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Divergence in mate choice systems: does evolution play by rules?

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Abstract

Understanding the genetic bases of phenotypes associated with the earliest stages of divergence will reveal a great deal about species formation. I review a number of model systems, most involving plant–insect interactions, that have already revealed genetic aspects of incipient speciation. It is suggested that progress in understanding the causal forces driving mating signal evolution and incipient speciation will be expedited in model systems where; (1) ecological and evolutionary information is available, (2) different aspects of mating behaviors that function in mate and/or species recognition are known, (3) genetic analysis of single phenotypes is undertaken, (4) analysis of sexual selection and isolation is performed under natural conditions (or in the wild), and (5) comparative data from related species are available to assess phylogenetic trends.

Introduction

The genetic basis of phenotypes involved in the initial stages of reproductive isolation between populations is still poorly known. There is great hope that the kinds of genetic systems involved in the very earliest stages of reproductive isolation will ultimately be revealed in a variety of organisms (Reinhold, 1998; Ritchie & Phillips, 1998). Identifying the number and kinds of genes involved with these early stages of speciation will tell us a great deal about the initial steps leading towards reproductive isolation and the basis of complex behavioral phenotypes thought to be involved in the remolding of mate choice systems (see Panhuis et al., 2001). An outstanding question is whether insights into these kinds of genetic systems will reveal how evolution shapes the genetic structure of traits that cause reproductive isolation. Also, are certain trait differences more likely to evolve early in the divergence of species than others?

Recent genetic studies of premating isolation have suggested that the influence of major genes may be common (Bradshaw et al., 1995; Schemske & Bradshaw, 1999; Doi et al., 2001). Thus, there is no compelling reason to expect that the genetic architecture of traits responsible for divergence in courtship behaviors should be any different than those involved in adaptive morphological divergence (Orr, 1992, 1998; Marshall, Orr & Patel, 1999) or the kinds of genetic differences revealed by genetic analysis of artificially selected varieties, such as tomatoes (Tanksley, 1993) and corn (Doebley & Stec, 1993). One might suppose that any sort of genetic system could be involved in the process of reproductive isolation even if by correlation with those genes that are subject to natural or sexual selection. Numerous loci influence mating behavior in *Drosophila melanogaster* (Hall, 1994; Yamamoto et al., 1997), but few if any are known to influence sexual isolation among species (Gleason & Ritchie, 1998; Kyriacou, 2002). Thus, one expectation, or rule, for genetic analysis is that there could be both major and minor genes influencing a particular trait.

Whether a new mutation has the effect of a major or minor gene might depend on the type of character being considered. For example, in moth pheromone systems, new mutations may have large effects if small chemical differences can be perceived by the receivers.

Such mutations will simply increase the spectrum of pheromone chemicals in the system. However, a major mutation influencing mating songs in *Drosophila* would presumably have deleterious effects because male song could be outside of the range of a female's preference spectrum. New mutations should be filtered out by selection if they decrease the ability of individuals to obtain matings, or recruited if they serve to increase the efficiency of finding high quality mates (Greenfield, 1997).

Can there possibly be common sets of genes among closely related taxa that evolve repeatedly during the initial stages of reproductive isolation, that is, are there genetic 'rules' that dictate how mating systems diverge? *Drosophila* behaviorists know a great about courtship behavior, its elements, and in some cases, the genetic basis of these traits. If we examine other groups, can we ever predict either the genetic basis for such traits or which types of traits should be generally involved in the initial stages of reproductive isolation? If these are species-specific or even clade-specific, can we predict which behaviors or components of signaling systems will be involved in the earliest stages of divergence? Does evolution play by rules?

The search for general causes of speciation has occupied biologists throughout the modern synthesis. Dobzhansky (1940) asked "isn't it a task of science to detect fundamental similarities concealed by apparent unlikeness? A fundamental but common property of species is the presence of isolating mechanisms." He then concluded, "The precise means whereby the interbreeding is eliminated are immaterial so long as the exchange of genes is precluded. Any gene that raises the effective barrier to the mingling of incipient species is adaptively valuable, and hence may become the basis of speciation." It may not be surprising that evolution can recruit different means of favoring and improving mechanisms preserving matings within groups as opposed to between groups. Certainly, mechanisms of postmating isolation in animals fall into a discrete number of genetic categories, that is, large X chromosome effects, numbers of genes influencing male sterility versus hybrid inviability, etc. (Dobzhansky, 1936; Zouros, Lofdahl & Martin, 1988; Coyne & Charlesworth, 1989; Coyne & Orr, 1989b; Wu & Palopoli, 1994). As for premating isolation, much of this might be organism or clade-specific in that diverse signaling systems are as much a by-product of the evolutionary process as adaptations are to past and current ecological conditions. We should not necessarily expect similar mechanisms for reproductive isolation in disparate groups, for example, pheromones in moths versus male advertisement calls in anurans, but we might come to understand the evolution of chemical signaling 'rules' in related moth species by comparing chemical signatures of these pheromones (Phelan, 1997) or the variation in acoustic qualities of male frog calls in sexual selection and species recognition (Gerhardt, 1982; Ryan & Rand, 1993; Pfennig, 1998).

Recent focus on understanding the driving forces of speciation, and not just the basis of species differences, has suggested that only a few mechanisms or rules may be responsible. First and perhaps most generally accepted is that the evolution of reproductive isolation can be a correlated response to adaptive divergence among populations. Muller (1942) suggested that postmating isolation might arise as a secondary consequence to the irreversible pleiotropic changes accruing as populations diverged in allopatry whether or not these genetic changes were influenced by natural selection or were solely a consequence of mutation and drift. Until speciation is 'complete', partially interfertile populations may coalesce depending on rates of gene flow, or natural selection might act to further strengthen isolation by reinforcing premating isolation (reviewed in Noor, 1999) or maintain reproductive character displacement (Hostert, 1997). Although still controversial, empirical support for reinforcement exists (Coyne & Orr, 1989a; Howard, 1993; Howard & Gregory, 1993; Higgie, Chenoweth & Blows, 2000). Laboratory experiments also tend to support Muller's (1942) hypothesis (Dodd, 1989; Rice & Hostert, 1993). Even in sympatry with low levels of gene flow, strong natural selection can maintain species distinctness (Feder et al., 1994; Filchak, Roethele & Feder, 2000) as long as those parts of the genome causing interspecific sterility are shielded from recombination (Noor et al., 2001).

Speciation research was re-energized by the challenge of an alternate view of the speciation process and a new view of species, the recognition concept (Paterson, 1993; Lambert & Spencer, 1995). This view has not been widely accepted (Coyne, Orr & Futuyma, 1988), but refocused attention on the role of sexual selection in the speciation process. By de-emphasizing isolating *barriers* in the study of speciation processes, Carson (1978) suggested that interactions between potential mates within demes must be the driving force of sexual selection with only secondary consequences for reproductive isolation (Carson, 2000).

Sexual selection can lead to accelerated diversification in mating preferences within (Turner & Burrows, 1995) and between populations (Lande, 1981; Lande & Kirkpatrick, 1988; Schluter & Price, 1993). Local conditions (i.e., natural selection) may mold mate recognition systems leading to divergence in courtship behaviors between isolated demes (Butlin, 1995). Thus, natural and sexual selection need to be untangled to identify causation in speciation studies (Blows, 2002).

This paper is an attempt to integrate knowledge of the genetics of sexual isolation to discover more general mechanisms causing speciation in its earliest stages. I hope that some of the better understood circumstances surrounding the evolution of nascent species will reveal the kinds of genetic transformations required to initiate reproductive isolation. I review a number of helpful empirical cases, and use cactophilic *Drosophila mojavensis* as a useful example for understanding the origins of intraspecific behavioral isolation causing incipient speciation. To facilitate a more comprehensive understanding of the causes of signaling system evolution and genetic analyses of the relevant phenotypes, progress will be made in studies that recognize one or more of the following considerations:

1. Considerable natural history information is required to know enough about the signaling system of the organisms studied *in nature*. Is sexual selection within local demes strong or did sexual isolation arise as a consequence of allopatry? Some knowledge of the biogeography and evolutionary history of the organisms is also helpful.
2. Some ranking of mating behavior phenotypes is required to know which are part of the sexual selection and/or sexual isolation systems.
3. Genetic analysis of *single* phenotypes driving divergence is required to estimate the genetic basis of these traits. Trying to compare the genetic architectures of components of sexual isolation is predicated on knowing that the traits analyzed are unitary or not. For example, assortative mating measured by multiple choice tests is likely to be a composite trait and so its genetic basis is likely to be complex.
4. Detailed ecological information is necessary to insure that phenotypic measurement and genetic analysis of characters thought to be involved in sexual isolation are carried out under realistic environmental conditions. G × E interactions are at least as likely with behavioral traits associated with mate choice as they are with any other phenotype, and thus trait expression may be biased unless phenotypes can be measured in conditions like those in nature.
5. Comparison of behavioral phenotypes relevant to sexual isolation with those in sister species may help to order behavioral components into those evolving earlier versus later during species divergence. If particular phenotypes tend to diverge earlier than others in particular groups, studies of the causes of divergence in these traits and their genetic bases may shed some light on the nature of incipient speciation.

The value of natural history

Considerable progress has been made into understanding the circumstances in which incipient species may arise by integrating ecological, geographical and historical information. It is likely that these cases will prove instructive in evaluating the genetic bases of phenotypes causing reproductive isolation. With scant ecological information or incomplete knowledge of the evolutionary history of populations thought to be undergoing reproductive isolation, it will not be possible to identify the causes underlying divergence. Host affinities in insects have provided some of the best insights into host specialization and reproductive isolation in fruit flies (Bush, 1975; Berlocher & Feder, 2002), tree hoppers (Wood & Guttman, 1983; Wood et al., 1999), aphids (Via, 1990; Hawthorne & Via, 2001), gall forming insects (Craig et al., 1993), cactophilic *Drosophila* (Markow, Fogleman & Heed, 1983; Etges & Ahrens, 2001), soapberry bugs (Carroll & Boyd, 1992; Carroll, Dingle & Klassen, 1997), and leaf beetles (Funk et al., 1995; Funk, 1998). Extensive ecological, chromosomal, and behavioral analyses of Hawaiian *Drosophila* species have provided understanding into the roles of interdemic isolation, extinction, and sexual selection in the explosive diversification of this group (Carson & Kaneshiro, 1976; Carson, 1978, 1987; Kaneshiro, 1980; Droney, 1992). Accurate information on geographic distributions of incipient or sibling species is a prerequisite for understanding the potential for gene exchange in regions of sympatry; several case studies would not have been possible without such background information (Butlin & Hewitt, 1988; Noor, 1995; Jiggins et al., 1997). Even basic information on species distributions in nature, for example, allopatry versus sympatry, can lead to inferences about species formation such as the evidence for reinforcement in *Drosophila* species (Coyne & Orr, 1989a, 1997).

If a fundamental understanding of the genetics of incipient speciation is to be achieved, the context in which such evolutionary changes occur is necessary so that causal factors may be identified, for example, Bradshaw et al. (1995). Descriptions of genetic differences at the chromosomal or nucleotide levels may provide detailed insights into the kinds of genetic changes that may be associated with the earliest stages of species divergence. The discovery of southern African populations of *D. melanogaster* that are reproductively isolated from all cosmopolitan strains has allowed detailed genetic analysis of sexual isolation (Hollocher et al., 1997a; Ting, Takahashi & Wu, 2001), but it has been so far impractical to gather field data on these populations. Unless these genetic differences can be assessed in an ecological context such that we can understand the conditions in nature in which they arose, we will be left with an incomplete synthesis of the speciation process in such cases.

Cactophilic *D. mojavensis* is a notable example of a well-studied species in which biogeography, genetics, and ecology have provided the necessary context for analysis of incipient speciation. For more than 25 years, significant assortative mating in laboratory mating tests between Baja California and mainland Mexico populations of *D. mojavensis* has been described as a case of incipient speciation (Zouros & d'Entremont, 1974) caused by reproductive character displacement (Zouros & d'Entremont, 1980; Markow, 1981a, 1991). In multiple choice tests, mainland females tend to discriminate against mating with Baja males, leading to the characterization of 'one-way premating isolation'. Causes for this sexual isolation centered on the history of speciation and biogeography of *D. mojavensis* and its sibling species, *D. arizonae*. Based on the extensive patterns of chromosomal evolution in this group (and other related members of the large *D. repleta* group) Wasserman and Koepfer (1977) suggested that *D. mojavensis* and *D. arizonae* evolved from a common mainland ancestor. Populations isolated by tectonic drift on the Baja peninsula became *D. mojavensis* and those on the mainland evolved into present-day *D. arizonae*.

Further analyses suggested that these Baja populations of *D. mojavensis* gave rise to a number of derived, geographically isolated populations located in southern California and mainland Mexico. Evidence includes the presence of a rare ancestral chromosome in central Baja California found nowhere else (Ruiz, Heed & Wasserman, 1990), significant levels of inversion polymorphism not found in mainland populations (Etges et al., 1999), and widespread use of a preferred host in Baja California even though secondary hosts are found there. *D. mojavensis* apparently colonized the mainland by switching host plants, and in Sonora and Sinaloa, Mexico became sympatric with *D. arizonae*. The presence of *D. arizonae* was hypothesized to have caused reproductive character displacement in these derived mainland populations of *D. mojavensis*, such that when subjected to laboratory assortative mating tests, mainland *D. mojavensis* now discriminate against mating with individuals from ancestral populations in Baja California (Zouros & d'Entremont, 1980).

Of the four endemic species of Sonoran Desert *Drosophila*, *D. mojavensis* uses a greater variety of host cacti across its species range than the others (Heed & Mangan, 1986). In Baja California, the islands in the Gulf of California, and a small zone on the western coast of Sonora, pitaya agria, *Stenocereus gummosus*, is the preferred host plant (Downing, 1985; Newby & Etges, 1998). In mainland Sonora, Sinaloa, and Arizona, organ pipe cactus, *S. thurberi*, is a host with occasional use of sina, *S. alamosensis*, that is sometimes shared with *D. arizonae* in southern Sonora. In southern California and in the Grand Canyon, Arizona, California barrel cacti, *Ferocactus cylindraceous*, are hosts except for populations on Santa Catalina Island, California, that use *Opuntia demissa* (Heed & Mangan, 1986; Etges et al., 1999). Thus, *D. mojavensis* is considered oligophagous, using different host cacti in different parts of its species range.

Variation in host use, in particular the switch from agria to organ pipe cactus since *D. mojavensis* invaded the mainland, has had a profound impact on life history evolution and, although largely ignored by earlier workers, influenced the evolution of sexual isolation. *D. mojavensis* populations separated by the Gulf of California are 'host races', that is, adapted to their respective host cacti, agria in Baja California and organ pipe on the mainland (Etges, 1989, 1990). Genetic shifts in several life history traits in derived mainland populations include increased egg to adult development time and adult body size. These fitness components have evolved in response to several key ecological features of organ pipe cactus, principally slower tissue fermentation rates and increased rot duration (Etges, 1989). Further, Etges (1993) revealed significant genetic variation within populations for these traits, suggesting ongoing life history evolution, and therefore continuing adaptation to the use of different host cacti. Within population G × E interactions revealed genotypic sensitivity influencing these life history components to the host cacti used in nature (see Measurement, ecological variation, and G × E interactions).

Such detailed historical scenarios for species undergoing the initial stages of reproductive isolation allow for specific hypothesis-testing concerning the causes for divergence between Baja and mainland populations of *D. mojavensis*. If the degree of sexual isolation resulted from only geographic isolation and genetic drift, then there should be chance variation in levels of premating isolation depending on which mainland and Baja populations are tested and no predictable effects on mate choice behaviors due to use of particular host plants in nature. In all studies to date, consistent and low levels of sexual isolation have been observed between any pair of Baja and mainland populations examined (except for those from southern California – they exhibit little isolation from Baja populations). The reproductive character displacement hypothesis, so far not directly tested, requires evidence that *D. arizonae* is the cause for shifts in mating preferences in mainland populations of *D. mojavensis* and that both species encounter each other during courtship on host cacti and perhaps share hosts as oviposition sites; with respect to the latter, direct evidence is meager (Markow, Fogleman & Heed, 1983; Etges & Jackson, 2001). No interspecific hybrids have ever been captured in nature, so reinforcement is unlikely (Etges et al., 1999). Further, the diagnostic epicuticular hydrocarbons that qualitatively differentiate all Baja and mainland Mexico *D. mojavensis* populations are quantitatively very similar in amounts in mainland *D. mojavensis* and *D. arizonae* populations (Etges & Jackson, 2001). Since these hydrocarbons are part of the mate recognition system in *D. mojavensis* (see below and Etges & Ahrens, 2001), these data falsify the hypothesis that reproductive character displacement has influenced hydrocarbon profiles in this system.

However, if sexual isolation between Baja and mainland populations of *D. mojavensis* is a correlated response to adaptation to different environments, then Muller's (1942) hypothesis predicts that one or more aspects of assortative mating should be genetically correlated with some aspect of adaptation to the use of different host cacti. I directly tested this hypothesis by performing artificial selection on egg to adult development time in Baja and mainland populations of *D. mojavensis* cultured on both agria and organ pipe cactus for 12 generations (Etges, 1998). Response to selection for development time resulted in heritabilities averaging 8–11% consistent with earlier results (Etges, 1993). In replicate lines selected for short or long development times, premating isolation between replicates of a Baja and mainland population decreased to non-significant levels by the end of the experiment (Figure 1). In the last generation, premating isolation was measured again with control flies from both populations and those selected for short versus long development times cultured on the same host cactus. Results showed that premating isolation was not significantly different from zero in any of the mating tests except for the controls cultured on organ pipe (as expected). Therefore, the correlated genetic responses in mate choice in both the fast and slow lines in both populations must have been caused by changes in the frequencies of genes with very similar effects on premating isolation. Thus, the life history differences that evolved in mainland populations of *D. mojavensis* in response to use of organ pipe cactus are not independent of shifts in mating preferences known to cause sexual isolation with populations from Baja California. Natural selection due to the shift in host plants is at least partially responsible as a cause for premating isolation between Baja and mainland Mexico populations of *D. mojavensis*.

Analysis of incipient speciation in *D. mojavensis* is far from complete. Further work is needed to reveal the relative contributions of character displacement and host plant-caused behavioral shifts to sexual isolation between allopatric populations. So far, there has been no work on the role of sexual selection within demes that may also contribute to measurement of sexual isolation. Most importantly, data are needed on the genetic basis of those traits responsible for behavioral isolation beyond documentation of genetic variation in these behaviors (Koepfer, 1987; Etges, 1998) and that factors influencing male and female mating success are on different chromosomes (Zouros, 1981; Krebs, 1990).

Components of the signaling system

Key to interpreting studies of speciation and its genetic architecture is knowing whether components of intraspecific signaling systems are the same or different

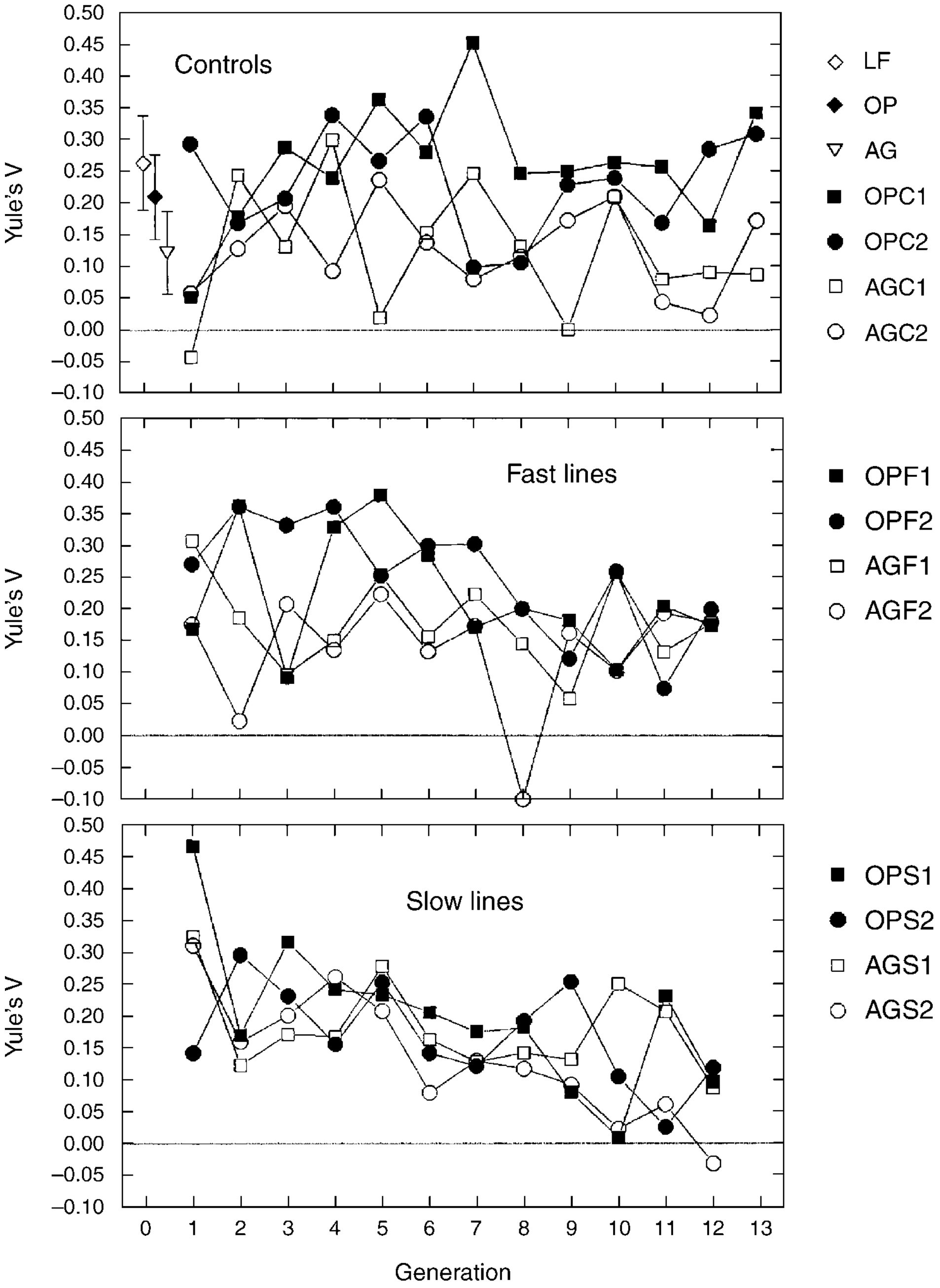

Figure 1. Changes in premating isolation (Yule's V) between a mainland and Baja population of *D. mojavensis* over the course of 12 generations of artificial selection on egg to adult development time. Individual line designations refer to replicate cultures of a mainland (Punta Onah, Sonora) and Baja California (Punta Prieta) population reared on organ pipe (OP) or agria (AG) cactus. C, F, or S refer to control, fast, or slow selection lines. Control lines were maintained without selection on development time. Numbers (1, 2) refer to replicate lines. For example, AGS1 refers to the observed level of premating isolation between mainland and Baja populations reared on agria, replicate line 1 artificially selected for slow development time. See Etges (1998) for details. The three estimates of Yule's V (±1 SE) indicated by LF (lab food), OP (organ pipe), and AG (agria) in the upper panel refer to the estimates of premating isolation for these two base populations described in Etges (1992). Figure reprinted with permission of the University of Chicago Press.

from species recognition systems. Two major unresolved problems in signaling system evolution are (1) what parts of signaling systems causing reproductive isolation evolve first and, (2) do these signaling components, perhaps parts of sexual selection systems, form the basis of signaling systems that once diverged, then give rise to reproductive isolation? In organisms such as *Drosophila* and birds with complex courtship behaviors, multiple signals may be required and the order in which they are presented to prospective mates may be important to the reinforcement of signal transmission (Spiess, 1987; Borgia, 1995; Cobb & Ferveur, 1996). If some signals vary among individuals more than others do, there is greater potential for these traits to be subject to sexual selection. It is an empirical problem to determine whether components of sexual selection systems also serve in species recognition, but the type of communication system may also provide answers.

The spectrum of signaling systems among taxa is both spectacular and sobering in its breadth. Acoustic advertisement calls are, in general, good examples of sexually selected traits that also play a role in species recognition (Blair, 1974; Ryan & Rand, 1993; Gerhardt, 1994; Henry, 1994; Gray & Cade, 2000). Male call duration (Woolbright & Stewart, 1987; Welch, Semlitsch & Gerhardt, 1998), chorus tenure (Murphy, 1994; Bertram, Berrill & Nol, 1996; Howard & Young, 1998), and call site selection (Fellers, 1979) are all important in sexual selection because they relate directly to the strength and frequency of male advertisement calls. Cryptic female choice has evolved in systems where male–female recognition and species recognition systems are the same (Hoikkala, Crossley & Castillo-Melendez, 2000). In organisms using visual cues, sexual selection can drive species divergence (Seehausen & van Alphen, 1998; Uy & Borgia, 2000; Boughman, 2001; Lande, Seehausen & van Alphen, 2001) and reinforce species isolation (Alatalo, Gusstafsson & Lundberg, 1994; Sætre et al., 1997), but see (Veen et al., 2001).

When shouldn't there be a relationship between sexual selection and sexual isolation between species? In some insect groups, courtship rituals involve a number of sequential behaviors and cues that are all necessary for successful courtship and female acceptance. Many *Drosophila* species, including *D. mojavensis*, exhibit a stereotyped series of behaviors (Spieth & Ringo, 1983; Alonso-Pimentel & Tobin, 1992) starting when males approach females to elicit copulation. The male extends his proboscis repeatedly to contact the female's genitalia, rubbing the sides of the female's vaginal plates, and taps his foretarsi in an upward motion on the ventral surface of the female's abdomen. Both sexes exchange chemical cues at this point through tactile stimulation caused by sex-specific cuticular hydrocarbons. At the same time, the male vibrates his wings producing a mating song to further stimulate the female, although sometimes this is omitted. Male courting usually continues until the female signals acceptance by elevating the tip of her abdomen, spreading her vaginal plates and both wings allowing the male to mount her and attempt intromission.

Because multiple cues are involved, it is possible that some are better as intersexual signals, whereas others vary less among individuals and are more important as interspecific signals. For example, experimentally altering cuticular hydrocarbon profiles by mechanically transferring hydrocarbons from mainland to Baja male *D. mojavensis* significantly increased mating success of these 'perfumed' males with mainland females. Altering female hydrocarbon profiles had no detectable effects on courtship success (Etges & Ahrens, 2001). Hydrocarbon transfers between male *D. arizonae* and Baja *D. mojavensis* had no effect on male mating success with *D. arizonae* females (Etges, unpubl. data) suggesting that cuticular hydrocarbons are important intraspecific signals, but may not be involved in species recognition (Noor & Coyne, 1996). Other signals, such as mating songs, are involved in species recognition (Byrne, 1999) as in some cricket species (Fitzpatrick & Gray, 2001). Because cuticular hydrocarbons are known pheromones in such a wide variety of insects (Cobb & Ferveur, 1996) and contribute to species recognition in some species (Coyne, Crittenden & Mah, 1994; Higgie, Chenoweth & Blows, 2000), their role in species recognition between *D. mojavensis* and *D. arizonae* needs further study.

The idea that multiple signals involved in mate recognition might evolve serially is critical to studies of the genetics of incipient speciation. We need to know which components evolve first and why. The way that signaling systems evolve matters because if behavioral evolution is a result of sexual selection, there is no necessary causal relationship between mate choice behaviors and isolating mechanisms. As predicted by Carson (2002), determinants of sexual selection may be unrelated to sexual isolation, as illustrated by the broad-headed male trait in *D. heteroneura*. Wider heads are related to male–male competition for females and play little role in species recognition with the closely related *D. silvestris* (Boake, DeAngelis & Andreadis, 1997). Evaluating the causes for sexual isolation will also require distinction between sexual and natural selection. Selection may operate independently on mate recognition systems in allopatry and only when those populations come into secondary contact will it be possible to observe whether mate choice within these populations is the same as the mechanism of sexual isolation between them. There can be a direct link between signal divergence and sexual isolation where behavioral isolation is a pleiotropic by-product of natural selection assuming detailed information is at hand concerning patterns of adaptation to local environments.

Assigning phenotypes

Interest in the genetic basis of those traits causing species divergence is high (Carson & Lande, 1984; Ritchie & Phillips, 1998; Butlin & Ritchie, 2001). Determining the genetic architecture of these traits and identification of the genes involved will reveal a great deal about the processes of species formation. To answer questions such as "is incipient speciation caused by one or a few genes or does it require many genes?", the traits chosen for genetic analysis should be unitary, that is, a single phenotype, unless pleiotropy is assumed.

Traits such as male mating success, sexual isolation, and assortative mating in *Drosophila* are not necessarily unitary traits because they may all depend on more than one causal phenotype. For example, Zouros (1981) determined that sexual isolation between *D. mojavensis* and *D. arizonae* was caused by factors on different sets of chromosomes, one set for males and another for females. The data were gathered by observing copulations in standard mating choice experiments, for example, a female of each species enclosed with a single male (and vice versa). The success of each type of male (and female) in achieving a copulation was scored in different trials. Since male mating success is influenced by male cuticular hydrocarbon profiles (in *D. mojavensis*, Etges & Ahrens, 2001) and mating song differences between species (Byrne, 1999), it is not surprising that different, independently assorting genes might be involved, that is, those influencing hydrocarbon differences and others influencing components of song. A number of other examples of this type of analysis also make it difficult to know how many phenotypes were being analyzed together (Tan, 1946; Welbergen et al., 1992; Wu et al., 1995; Hollocher et al., 1997b; Ting, Takahashi & Wu, 2001).

By focusing on single traits it should be possible to localize the effect of major genes using the appropriate crossing schemes or QTL analysis. Doi et al. (2001) carefully eliminated all but courtship song as the cause for sexual isolation between *D. pallidosa* and *D. ananassae* and isolated a very small genomic region near the *Delta* locus that influenced female mating preference (although minor effects of other chromosomes were also reported). Genetic analysis of single traits in cases like this is likely to be far more informative about the architectures of premating isolation (for a review, see Ritchie & Phillips, 1998). Analysis of cuticular hydrocarbon polymorphisms (Coyne, Wicker-Thomas & Jallon, 1999; Dallerac, Labeur & Wicker-Thomas, 2000; Takahashi et al., 2001), mating song differences (Henry, 1985; Shaw, 1996; Ritchie, 2000), and pheromone production in moths (Roelofs et al., 1987) have already revealed a number of insightful cases describing the genetic bases of single traits influencing sexual isolation.

Measurement, ecological variation, and G × E interactions

Components of mate recognition systems, particularly behavioral traits, are phenotypes, and as such, can be expected to be influenced by the environments in which they are expressed. Because observations of courtship behaviors and mate choice are so often conducted under controlled laboratory conditions, it is essential that these conditions are related to those experienced in nature by the organisms under study. In insects, preimaginal conditioning of adult learning and behavior has long been of interest (Barron & Corbet, 1999), so we might expect environment-dependent expression of signaling phenotypes. Differences in mate signaling systems and mating success can be influenced by variation in temperature (Markow & Toolson, 1990), variation in larval densities (Ehrman, 1990; Kim, Ehrman & Koepfer, 1996), presence of closely related species during larval development (Kim, Ehrman & Koepfer, 1992), food stress (David et al., 2000), physical condition (Bakker, Kunzler & Mazzi, 1999; Gray & Eckhardt, 2001), hatching date (Griffith, Owens & Burke, 1999), degree of sexual experience (Markow, Quaid & Kerr, 1978; Schwartz, 1991), photoperiod (Barth, Hirsch & Heisenberg, 1997), and different larval substrates (Spiess & Spiess, 1967; Etges, 1992, 1998; Howard, 1998). While this is certainly an incomplete survey, it does suggest that these effects may be more common in organisms with complex life cycles.

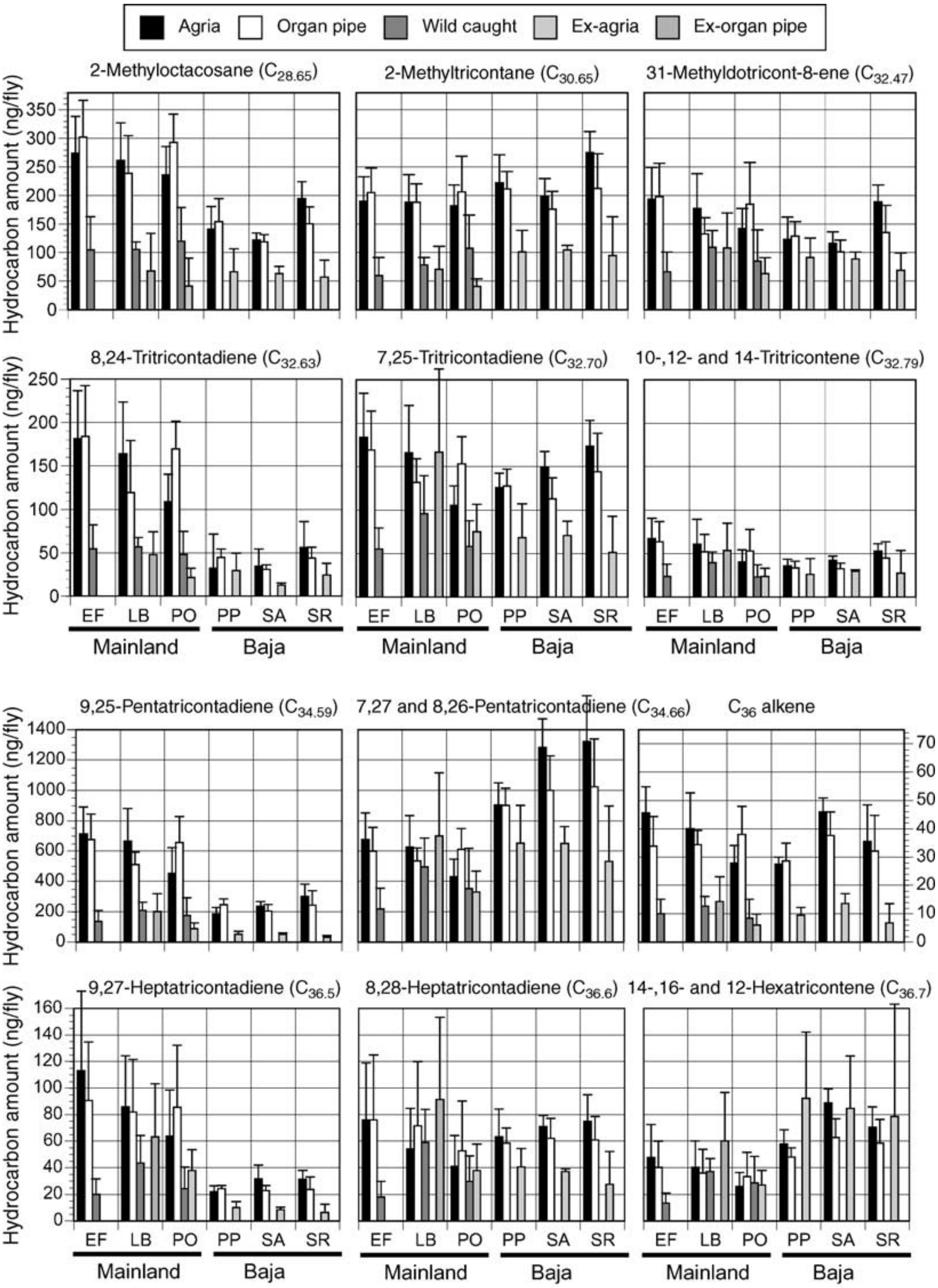

Figure 2. Comparisons of epicuticular hydrocarbon amounts for *D. mojavensis* populations from mainland Mexico (EF – El Fuerte, Sinaloa, LB – Las Bocas, Sonora, and PO – Punta Onah, Sonora) and Baja California (PP – Punta Prieta, SA – San Augustin, and SR – Santa Rosalia) reared on agria and organ pipe cactus in the lab, wild-caught adults, and adults reared from cactus rots returned to the lab from the field. See the text for details and Etges and Ahrens (2001) for a map of these localities.

Larval experience in *D. mojavensis* provides an interesting example of the necessity for examining relevant ecological influences on adult behavior. Brazner (1983) first observed that courtship latency, or time to copulation, was significantly extended in male *D. mojavensis* from Baja California when cultured on laboratory media versus the fermenting tissues of their principal host plant, agria cactus. Further observations showed that in assortative mating trials involving mainland and Baja populations of *D. mojavensis*, mainland female discrimination and sexual isolation was strengthened when flies were reared on organ pipe cactus, and even more so when reared on lab food. Premating isolation in agria-reared flies is rarely statistically significant, and the 'one-way' premating isolation observed by earlier workers (Zouros & d'Entremont, 1980; Markow, 1981a) is not always observed (Etges, 1998). Comparing all types of lab food used in earlier studies, Brazner and Etges (1993) showed that premating isolation between Baja and mainland populations was significantly greater than when cactus-reared flies were used.

A likely explanation for these substrate-induced changes in sexual isolation is that adult epicuticular profiles are influenced by larval rearing substrates, particularly the difference between lab food and cactus (Stennett & Etges, 1997). Adult hydrocarbon profiles are not strongly influenced by differences in agria and organ pipe tissues in the laboratory, yet almost all hydrocarbon components are increased when flies are reared on *Opuntia* tissues (Etges & Jackson, 2001). Varying concentrations of several triacyglycerols in larval substrates was found to influence adult epicuticular hydrocarbon profiles (Etges, Veenstra, & Jackson, unpubl. data), so the chemical composition of cactus rots in nature is likely an important determinant of adult epicuticular hydrocarbon profiles.

An obvious question is whether these differences are expressed in the wild. Data from natural populations is crucial. So, hydrocarbon profiles of wild-caught adult *D. mojavensis* and adults that emerged from cactus rots returned to the lab were compared with their cactus-reared descendents in the laboratory. A balanced experimental design was not possible because sufficient numbers of wild-caught adults and those reared from both host cacti were not available from each location (see Table 1 in Etges & Ahrens, 2001). Amounts of 12 hydrocarbon components were clearly lower in wild-caught and cactus-reared flies from these natural populations than their lab-reared descendants in most cases (Figure 2; statistical analyses available from the author). The diagnostic peaks that differentiate all Baja California and mainland Mexico populations ($C_{32.63}$, $C_{34.59}$, $C_{36.5}$) were consistently expressed in the wild flies, although amounts were very much lower. Since these samples were not separated by sex, we could not estimate male–female differences in wild-caught adults. We are clearly in need of more data like these to insure that the causal mechanisms of sexual isolation studied in the lab are comparable to those we think may operate in nature.

Phylogenetic analysis

Systematic methods have provided well-resolved patterns of phylogenetic relationship for many groups which will provide insights into the types of changes that accompany speciation. If patterns of mating signal divergence show regular or non-random patterns of divergence among clades of related species, then hypotheses concerning mating signal evolution are falsifiable. Hypotheses concerning pre-existing sensory bias (Basolo, 1995) and sensory exploitation (Ryan & Rand, 1999) required robust phylogenetic information in order to be tested. Kaneshiro (1980, 1983) hypothesized that there should be a direction of behavioral evolution due to loss of courtship behaviors in derived species of Hawaiian *Drosophila* after experiencing founder events. He predicted that individuals from these younger, derived populations should be less choosy when confronted with their closely related ancestors, and be discriminated against during courtship. Although Kaneshiro's hypothesis was found to be inconsistent with a number of other case studies (Markow, 1981b; Ehrman & Wasserman, 1987), including *D. mojavensis* (Wasserman & Koepfer, 1980), but see (Giddings & Templeton, 1983), it stands as a thought-provoking prediction of behavioral evolution for a wide variety of closely related species.

Comparative data are essential for unraveling sequential evolution of mating signals providing some species' recognition behaviors are shared among daughter species. For example, when complex courtship songs in *D. willistoni* sibling species were mapped onto a species phylogeny based on the *per* locus, the majority of song pattern evolution was found in a single species, *D. equinoxialis*. Gleason and Ritchie (1998) concluded that songs in this group evolve too rapidly to be of much phylogenetic use

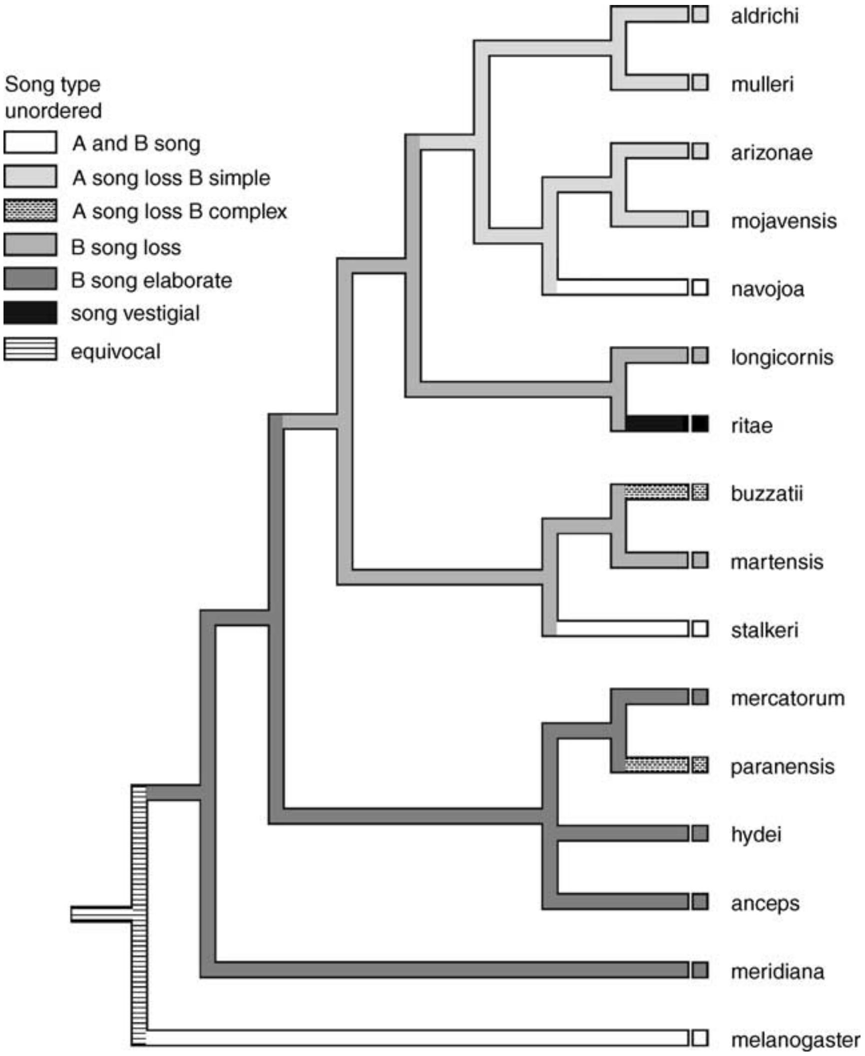

Figure 3. Song characters analyzed by Ewing and Miyan (1986) mapped onto a phylogeny of selected *D. repleta* group species (from Durando et al., 2000).

and thus unlikely to be species recognition signals. Here, the comparative method allowed rejection of the hypothesis that there was some direction to song evolution. In another study, patterns in the behavior courtship sequence in members of *Desmognathus ochrophaeus* complex were mapped onto their phylogeny based on cytochrome *b* sequences. Mead and Verrell (2002) concluded that courtship behaviors had been lost or gained independently contributing to reproductive isolation among members of the species complex.

Using members of the *D. repleta* group (including *D. mojavensis* and *D. arizonae*), Ewing and Miyan (1986) assessed song variation among 22 species for which there is a well-resolved phylogeny based on chromosome inversions (Wasserman, 1992). Because of the large number of species that overlap in major song characteristics, they were able to reconstruct the evolution of song types, but came to few conclusions about phylogenetic patterns of song evolution. They considered the ancestral condition to be shared by species that produce A songs with short pulse trains followed by a more complex B song. They grouped species into those that have lost the B song, lost the A song, and whether A or B songs were simple or complex.

Using the *D. repleta* phylogeny from Durando et al. (2000), I mapped these song characters onto the fly phylogeny with MacClade (Maddison & Maddison, 1992) using *D. melanogaster* as the outgroup. Although some of the species analyzed by Ewing and Miyan (1986) were not included in this recent phylogenetic analysis, mapping these song characters produced a regular, if not perplexing pattern of mating song evolution (Figure 3). Assuming *D. melanogaster* shares the ancestral condition with other 'primitive' members of the genus (Ewing & Miyan, 1986), elaborate B songs (with doublet pulses, polycyclic pulses, or short bursts) are shared among the more basal species in the *repleta* group. The sister group containing the monophyletic *buzzatii* complex and *D. longicornis*, a member of the *mulleri* complex, have lost the B song. *D. ritae* seems to have lost most song elements altogether. However, the more derived members of the *mulleri* subgroup, including *D. mojavensis* and *D. arizonae*, have reacquired the B song but have lost the A song (Figure 3). Two species, *D. navojoa* and *D. stalkeri*, seem to have independently reaquired lost song elements, but *D. navojoa* do not always sing the A song, prompting Ewing and Miyan (1986) to suggest this species 'is in the process of losing the A song and therefore intermediate between the ancestral condition' and that of *D. mojavensis* and *D. arizonae*. Song evolution in the *D. repleta* group shows a complex pattern of diversification, character loss, and reverse evolution not unlike the patterns found among species of salamanders in the *D. ochrophaeus* complex (Mead & Verrell, 2002). While the phylogenetic trends are strongly patterned, further work will be required to verify these results by including more species. Addition of other characters into this phylogenetic framework, such as epicuticular hydrocarbon profiles, may help to resolve the patterns of diversification of species-specific mating signals.

Conclusions

The 'problem' of speciation would have been already solved if it was simple and the mechanisms shared amongst most organisms. Revealing the causes driving genetic changes underlying incipient speciation is ongoing in a number of model systems. Whether conditions forcing incipient speciation are general and abstract evolutionary 'rules', or in those cases where behavioral diversification is involved will common sets of genes influencing similar phenotypes be found, future progress will likely accelerate by including knowledge of the ecological and evolutionary circumstances surrounding incipient speciation. Evaluation of the genetic differences, the number of loci and alleles involved, etc. in characters involved in courtship signaling systems should reveal the nature of speciation when we have knowledge of the forces causing reproductive divergence.

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