SEX-LINKED GENES AND SPECIES DIFFERENCES IN LEPIDOPTERA

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Abstract

The Canadian Entomologist **126**: 807–818 (1994)

I review reports on the genetic basis for species differences in the Lepidoptera. In the six best-studied species complexes, more than half of all ecological, behavioral, or physiological differences among species are controlled by X-linked genes. Because Lepidoptera have about 30 pairs of chromosomes, this finding clearly indicates strong bias toward X-linkage of genes for species differences. The proportion of X-linked species differences ranges from complete X-linkage in *Colias* butterflies, to almost none in *Yponomeuta* moths. Four other complexes all have at least one X-linked gene that is crucial to species differences, including the *Choristoneura fumiferana* Clemens, *Papilio glaucus* L., and *Papilio machaon* L. species groups, and *Ostrinia nubilalis* Hübner pheromone strains. The mechanisms that account for this phenomenon are open to speculation. Nonetheless, an interesting implication of disproportionate X-linkage is that reproductive isolation may frequently arise by selection on linkage complexes, rather than as a random byproduct of evolution in geographically isolated populations. If confirmed, the bias toward X-linked species differences may also help efforts to find characters that distinguish host races and sibling species, as well as provide an avenue by which genes crucial to speciation can be more easily mapped and characterized at the molecular level.

Sperling, F.A.H. 1994. Gènes reliés aux chromosomes sexuels et différences spécifiques chez les Lépidoptères. *The Canadian Entomologist* **126**: 807–818.

Résumé

J'ai fait la synthèse des données sur l'origine génétique des différences spécifiques chez les Lépidoptères. Chez les six complexes d'espèces les plus étudiés, plus de la moitié de toutes les différences écologiques, comportementales et physiologiques entre les espèces sont attribuables à des gènes liés au chromosome X. Comme les Lépidoptères ont environ 30 paires de chromosomes, cette constatation indique clairement que les différences spécifiques sont fortement reliées au linkage à l'X des différents gènes. La proportion des différences spécifiques liées à l'X va de 100% chez les espèces de *Colias*, à près de 0% chez les papillons nocturnes du genre *Yponomeuta*. Quatre autres complexes ont tous au moins un gène lié à l'X qui est essentiel aux différences spécifiques, notamment les groupes d'espèces de *Choristoneura fumiferana* Clemens, de *Papilio glaucus* L. et de *Papilio machaon* L., et les lignées d'*Ostrinia nubilalis* Hübner différenciées par leurs phéromones. Les mécanismes responsables de ce phénomène sont mal connus. Cependant, l'un des corollaires intéressants de l'hypothèse d'un linkage à l'X disproportionné suppose que l'isolement génétique est souvent le résultat de la sélection de complexes de linkage plutôt que le produit aléatoire de l'évolution chez des populations isolées géographiquement. Si cette hypothèse est confirmée, l'attribution des différences spécifiques aux gènes liés à l'X peut également rendre plus facile la détermination de caractères qui distinguent les races hôtes et les espèces soeurs; elle peut également faciliter la cartographie des gènes essentiels à la spéciation et permettre de les caractériser au niveau moléculaire.

[Traduit par la rédaction]

Introduction

Sex-linked genes play an intriguing role in speciation. In most animals, hybrid males are more likely to be infertile or sterile than are hybrid females. This phenomenon led Haldane (1922) to hypothesize that hybrid sterility or infertility is associated with the heterogametic sex, which is the sex characterized by an XY or X0 chromosome combination. Lepidoptera and birds are apparent exceptions, in showing greater sterility or inviability in female hybrids. However, they provide the best support for Haldane's rule, because in these groups females are the heterogametic (XY) sex. A related phenomenon, discussed as a separate rule of speciation by Coyne and Orr (1989), is that the genes having the greatest effect on hybrid sterility or inviability are usually X-linked. One plausible explanation for both Haldane's rule and the "X effect" (Coyne and Orr 1989) is that hybrid infertility or inviability is the result of pleiotropic interactions between X-linked genes and autosomal genes. However, the mechanisms underlying either phenomenon remain unresolved (Coyne et al. 1991; Wu 1992; Orr 1993).

Coyne and Orr (1989), relying primarily on studies of *Drosophila*, restricted their definition of the X effect to genes for postzygotic reproductive isolation. In contrast, they stated that genes for morphological and behavioral differences between species, which presumably include genes for prezygotic isolation, are not found disproportionately frequently on sex chromosomes. If this finding is generally true, then sex-linked genes controlling prezygotic reproductive isolation should be uncommon in the Lepidoptera. In butterflies and moths, sex chromosomes comprise one of approximately 30 pairs of similarly sized chromosomes (Robinson 1971), and therefore about 3% of species differences should be sex-linked.

However, Coyne and Orr (1989) cited only a single lepidopteran example (*Colias philodice* Godart/*C. eurytheme* Boisduval) in their tabulation of X-linked genes for postzygotic isolation, and X-linked genes for prezygotic isolation have also been demonstrated in this case (Silberglied and Taylor 1973, 1978; Grula and Taylor 1980*a*). Three other cases of sex-linked genes controlling morphological and behavioral differences between species have been reported in the Lepidoptera (Roelofs et al. 1987; Thompson 1988; Hagen and Scriber 1989). These raise the possibility that sex-linked genes controlling prezygotic isolation between species may be more common in the Lepidoptera than the X effect predicts.

The purpose of this paper is to review reports of genes controlling species differences in Lepidoptera, in order to assess the frequency with which these are sex-linked. I examine only cases for which a wide array of genetic information is available, including morphological, behavioral, and physiological differences. I then discuss mechanisms and potential consequences of disproportionate sex-linkage.

Linkage Relationships of Species Differences

For this paper, species are considered to be populations that maintain their genetic integrity when they contact each other. Some species with incomplete reproductive isolation maintain their genetic integrity in spite of limited gene exchange. Traits that differ between such species are likely to provide information on the kinds of genes that are most important in allowing species to maintain their integrity.

Unlike most other animals, lepidopteran females are the heterogametic sex and males are the homogametic sex (Robinson 1971). The sex chromosomes are sometimes referred to as Z (=X) and W (=Y) in Lepidoptera, but, for consistency, are referred to here as X and Y. Because lepidopteran females receive their X chromosome from their father and their Y chromosome from their mother, X-linkage is detected when F1 females from reciprocal crosses differ from each other and resemble their respective fathers. In contrast, males receive one X from their mother and one X from their father, and F1 males from reciprocal crosses end up with the same combination of X chromosomes and phenotype. Y-linkage or cytoplasmically determined traits are indicated by F1 females that resemble their mother. Males do not have Y chromosomes, and hence exhibit no Y-linked traits, but, like female hybrids, should have the same cytoplasmically controlled traits as their mother. For allozymes and other traits in which heterozygotes can be distinguished, X-linkage may be inferred if heterozygotes are common in males but absent in females.

I have found six lepidopteran species groups whose allozymic, behavioral, and morphological species differences have received extensive genetic study. Each of these groups is discussed separately below. The traits that distinguish the most extensively studied pair of species in each group are listed in Table 1. Numerous additional studies of interspecific hybrids in Lepidoptera are compiled by Robinson (1971), but most of these report on the inheritance of only one or two genes controlling species differences.

1. *Choristoneura fumiferana* Clemens Group. Eight species are recognized in the *Choristoneura fumiferana* group of budworm moths (Harvey 1985). Most of these have parapatric distributions, but *Choristoneura fumiferana*, the spruce budworm, and *C. pinus* Freeman, the jackpine budworm, are extensively sympatric. Species in the *C. fumiferana* group are primarily distinguished by ecological traits related to larval host associations.

Hybrids between *C. fumiferana* and *C. pinus* are unknown in nature (Smith 1954). Although intraspecific hybridization is easily achieved in the lab, and no greater inviability or sterility has been reported in interspecific hybrids than in intraspecific crosses (Smith 1953; Campbell 1961; but see Stehr 1959), investigation of pheromones shows that many female hybrids do not attract males of either parental species (Sanders et al. 1977). Thus pheromone composition contributes to postzygotic inviability as well as to prezygotic isolation.

Of 21 allozyme loci surveyed (Castrovillo 1982), only adenosine aminotransferase (AAT) shows a major allele frequency difference between *C. fumiferana* and *C. pinus*. AAT is the only locus shown to be X-linked (May et al. 1977), and most other species differences are also X-linked (Table 1). Differences in egg weight, larval diapause, and female size or fecundity all probably reflect a tradeoff between rate of population increase and ability to survive overwintering stress (Harvey 1985). Differences in the color of pupal hemolymph and adult wings may be due to selection for crypsis (Stehr 1964). Differences in larval–pupal development rate relate to the phenology of bud break of the host trees, and also influence the timing of adult eclosion and the amount of overlap in the flight season of the two moth species (Smith 1953).

At least three traits contribute to prezygotic isolation between *C. fumiferana* and *C. pinus*: development rate, pheromone composition, and female calling time; all are substantially controlled by X-linked genes.

2. *Ostrinia nubilalis* Hübner Pheromone Strains. Two pheromone strains, labeled E and Z, are known in *Ostrinia nubilalis*, the European corn borer (Kochansky et al. 1975). The pheromone strains of *O. nubilalis* are not generally considered valid species, but are included here because they have been particularly well studied and may provide an example of an early stage of speciation (Cardé et al. 1978). Differences between the strains probably reflect differences in the original source populations in Europe (Klun et al. 1975; Hudon et al. 1989). The Z strain differentiates rapidly in response to new habitats (Lee and Spence 1987) and, in some areas, is divided into two voltinism races that show partial overlap (McLeod 1978; Roelofs et al. 1985).

The two pheromone strains have different hostplant specificities, voltinism, and female calling times, and show considerable prezygotic isolation (Liebherr and Roelofs 1975). However, hybrids have better growth rates under lab conditions than do the parental strains, and hybrid females do not appear to be less fecund, fertile, or viable (Liebherr and Roelofs 1975). Hybrid females produce a mixture of both pheromones (Klun and Maini 1979) and hybrid males, unlike the parental strains, respond to the full range of pheromone blends (Roelofs et al. 1987). Thus there is no evidence for postzygotic isolation.

Two traits that contribute to prezygotic isolation are X-linked: voltinism and male flight response to pheromones (Table 1). Only one allozyme, triose phosphate isomerase (TPI), shows substantial differences in allele frequency between sympatric pheromone strains, and this locus is X-linked (Glover et al. 1990). Four autosomal allozyme loci show only minor differences in allele frequency between the pheromone strains (Harrison and Vawter 1977). Two autosomally controlled differences between the strains also contribute to prezygotic

TABLE 1. Genetically investigated traits that distinguish species or strains. Most traits listed as X-linked are also partially controlled by autosomal genes

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Choristoneura fumiferana/C. pinus (Tortricidae)
- **X-linked:**
  - Egg weight (Campbell 1958, 1962)
  - Pupal weight (Campbell 1958)
  - Pupal hemolymph color (Stehr 1959)
  - Female fecundity and body size (Campbell 1962)
  - Adult wing color (Stehr 1955, 1964)
  - Larval–pupal development rate (Smith 1953; Campbell 1962)
  - Pheromone composition (Sanders et al. 1977)
  - Female calling time (Sanders et al. 1977)
  - AAT allozyme (Castrovillo 1982)
- **Cytoplasmic:**
  - Mitochondrial DNA (unpublished data)
- **Autosomal:**
  - Larval diapause (Harvey 1967)
  - Body weight available for egg production (Campbell 1958)

Ostrinia nubilalis pheromone strains (Pyralidae)
- **X-linked:**
  - Voltinism (Showers 1981; Glover et al. 1992)
  - Male response to pheromones (Roelofs et al. 1987)
  - TPI allozyme (Glover et al. 1991)
- **Autosomal:**
  - Pheromone composition (Klun and Maini 1979)
  - Detection of pheromones by males (Roelofs et al. 1987)

Yponomeuta padellus/Y. malinellus (Yponomeutidae)
- **X-linked:**
  - ?Web structure of cocoons (Hendrikse 1988)
- **Autosomal:**
  - ?Larval APH allozymes (Menken 1980)
  - ?Gustatory sensitivity of larvae (van Drongelen and van Loon 1980)
  - EST-1 and CA-1 allozymes (Arduino and Bullini 1985)
  - Pheromone composition of females (Hendrikse 1988)

Colias philodice/C. eurytheme (Pieridae)
- **X-linked:**
  - Egg-to-adult development rate (Grula and Taylor 1980*a*)
  - Male ultraviolet wing color (Silberglied and Taylor 1973)
  - Mate selection by females (Silberglied and Taylor 1978)
  - Pheromone composition of males (Grula and Taylor 1978)
  - Mating vigor of female hybrids (Grula and Taylor 1980*a*)
  - Female fertility–fecundity (Grula and Taylor 1980*a*)
  - Adult size (Grula and Taylor 1980*a*)
  - Wing pigmentation and black wing border (Grula and Taylor 1980*a*)

Papilio glaucus/P. canadensis (Papilionidae)
- **X-linked:**
  - Pupal diapause (Rockey et al. 1987)
  - Oviposition preference (Scriber 1992)
  - Female mimetic morph suppression (Hagen et al. 1991)
  - LDH and PGD allozymes (Hagen and Scriber 1989)
- **Y-linked or cytoplasmic:**
  - Female mimetic morph determination (Hagen et al. 1991)
  - Mitochondrial DNA (Sperling 1993*b*)
- **Autosomal:**
  - Larval feeding (Hagen et al. 1991)
  - HK allozyme (Hagen and Scriber 1991)

TABLE 1. *(Concluded)*

Papilio machaon/*P. zelicaon* (Papilionidae)
  X-linked:
    Oviposition preference (Thompson 1988)
  Cytoplasmic:
    Mitochondrial DNA (Sperling 1993*a*)
  Autosomal:
    Larval feeding – development rate (Thompson et al. 1990)
    Larval spot color (Clarke and Sheppard 1955; Sperling 1987)
    Four adult color traits (Clarke and Sheppard 1955; Clarke et al. 1977)
    G6PD and Est4 allozymes (Sperling 1987)

isolation: pheromone composition and the physiological basis for detection of pheromone by males. Thus two of four genetically investigated traits contributing to prezygotic isolation in pheromone strains of *O. nubilalis* are X-linked.

3. *Yponomeuta padellus* (L.) Species Complex. The five species of ermine moths recognized in the *Y. padellus* complex have been the subject of substantial study (Menken et al. 1992). All five species are sympatric in western Europe, where they are primarily distinguished by larval hostplant and adult pheromones. The most closely related pair are *Y. padellus* and *Y. malinellus* Zeller, for which Menken (1982) found only minor frequency differences at 51 allozyme loci, including three loci that were X-linked in at least one of the two species. Menken (1980) also found complete allelic substitution between the species at a larval alkaline phosphatase locus, but the enzyme was found to be unstable and was not used in further analyses (Table 1). In a study specifically designed to distinguish *Y. padellus* and *Y. malinellus*, Arduino and Bullini (1985) found three more loci that showed major allelic substitutions, two of which were diagnostic, and their analysis of these additional loci implies that they are autosomal.

In a study of artificial hybrids among four species in the *Y. padellus* group, Hendrikse (1988) found that the F1 progeny of female *Y. malinellus* and male *Y. padellus* developed to the adult stage, but those of the reciprocal cross died in the second larval instar. If confirmed, such asymmetric viability suggests antagonism between cytoplasmic or Y-linked factors and other nuclear genes. Arduino and Bullini (1985) found only a very low frequency of natural hybrids between *Y. padellus* and *Y. malinellus*.

Female hybrids in the *Y. padellus* group generally had intermediate pheromone composition, indicating autosomal inheritance, but web structure of cocoons resembled that of the paternal species and may indicate X-linkage (Hendrikse 1988). Hendrikse (1988) also investigated development rate, pupal weight, calling time, and male response in hybrids but did not comment on linkage nor report results separately for reciprocal crosses. Autosomal inheritance was reported for gustatory sensitivity of larval hybrids between *Y. malinellus* and *Y. cagnagellus* (Hübner) (van Drongelen and van Loon 1980).

Yponomeuta species show unusual sex chromosome inheritance, where the Y chromosome has been translocated to an autosome and the first meiotic division in females is characterized by a trivalent association between the sex chromosomes and a pair of autosomes (Nilsson et al. 1988). However, because the X chromosomes pair normally in male *Yponomeuta* (Nilsson et al. 1988), and Y chromosomes usually carry very few genes in the Lepidoptera (Robinson 1971), this pattern may have little consequence for the evolution of X-linked genes.

4. *Colias philodice* and *C. eurytheme*. These two pierid butterflies, commonly known as the clouded sulphur and the alfalfa butterflies, are the most dramatic example of X-linked species differences reported for the Lepidoptera (Table 1). They have widespread, sympatric ranges and the larvae of both species feed on a variety of leguminous herbs (Scott 1986). In nature, the two species hybridize often but not randomly (Taylor 1972). Female hybrids show significantly reduced viability, fertility, and mating vigor, resulting in a substantial degree of postzygotic isolation (Grula and Taylor 1980*b*).

There is significant X-linkage for all species differences that have been tested. At least three X-linked traits contribute to prezygotic isolation, including male ultraviolet wing color, mate selection by females, and composition of male pheromones (Table 1). The overwhelming predominance of sex-linked species differences prompted Grula and Taylor (1980*b*) to interpret X chromosomes in *Colias* as a closed system of invariable, species-specific blocks of genes, whereas the autosomes form an open system of polymorphic genes that are free to recombine and contribute to local adaptation.

5. ***Papilio glaucus* L. Species Group.** *Papilio glaucus* and *P. canadensis* Rothschild and Jordan comprise two of the six species recognized in the tiger swallowtail species group (Hagen et al. 1991). These two species have large geographic ranges that meet in a narrow line of hybridization along the northern United States. Ecological differences between the species include hostplant of the larvae, pupal diapause, and mimetic color pattern in females. Larvae of *P. glaucus* do not survive well on the hostplant of *P. canadensis*, and vice versa, but hybrid larvae show good growth on both hostplants (Scriber 1986). However, there is a small but significant deficiency of female hybrids, and therefore some postzygotic inviability, in interspecific crosses (Hagen et al. 1991).

Two X-linked ecological traits may contribute to prezygotic isolation between *P. glaucus* and *P. canadensis* (Table 1). The obligatory pupal diapause of *P. canadensis* eliminates the potential for hybridization with the second generation of *P. glaucus*. In addition, it is plausible that the black mimetic morph of *P. glaucus* females may reduce courtship by *P. canadensis* males (Brower 1959), although courtship between these species has not yet been studied.

Three of 26 allozyme loci surveyed by Hagen and Scriber (1991) were considered to be diagnostic between *P. glaucus* and *P. canadensis* (i.e. the loci shared less than 10% of their alleles). Two of the diagnostic loci are X-linked, but the third is autosomal. More X-linked than autosomal loci are listed as diagnostic in the three comparisons among a clade of closely related species including *P. glaucus*, *P. canadensis*, and *P. alexiares* Hopffer. In contrast, in the nine comparisons of these three species with more distantly related species outside this clade, diagnostic autosomal loci outnumber X-linked loci in seven comparisons, and the two types of loci are listed the same number of times in two comparisons. Thus X-linked species differences are most evident in comparisons among more closely related species. These results suggest that rapid evolution of X-linked loci may not be as easy to detect for older divergences, perhaps due to saturation of potential non-lethal mutations.

6. ***Papilio machaon* Species Group**. Natural hybrization is believed to occur among six of the eight species recognized in the Old World swallowtail species group (Sperling 1990). The most complex interaction is that of *P. machaon* and *P. zelicaon* Lucas in western North America (Sperling 1987). These two species are broadly sympatric in this region and differ in hostplant of the larvae, voltinism, larval and adult color pattern, allozymes, and mitochondrial DNA (Table 1).

The extent of hybridization between *P. machaon* and *P. zelicaon* appears to be related to the degree of ecological divergence between parental populations when they contact each other (Sperling 1990). In particular, differences in the location of larval hostplants probably result in separation of mating sites. Because differences in oviposition preference are partially X-linked in these species (Thompson 1988), oviposition preference may also result in some X-linked control of prezygotic isolation. No other traits influencing prezygotic isolation have been mapped, although several ecological, morphological, and physiological species differences are all autosomal (Table 1).

There appears to be significant postzygotic isolation between allopatric populations of *P. machaon* and *P. zelicaon*, as indicated by disproportionate mortality of female hybrids (Clarke and Sheppard 1955; Ae 1979). However, there are no reports on the fertility or viability of hybrids between sympatric populations of these species.

Other Species. There are several other species groups of lepidopterans for which a limited amount is known about the linkage relationships of genetic differences between closely related species, such as *Limenitis* species (Platt 1983), the *Papilio phorcas* Cramer group (Clarke et al. 1991), *Pieris napi* (L.) and *P. bryoniae* (Hübner) (Lorkovic 1986; Geiger 1988), and members of several families of moths (Robinson 1971). However, there is little information on the location of genes contributing to reproductive isolation among these species.

A few lepidopterans are currently receiving intensive genetic and evolutionary study, including species groups in *Heliothis* and *Helicoverpa* (Mitter et al. 1993), *Spodoptera* (Pashley et al. 1992), and *Pieris (Pontia)* (Kingsolver and Wiernasz 1991). In *Helicoverpa armigera* (Hübner), prereproductive period is X-linked (Colvin and Gatehouse 1993). A number of X-linked genes have also been mapped in *Heliothis* species (Heckel 1993), but the details remain unpublished.

Are Species Differences Disproportionately X-linked?

In contrast to Coyne and Orr's (1989) expectation, the six lepidopteran cases in Table 1 show that a large number of sex-linked genes contribute to prezygotic isolation. The most pronounced cases in *Choristoneura* and *Colias* show complete or almost complete X-linkage. The pattern is less pronounced in *Ostrinia*, whose pheromone strains appear to maintain their genetic integrity to a lesser degree than do the other four cases (Glover et al. 1991; Sorenson et al. 1992), and *Yponomeuta*, for which few genes controlling species differences have been explicitly mapped. The genetic linkage of genes contributing primarily to postzygotic viability has still only been determined in *Colias*. As Coyne and Orr (1989) pointed out, this case supports their observation that genes causing postzygotic isolation tend to be sex-linked.

Thus species differences, whether prezygotic or postzygotic, generally show a significant degree of X-linkage. Although many of the traits listed in Table 1 also have some control by autosomal genes, it is clear that a much larger proportion of genes for species differences are X-linked than might be expected from the fact that each of these species also has at least 29 pairs of autosomes.

Species differences in mitochondrial (mt) DNA may be correlated with sex-linked species differences (Sperling 1993*b*). Since mtDNA is maternally inherited, and Haldane's rule and the X effect both imply disproportionate elimination of females in the Lepidoptera, mtDNA can be expected to be disproportionately eliminated in hybrid interactions. *Choristoneura* and the two *Papilio* cases all show clear differences in mtDNA between species (Table 1). In fact, *Choristoneura fumiferana* and *C. pinus* have different mtDNA in spite of very little hybrid inviability or sterility. Thus the processes that cause a bias toward X-linked species differences may also cause a bias toward early divergence of the cytoplasmic genome.

However, it is important not to interpret too literally the frequency of sex-linked species differences in Table 1. One potential bias that should be considered is that sex-linkage is relatively easy to establish, because sex provides a convenient marker for the X and Y chromosomes, whereas no consistent markers are known for any lepidopteran autosomes (Heckel 1993). In addition, autosomal inheritance may not be considered remarkable and hence may be less likely to be reported. For example, *Yponomeuta* species may show an even lower frequency of sex-linked species differences than is indicated in published reports; given the detailed nature of Hendrikse's (1988) investigations, it seems likely that X-linkage would have been reported if it had been found for more behavioral and morphological traits.

Nonetheless, three lines of evidence suggest that X-linked differences tend to be associated with the species boundary. First, classic studies show only very low frequencies of X-linked genes in mutants within *Ephestia kuhniella* Zeller and *Bombyx mori* (L.) (Caspari and Gottlieb 1975; Tazima et al. 1975), and no X-linked genes are known from extensive investigations of mimetic variation within species of *Papilio* and *Heliconius* (Clarke et al. 1968; Clarke and Sheppard 1972; Sheppard et al. 1985). Second, loci showing allozyme polymorphism within species of Lepidoptera show no noticeable tendency to be X-linked (e.g. May et al. 1977; Menken 1980; Harrison et al. 1983), and X-linked allozymes are generally rare (Menken and Ulenberg 1987). Yet, as shown by *Choristoneura*, *Ostrinia,* and *Papilio glaucus*/*P. canadensis*, concerted efforts to find diagnostic characters have led in each case to X-linked loci. Third, as is illustrated by the *P. glaucus* species group, X-linked loci may be more likely to be diagnostic for closely related species than for more distantly related species.

Mechanisms

One mechanism causing species differences to be X-linked may simply be that recessive genes on X chromosomes are more exposed to selection in the heterogametic sex (Charlesworth et al. 1987). If this is true then X-linked genes may generally evolve quickly within populations as well as between species. An example in the Lepidoptera may be the genes controlling prereproductive period in females. These determine the length of time between eclosion and mating, and a longer prereproductive period appears to lead to longer dispersal distances. Prereproductive period is polymorphic within species and strongly influenced by genes on the X chromosome in four genera of noctuid moths (Han and Gatehouse 1991; Hill and Gatehouse 1992; Wilson and Gatehouse 1992; Colvin and Gatehouse 1993). However, this mechanism does not explain the presence of X-linked genes whose expression is limited to males, because in this case two X chromosomes are present just as for autosomes. Thus, as noted by Löfstedt (1990), it is difficult to explain X-linkage in traits like male response to pheromones or male-limited wing color.

Johnson and Turner (1979) suggest a mechanism that may lead to disproportionate X-linkage of male-limited traits. They report that dosage compensation does not occur for an X-linked enzyme in *Heliconius* butterflies, because females produce only half as much of the enzyme as do males. They suggest that this phenomenon may be widespread in the Lepidoptera and helps to explain why female-limited mimicry is rarely sex-linked, whereas male-limited coloration characters are commonly sex-linked. They envision a mechanism by which expression of a trait is only triggered by a large dose and not by a small one. In this situation, male-limited traits are easily turned on by the double dose produced by an X-linked locus, but female-limited traits are not. The primary genes for a male-limited trait are expressed because they are (1) on the X chromosomes or (2) they are autosomal but triggered by interaction with the double dose of an X-linked gene product. Because the X chromosomes are involved in both alternatives, approximately half of the genes controlling male-limited traits should be X-linked.

Given the wide range of traits that may cause reproductive isolation, and the complexity of genetic interactions involved, it seems unlikely that any single mechanism will explain a preponderance of X-linked species differences (Wu 1992). In *Drosophila,* for example, it has now been demonstrated that hybrid inviability and hybrid sterility are two separate genetic phenomena (Orr 1993).

Consequences

If X-linkage tends to occur for genes controlling both behavioral and ecological species differences, then there is obvious potential for linkage interactions among these traits to shape the evolution of reproductive isolation among populations. In particular, biotypes that have an association between ecological traits and mating traits have long been considered to have the potential for sympatric speciation (Diehl and Bush 1984; Tauber and Tauber 1989).

X-linkage has been demonstrated for important species differences in all the lepidopteran examples detailed above, except *Yponomeuta*. Linkage maps for the location of genes on the X chromosome have been constructed only for *Papilio glaucus* and *P. canadensis*. Thus the association between ecological traits and mating traits in some of the remaining cases may be due to pleiotropy rather than physical linkage. For example, in *Choristoneura*, *Yponomeuta*, and the *Papilio machaon* group, the location of hostplants of larvae may affect the location of mating sites, and in *Choristoneura*, *Ostrinia*, the *Papilio glaucus* group, and the *P. machaon* group, voltinism differences impose phenological constraints that increase the likelihood of assortative mating (Table 1; Hendrikse and Vos-Bünnemeyer 1987; Sperling 1990).

In any case, it is often observed that a number of factors act simultaneously to produce full reproductive isolation between closely related species of Lepidoptera (e.g. Hendrikse 1979; Harvey 1985; Sperling 1990; Pashley et al. 1992). Physical linkage on the X chromosome provides a means by which associations between two or more genes are maintained, at least for *Choristoneura* and *Colias*. Mapping genes for species differences thus provides insight into one of the most contentious problems in the study of speciation (Coyne 1992): does reproductive isolation arise by drift, as a byproduct of evolution in geographically isolated populations, or does it arise by selection, particularly on polymorphisms among linkage complexes of genes controlling ecological, mating, viability, or sterility traits? The examples in *Choristoneura* and *Colias* suggest that selection on X-linked complexes of ecological and mating traits may be an important mode of speciation in the Lepidoptera. In *Ostrinia* pheromone strains, however, there is still high incidence of X-linked species differences, but two important traits that affect reproductive isolation are primarily controlled by unlinked autosomal genes. Thus the correlation between X-linkage and species differences is by no means a strict rule.

Nonetheless, this correlation may have practical applications. If confirmed, the phenomenon could help to focus efforts to find diagnostic characters that distinguish host races and sibling species. In such cases it may be profitable to survey allozyme loci that are known to be X-linked in related taxa, or to look for differences between female F1 hybrids from reciprocal crosses. In addition, X-linked marker genes, like TPI in *Ostrinia* and AAT in *Choristoneura*, can be used to localize genes that allow populations to maintain their genetic integrity (e.g. genes for control of diapause and pheromone production). The fact that these genes are concentrated on the X chromosomes of many Lepidoptera provides an exciting new avenue for investigating host race formation and speciation.

Acknowledgments

I thank J. Spence, D. Hickey, A. Ewen, and two anonymous reviewers for comments on this paper. This work was supported by a grant from the Canadian Network of Centres of Excellence to Insect Biotech Canada. I am grateful to George Ball for encouraging me to embark on the path that has led to this paper.

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(Date received: 31 January 1993; date accepted: 17 July 1993)