribution to the differences between species. Reprinted from Pugh and Ritchie (1996) with permission of Blackwell Science Ltd.

backcross matings being at least partially viable and fertile (Ae 1979). There is significant Z-linkage for a dramatic number of interspecific differences between these two species. At least three of these contribute to premating isolation: mate selection by females, via female response to species-specific male visual and olfactory cues (Silberglied and Taylor 1978); composition of male pheromones (Grula and Taylor 1980): and male ultraviolet wing color, an important visual signal (Silberglied and Taylor 1978).

The two pheromone races of the European corn borer moth *Ostrinia nubilalis* Hübner (Kochansky et al. 1975) are not considered biological species but have been extensively studied and probably represent an early stage of speciation (Cardé et al. 1978). They show different female calling times, voltinism, and host-plant specific-

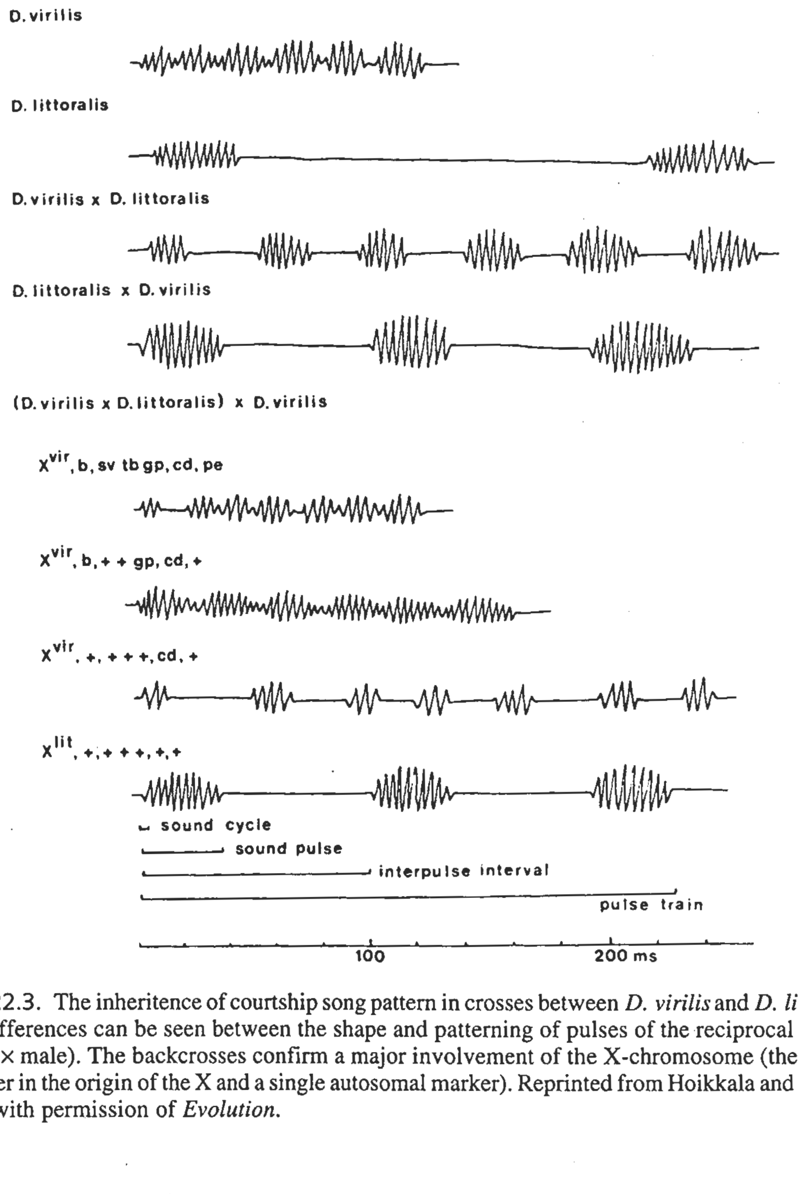

Figure 22.3. The inheritence of courtship song pattern in crosses between *D. virilis* and *D. littoralis*. Clear differences can be seen between the shape and patterning of pulses of the reciprocal hybrids (female × male). The backcrosses confirm a major involvement of the X-chromosome (the bottom two differ in the origin of the X and a single autosomal marker). Reprinted from Hoikkala and Lumme (1987) with permission of *Evolution*.

ity leading to considerable premating isolation (Liebherr and Roelofs 1975). Hybrids can easily be produced in the laboratory. Voltinism (Showers 1981; Glover et al. 1992) and male flight behavioral response to pheromones (Roelofs et al. 1987) have been shown to be Z-linked. However pheromone composition (Klun and Maini 1979) and the physiological basis of pheromone detection by males (Roelofs et al. 1987; Löfstedt et al. 1989) are autosomally controlled.

Two species of tortricid moth, *Choristoneura fumiferana* and *C. pinus,* are sympatric over much of their ranges yet hybridization is unknown in the wild (Smith 1954). Premating isolation is due to at least three factors, female calling time (Sanders et al. 1977), larval-pupal development rate leading to phenological separation (Smith 1953; Campbell 1962), and female pheromone composition (Sanders et al. 1977). All of these have been shown to be significantly Z-linked.

Papilio machaon and *P. zelicaon* are sympatric across much of western North America (Sperling 1987). The major interspecific differences include adult and larval color pattern (Clarke and Sheppard 1955; Sperling 1987), voltinism (Sperling 1994), and larval host-plant (Thompson 1988). The extent of premating isolation in the wild is primarily related to the spatial geography of the different larval host plants. Oviposition preference is partially Z-linked in these species and may thus result in some Z-linked control of premating isolation.

As a counterexample, the most closely related species pair of the *Yponomeuta* complex of ermine moths (consisting of several sympatric cryptic species) are *Yponomeuta padellus* complex and *Y. malinellus*. Premating isolation is controlled by species specific pheromones. F_1 hybrid females generally demonstrate intermediate pheromone composition, suggesting autosomal inheritance (Hendrikse 1988).

Orthoptera

The major component of the mating system of Orthoptera is female phonotaxis to relatively easily analyzed male song, making this group an excellent model for the study of sexual communication. Male songs and female phonotaxis must provide a major component of premating isolation in many sympatric cricket species, as they enable receptive males and females to find each other. Moreover, male songs can provide cues upon which females discriminate among conspecific males, so these songs are also interesting from a sexual selection viewpoint. However, Harrison and Bogdanowicz (1995) recently showed that major structural details of *Gryllus* chirp patterns need not change during speciation (see also Bigelow 1965), and Howard et al. (this volume) have shown how two hybridizing species of cricket can have high assortative fertilization without overt behavioral differentiation. The importance of the song preference system to sexual isolation needs to be considered carefully for individual studies of species pairs.

Two major early studies of orthopteran mating systems gave rise to a substantial literature on the genetics of premating isolation, because they stimulated debate about the "Genetic Coupling" hypothesis (Alexander 1962). Studies of F_1 hybrids between *Teleogryllus oceanicus* and *T. commodus* showed that, although some song traits were intermediate, reciprocal males strongly differed in song pattern. This suggests significant sex-linkage despite a high chromosome number of 29 (Bentley 1971; Bentley and Hoy 1972). Hoy et al. (1977) studied the phonotactic preferences of reciprocal female hybrids and found they preferred song of the appropriate reciprocal hybrid type. Thus, despite the fact that females are heterozygous for sex-linked genes, they still showed a behavioral preference for males from the same reciprocal cross as themselves. In contrast, von Helversen and von Helversen (1975a,b; see also Elsner and Popov 1978) studied F_1 hybrids between the acridid grasshoppers *Chorthippus mollis* and *C. biggutulus* (using female receptive stridulation rather than phonotaxis). Males and females produced songs that could be either intermediate or parental-like in structure, and females could express parental-like patterns of preference (though, e.g., a hybrid female could sing with mollis-like song but respond preferentially to biguttulus-like song!). It has been argued that the reciprocal specificity of preference and trait within the gryllid hybrids suggests a common inheritance, possibly even pleiotropy (hence the traits are genetically "coupled"), whereas the acridid hybrids suggest independent inheritance. In truth, neither of these analyses is sophisticated enough to distinguish these alternatives (Elsner and Popov 1978; Butlin and Ritchie 1989). The ability of the F_1 grasshoppers to behave like the parental species is intriguing and might occur if each haploid complement of genes is sufficient to produce almost parental behaviors. An F_1 female possesses one complete haploid complement from each parental species. Females of crosses between *D. melanogaster* and *D. simulans* show preferences for song that has a combination of traits appropriate for each species; hence, they also show an ability to behave like either parental species (though they also prefer exactly intermediate song; see Kyriacou and Hall 1986; see also figure 22.4). Hybrid males lack a complete haploid complement from the paternal species—this could be a substantial proportion of the relevant genes if there is strong sex linkage or few chromosomes. This does not seem to result in difficulties in expressing parental-like male behaviours in the hybrid grasshoppers. In contrast, in the *Drosophila* example, hybrid males cannot produce the most preferred intermediate song as one of the traits is sex-linked. In the dominance theory of Haldane's Rule, the breakdown of fertility in male hybrids could involve the lack of the sex-linked component of one haploid complement of the paternal genome (Muller 1940; Turelli and Orr 1995; Davies and Pomiankowski 1995).

There is a suggestion in the research we have reviewed that the inheritance of calls and preferences in Orthoptera is unusual. Consider *Teleogryllus* again. Females would be expected to prefer intermediate songs if the genes acted additively (whether or not the relevant genes are sex-linked). Intermediate songs were not presented to these females as the males did not produce them. Hoy et al. (1977) suggested the preference for appropriate reciprocals seen in the homogametic sex might occur if the genes for female preference were sex-linked and a system of dosage compensation involving preferential inactivation of the paternal X-chromosome occurred. Another alternative is maternal (cytoplasmic) inheritance. Ritchie (1996) has found evidence for reciprocal differences for both song and preference in the tettigoniid bush-cricket *Ephippiger ephippiger*, as in *Teleogryllus*. Maternal effects were also sometimes seen in the *Chorthippus biguttulus/mollis* studies, and some song traits within the *C. parallelus* group show inheritance patterns more compatible with maternal than nuclear inheritance (Butlin and Hewitt 1988). Reinhold (1994) found X or maternal effects and a curious paternal effect in sexually selected traits in the tettigoniid *Poecilimon*. Bigelow (1960) found that hybrids between *Acheta* species were intermediate in pulse rate, but that the chirp structure was determined by sex-linked genes (not maternal). Ross (1992) found evidence for linkage or maternal effects in male mate choice in the cockroach *Blatella* (though morphological differences were confirmed to be X rather than maternal). Against these examples, Shaw (1996) recently

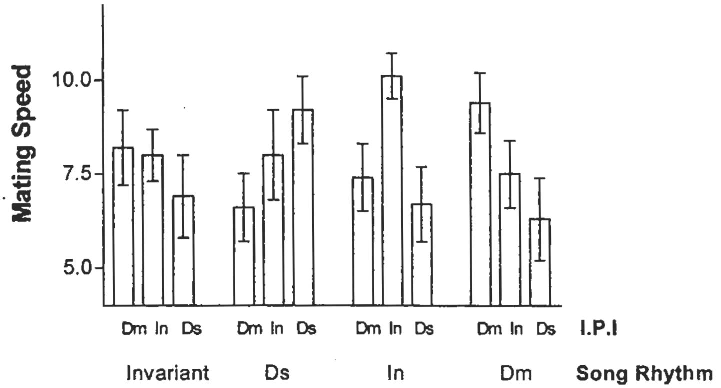

Figure 22.4. Responses of F_1 females produced by crossing *D. melanogaster* females with *D. simulans* males to synthetic song. The synthetic song varies both IPI (which is around 34 ms in *D. melanogaster*, 48 ms in *D. simulans*) and rhythm length (55 s in *D. melanogaster*, 35 s in *D. simulans*). Hybrid males produce an intermediate IPI but their rhythm is determined by the sex-linked *period* gene. Hybrid females can behave like either parental species in that, when presented with song with a normal rhythm length, they prefer the appropriate parental IPI (second and fourth group of histograms). But, when presented with an intermediate rhythm length, they prefer intermediate IPI (third group of histograms). The first group of histograms is for song lacking a rhythm. Reprinted with permission from Kyriacou and Hall (1986). Copyright 1986 American Association for the Advancement of Science.

completed a detailed study of song in crosses between *Laupala* species. She expected to find a simple inheritance pattern (possibly involving single genes) because this is a very recent speciation event and the behavioral basis of the song pattern difference seemed simple (a change in pulse rate). In fact, she found on average about 11% of the difference was sex-linked (though there were surprising differences in this between reciprocals). This corresponds very well with polygenic expectations given n = 8 in these species. Similarly, the number of "effective factors" was found to be 8.4. So, in contrast to the expectations, this study probably provides the best example of a quantitative, additive, polygenic inheritance in the Orthoptera.

Although few of the studies described above are sufficiently detailed to provide more than tentative evidence there is a clear suggestion of sex linkage or maternal effects in several. There have been few hypotheses proposed that could explain maternal effects. One intriguing possibility arises from sexual selection. Hastings (1994) suggested that ornate male traits were more likely to evolve in birds and butterflies because the sex determining system causes females to be the heterogametic sex. If the preference gene is on the sex-determining chromosome, it will therefore only be passed on to daughters. This will facilitate viability indicator processes of sexual selection because the preference gene avoids any association with the negative viability selection imposed on males, who carry the expensive indicator trait. Currently this simple idea lacks supporting empirical evidence, but a pattern of cytoplasmic inheritance would have the same effect. Hoy et al.'s (1977) suggestion of paternal X-chromosome inactivation is not supported by the evidence available suggesting conventional random inactivation in Orthoptera, though this does rely on a single study of *Gryllotalpa* (Rao and Padmaja 1992). At any rate, we should probably be cautious in concluding unusual patterns exist within Orthoptera because the most detailed study available, Shaw (1996) with *Laupala*, shows conventional inheritance.

Other Groups

Few groups have been studied in as much detail as *Drosophila*, Lepidoptera, and Orthoptera. Henry's (1985) studies of neuropteran lacewing *Chrysoperla* seem to show conventional inheritance of song pattern (but see Henry 1994). Wells and Henry (1994) showed that some F_1 song parameters show reciprocal differences. F_1 females prefer hybrid song over one parental, but not the other (curiously, the one that seems most different from an intermediate pattern is not discriminated against). In the hemipteran *Nilaparvata* initial crosses between host races (probably isolated species) implied that differences in vibrational signal are polygenic (Claridge et al. 1985), though subsequent work shows that the number of loci is less than the recombination index, and could be quite low (Butlin 1996).

There are virtually no detailed studies of the genetics of sexual isolation outside of the insects. Important behaviours and morphological traits in *Schizocosa* spiders show apparently simple inheritance patterns (Stratton and

Uetz 1986). Doherty and Gerhardt (1983, 1984) have shown intermediate traits and preferences in crosses between species of tree frog, and a curious example is that of Beiles et al. (1984), who found evidence of a major gene effect for female mating preferences in subterranean mole rats.

Discussion

Surveying the literature reinforces the impression that our knowledge of the genetics of sexual isolation lags behind that of postmating isolation. Limited data are available from a range of species, but only a few have been studied in sufficient depth to give real confidence about the genetic architecture of the species differences. We are therefore cautious about inferring general trends from this survey, but there are some conclusions.

Major Genes

The frequency with which major genes are implicated in the control of sexual isolation differs depending on the trait involved in the sexual signaling system. Song differences are usually polygenic in origin, even between very closely related species with superficially "simple" differences, and in selection experiments. This contradicts Henry's (1994) recent suggestion that major genes may be common in such systems. Polygenic determination may be common in acoustic signals because such behaviors involve the coordinated action of multiple morphological traits and neurons. In contrast, changes in pheromone components or blends are much more commonly dictated by major genes. Again, one can imagine why this might be the case—pheromone synthesis is a complex multistage pathway, but there is a clearer potential for blockage at specific points, producing large changes in the final chemical product. Similarly, it may also be easier to envisage a single gene influencing female preference for a pheromone detection system as opposed to a neural signal processing system. For example, a single protein may have a large effect on the sensitivity or permeability of a surface membrane of an antennal receptor to a specific pheromone. Preferences for pheromones show numerous examples of major genes. Similar results are not found in studies of acoustic preferences, though there are many fewer such studies, and one could imagine the detection of frequency differences having a simple genetic basis (e.g., involving "clock" genes that are known to be expressed in neural tissues). Assortative mating between *Drosophila* species can show major gene effects, and pheromones are probably the most important trait determining the initiation of courtship (via mate recognition). Subterranean mole rat recognition is presumably pheromonal.

Another trend is that major genes are more likely to be found for qualitative than quantitative trait differences, even for traits normally subject to polygenic determination (e.g., the presence and absence of song types in *D. pseudoobscura* and *D. persimilis* is possibly subject to major genes whereas differences in IPI are not; Ewing 1969). Again, this could reflect the possibility of single genes "blocking" the expression of a polygenically controlled behavioral trait rather than a single gene encoding a complex behavior.

Sex Linkage

There are phylogenetic differences in the likelihood of finding sex linkage of genes influencing sexual isolation. There is little convincing evidence that sex-linked genes commonly provide a disproportionate effect except in the Lepidoptera and perhaps the Orthoptera. Moths primarily communicate by pheromones and so possibly will be predisposed to major gene effects. Sex linkage in Lepidoptera is also evident in morphological and other behavioral traits, and may only be part of the phenomenon whereby most traits differing between closely related species are sex-linked (Sperling 1994; Prowell, this volume). The pattern within the Orthoptera is more ambiguous and somewhat confusing. In the absence of further detailed study, we should probably consider sex linkage of genes influencing sexual isolation unproven as a general pattern in the Orthoptera.

Speculation

The trends described above support the idea that the method of chromosomal sex determination and sex roles influences the evolution of communication systems (e.g., Hastings 1994; Butlin and Trickett 1996). In theory, the principal selection pressures in sexual signaling systems act against novel preferences and signals, resulting in strong stabilizing selection for maintenance of the coordinated status quo. Yet rapid divergence occurs during speciation. Certain permutations of signaling systems may be more prone to changes in signals and preferences by mutation than others. In particular, there are three parameters influencing the probability of shifts in sexual signaling systems, which must also influence the expected incidence of sex linkage: nonequal selection pressures acting on the two sexes, nonequal selection pressures acting on rare/mutant signals and preferences, and autosomal versus sex-linked loci.

Nonequal Selection Pressures Acting on the Two Sexes

Trivers (1972) showed that the asymmetry in parental investment in offspring between males and females leads to increased sexual selection acting on males. The fitness cost to a male with a variant sexual trait is likely to be very high due to greatly reduced or lack of mating success. The fitness cost to females with mutant sexual traits is probably lower. De Jong and Sabelis (1991) describe this as the "wallflower" effect where mutant females are likely to be mated eventually but do suffer the cost of increased predation risk prior to reproduction, and the reduced survival chances of offspring produced late in the season.

Nonequal Selection Pressures Acting on Rare/Mutant Signals and Preferences

The "response window" for many species is often wider than its corresponding range of signals (Basolo 1990; Ryan and Rand 1995; Collins and Cardé 1989; Löfstedt 1993; Butlin 1993), due either to arbitrary sensory biases or differences in sexual selection. The specieswide response window could result from either a range of narrow response windows of different centres, or each individual having a wide response window (Butlin and Trickett 1996). Differences in the width of the distribution of signal and preference would influence the selection against mutants affecting either trait (Löfstedt 1993). In figure 22.5 the mean and most preferred signal match (at X), but the preference function has a much greater width. A shift of signal from X to (x – y) causes the new signal to cross the preference curve at (l – m). The corresponding decrease in preference (m) is small. A similar shift of preference from X to (x – y) causes the preference function to cut the signal distribution at (l – n). The corresponding decrease in signal frequency (n) is much larger than (m). A shifted signal therefore remains relatively closer to the preference optimum than a shifted preference does to the peak of the signal distribution. Hence, in the absence of sex differences, the strength of selection against small shifts in signal will be weaker than that for preference. This may affect the expected genetic architecture of signal and preference traits. Mutations of large effect in signals will be more likely to be "tolerated" with the reestablishment of coordination coming through a more gradual polygenic retuning of preference genes.

Autosomal versus Sex-Linked Loci

Recessive mutations at autosomal loci are not initially expressed. For expression to occur it is necessary for the allele to increase via drift to a sufficiently high frequency that homozygotes occur. Dominant mutations will be expressed immediately, but most mutations are recessive. Recessive mutations at sex-linked loci will be expressed immediately and thus, if favored, have a reduced likelihood of being lost stochastically than favored but unexpressed autosomal recessives. This may be particularly true for sex-limited traits that are not available to selection in the homogametic sex.

Putting these three parameters together allows crude predictions to be made about the incidence of finding disproportionate contributions of sex-linked genes in sexual communication systems (figure 22.6). The most likely scenario for a shift is in an organism where the female is the signaler and the heterogametic sex, and where the signal locus is sex-linked. This fits the observation that the Lepidoptera show linkage most clearly. However, apart from the Lepidoptera, our survey provides little evidence of the above patterns. This may be for several reasons. Many examples of homogametic sex-limited characters were found, contrary to predictions, to be sex-linked (e.g., male ultraviolet wing color in *Colias*).

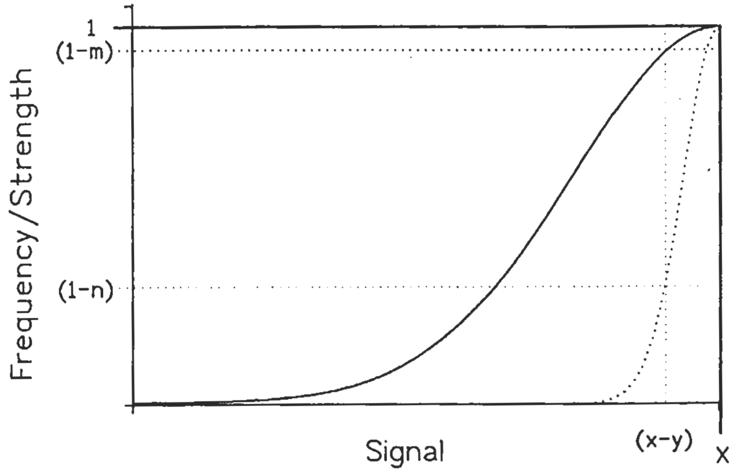

Figure 22.5. Schematic diagram of the consequences of differing widths of signal and preference distributions for the selection against a mutant. Horizontal axis is the value of a signal; vertical axis is the frequency of that signal value in the population (for trait; dotted curve) or strength of the preference for that signal value in the population (for preference; solid curve). For simplicity, only the left sides of the distributions are shown. See text for further details.

	Female *	Female *	Male	Male
	Homo	Hetero	Homo	Hetero
Signaller *	= **	x ***	= *	x **
Receiver	=*	x **	=	x *

Figure 22.6. Possible predictions for the likelihood of linkage in sexual isolation. =, alleles affecting premating isolation are equally likely to be present on any chromosome; x, alleles affecting premating isolation are more likely to be X-linked. The number of asterisks correlates with the ease of a shift in the signaling behaviors (e.g., easier for females, for signalers, and with linkage).

In Lepidoptera possible confounding factors are mechanisms of correctly expressing and developing sex-limited sexually dimorphic traits (Sperling 1994). In fact, most species-specific differences in Lepidoptera seem to be sex-linked, not just those associated with sexual isolation (Prowell, this volume). Reasons for this are obscure, but clearly Lepidoptera represent an important resource for understanding the genetics of speciation.

The generalizations above do allow some predictions to be made. In birds, sex-linked genes may be expected for preferences rather than male traits (perhaps even W-linked; Hastings 1994). Wax moths and a few other Lepidopterans that have secondarily evolved "conventional" sex roles (usually involving males singing or producing pheromones) might have a similar architecture due to the other two effects described above. We would also predict pheromone differences in *Drosophila* to be sex-linked (not apparent in the few detailed studies currently available).

Why No Rule?

Haldane's Rule provides a general framework for studies of the genetics of postmating isolation. The phenomenon, heterogametic sex-limited sterility or inviability, remains constant across taxa, even though the underlying explanations remain elusive and are probably heterogeneous. There does not seem to be any analogous cross-taxa generalization in sexual isolation. Why not? This may be due to an empirical bias in the few studies currently available, but is more likely to be due to fundamental differences in the selective forces that act on pre- and postmating isolation. The genes responsible for post mating isolation are not expressed except upon rare hybridizations. On the whole, they are therefore not subject to natural selection because of their affects on this trait (except for rare cases such as selection for the amelioration of hybrid unfitness in hybrid zones). In contrast, natural and sexual selection must constantly be acting on behaviors influencing mating behavior and, directly or more likely indirectly, sexual isolation. Shifts in signals and preferences seem to occur commonly and rapidly (there are numerous examples of substantial variability despite probable stabilizing selection on communication systems). Reproductive character displacement, abiotic environmental factors, or sexual selection can produce strong selection. Postmating isolation is only a pleiotropic consequence of divergence which occurs in another context. This must produce a more heterogeneous set of genetic systems underlying changes in sexual isolation, militating against common "rules" (Turelli and Orr 1995). Aberrant courtship behaviors seem likely to show more compelling analogies with Haldane's Rule, and there is some evidence that this is indeed the case (Davies et al. 1997; Noor 1997).

Conclusions

Detailed studies of the genetics of sexual isolation are rare. In contrast to studies of postmating isolation, sexual isolation does not have major patterns that are consistent across taxa. Some generalizations do seem to be present, however. Lepidoptera show a preponderance of sex-linked genes involved in sexual isolation, and probably general divergence between closely related species. Reasons for this are not clear, though the combination of role-reversed sexual communication and chromosomal sex determination is probably a factor. Another generalization is that the likelihood of major genes (and hence possibly of rapid speciation) varies with the mode of signaling—pheromonal systems showing many more examples of major gene effects than other systems such as acoustic systems, even where the differences superficially appear simple. The same is probably true of female signal detection systems, though more studies are needed of these. Moths and *Drosophila* provide the most promising systems for finding and characterizing major genes involved in sexual isolation.

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