

Reproductive Isolation in Sonoran Desert *Drosophila*

Testing the Limits of the Rules

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Efforts to elucidate the mechanisms of speciation have focused on broadly observed patterns from which rules or hypotheses about these mechanisms are inferred. In one description of such patterns (Coyne and Orr 1989a), the vast data on hybrid sterility and inviability are distilled and the ubiquity of Haldane's Rule, in which the heterogametic sex is the one most seriously compromised when sex differences exist in hybrid fitness, is underscored. These authors argue convincingly for the importance of the X-chromosome in producing this pattern, utilizing the impressive data accumulated on species of *Drosophila* (Coyne and Orr 1989a; Charlesworth et al. 1987). In a second contribution and its recent update (Coyne and Orr 1989b, 1997), the *Drosophila* literature again is surveyed exhaustively, providing the basis for additional and robust patterns: an increase in pre- and postzygotic isolation with time, the evolution of sterility and inviability at similar rates in accordance with Haldane's Rule, the appearance of postzygotic isolation in hybrid males before females (Haldane 1922), and the apparent evolution of stronger prezygotic isolation compared to postzygotic isolation when in sympatry, but not allopatry, with a closely related species.

The identification of these patterns is extremely valuable because it provides a series of predictions that can be tested with observations on species not included in the original surveys. Below we describe the nature of reproductive isolation observed for *D. arizonae* and its sibling species *D. mojavensis*, as well as between geographic populations of *D. mojavensis*, some of which are sympatric with *D. arizonae*. We evaluate these observations in the context of the more general patterns for *Drosophila*, especially Haldane's Rule and the role of reinforcement in explaining patterns of sexual isolation in these two species.

Drosophila arizonae and *D. mojavensis*

Drosophila arizonae and *D. mojavensis* are cactophilic species found in North America. These two species provide the numerous experimental advantages of *Drosophila*, in addition to being among the most thoroughly studied flies of this genus with respect to their ecology. Qualities that make them attractive for studies of speciation include their recent divergence as well as the existence of geographically separated populations of *D. mojavensis* that utilize different host cacti and are considered to be in the early stages of speciation. *Drosophila arizonae* (Ruiz et al. 1990), formerly *D. arizonensis* (Patterson and Wheeler 1942) and *D. mojavensis* (Patterson and Crow 1940), are members of the mulleri complex of the repleta group (Wasserman 1982). The species were first described by Patterson and his associates at the University of Texas, during a highly productive era in *Drosophila* evolutionary biology.

Drosophila arizonae is a fairly widespread species (figure 17.1). It is sympatric with *D. mojavensis* in Sonora, southern Arizona, and the cape region of the Baja peninsula, although *D. arizonae* apparently occur only in low densities in the cape region. In Sonora it primarily uses cina cactus (*Stenocereus alamosensis*) as its host; however, *D. arizonae* is a generalist and its distribution is by no means limited to the occurrence of cina. It occasionally has been reared from saguaro (*Carnegiea gigantea*), organ pipe (*S. thurberi*), agria (*S. gummosus*), and various opuntias (Ruiz and Heed 1978) and from soil soaked with the juice of necrotic cardon (*Pachycereus pringlei*) (Breitmeyer and Markow, unpublished). In Arizona it has been collected from rotting citrus (Markow, unpublished).

Shifts in host utilization in different geographic regions are well characterized for its sibling species,



Figure 17.1. Distributions of sister species *Drosophila arizonae* and *D. mojavensis*.

D. mojavensis. These shifts underlie the ability of *D. mojavensis* to inhabit different regions of the desert (figures 17.1 and 17.2). Organ pipe is the principal host in Sonora and southern Arizona, but in Baja California, agria is preferred even though organ pipe is present. Agria is also found and utilized in a small area in coastal Sonora. Neither of these two hosts is present in southern California or northwestern Arizona, where *D. mojavensis* breed in barrel cactus (*Ferocactus cylindraceus*). *Drosophila mojavensis* has also been reared occasionally from cina, saguaro, and opuntia in Sonora (Ruiz and Heed 1978), creating the opportunity for host overlap with *D. arizonae*.

Genetic differentiation between *D. arizonae* and *D. mojavensis* has been assessed at the morphological, physiological, chromosome, protein, and DNA levels. Original clues to their being different species were the slight differences between them in their coloration patterns and more obvious differences in male genitalia (Patterson and Wheeler 1942; Patterson and Crow 1940). Chromosomal differences allow the direction of evolutionary relationships to be inferred (Wasserman 1982; Ruiz et al. 1990). Based on allozyme variation, Zouros (1973) estimated the genetic distance between them to be 0.212. The date of divergence is unclear. Mills et al. (1986) used ADH sequence data to

estimate a divergence time of about 2–2.5 mya, but Pitnick et al. (1995) gave an earlier date, 6 mya, based on a combination of ADH and mitochondrial DNA (mtDNA) sequences. In contrast, based on mtDNA data, Park (1989) estimated a divergence time of between 0.15 and 1 mya depending on the genetic distance used to obtain the estimate. Inference is complicated by the fact that the ADH locus is duplicated and contains another coding region (Begun 1996), previously thought to be a pseudogene, and different strains of *D. arizonae* give different mtDNA results (Spicer, personal communication).

Chromosomal differences allow the direction of evolutionary relationships to be inferred (Wasserman 1982; Ruiz et al. 1990). Ruiz et al. (1990) describe a scenario for the evolution of these two species in southern Mexico and the subsequent colonization of Baja by *D. mojavensis* and mainland Mexico by *D. arizonae*. The evolutionary sequence is thought to have involved host shifts from an ancestral species using platyopuntia, or prickly pear cacti, to *D. arizonae* and *D. mojavensis* utilizing several different columnar cactus species. The ability to shift hosts enabled *D. mojavensis* to expand its range to southern California and the offshore islands and to Sonora, where it established contact with *D. arizonae*. A second zone of sympatry occurs in Baja California in

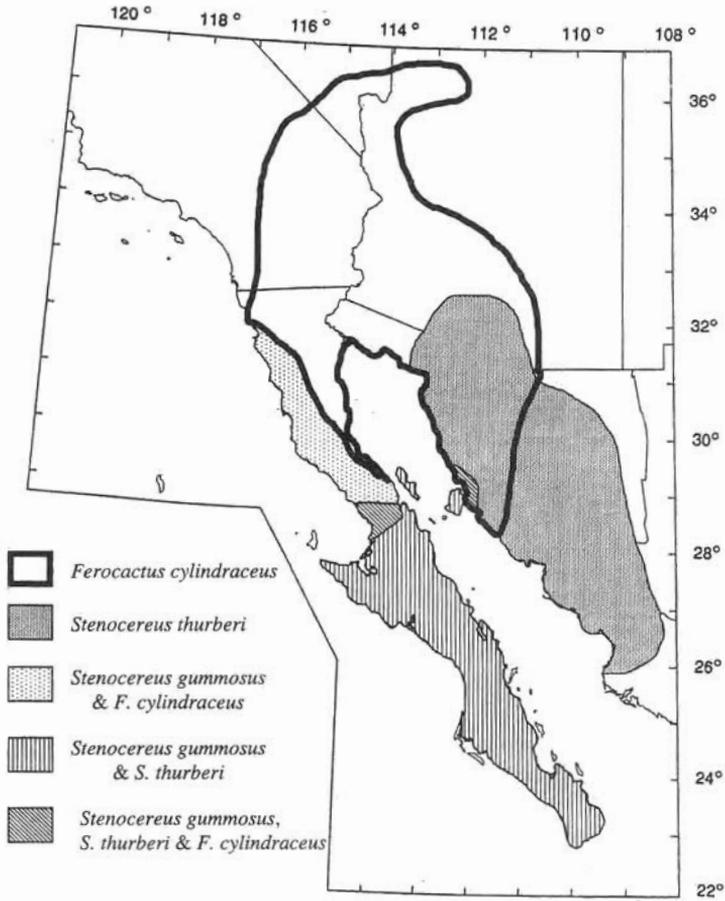


Figure 17.2. Distribution of major host cactus species of *Drosophila mojavensis* showing broad areas of overlap (compiled from Turner et al. 1995).

the region of La Paz. For purposes of this chapter, however, sympatric *D. mojavensis* will refer only to the population of the mainland Sonoran desert.

Interspecific Isolation: *D. arizonae* and *D. mojavensis*

Prezygotic Isolation

Sexual isolation between *D. arizonae* and *D. mojavensis* has been studied using a number of different experimental designs. Early studies of isolation between *D. arizonae* and *D. mojavensis* by Patterson (1947a) and by Baker (1947) were based on the numbers of females inseminated in reciprocal, no-choice pairings. Both studies utilized *D. mojavensis* from southern California and showed a significant degree of asymmetry in which fewer *D. arizonae* females mated with *D. mojavensis* males than the reverse,

although the magnitude of the difference was less in the Baker (1947) study.

Asymmetry is quite striking also in the results of "male choice" tests (Patterson 1947a). Using the same design, Wasserman and Koepfer (1977) not only confirmed the asymmetrical sexual isolation between *D. arizonae* and *D. mojavensis*, but reported character displacement for sexual isolation in regions where the two species are sympatric. The greatest influence of sympatry on sexual isolation is observed when the *D. mojavensis* female is from a region of sympatry. The situation is summarized in table 17.1.

This increase in prezygotic isolation in sympatry conforms to the pattern described by Coyne and Orr (1989b, 1997). The hypothesis that increased sexual isolation observed with sympatric *D. mojavensis* has been created by reinforcement generates several predictions. These do not apply to *D. arizonae*, since it does not change its already strong sexual isolation in sympatry. First, sym-

Table 17.1. Influence of sympatry on sexual isolation.

Source of <i>mojavensis</i>	Isolation Direction	
Allopatric	AZ female \times MO male $>$ MO female \times AZ male (I = 0.85)	(I = 0.57)
Sympatric	AZ female \times MO male = MO female \times AZ male (I = 0.94)	(I = 0.92)

Isolation indices (I) averaged from the male choice data of Wasserman and Koepfer (1977) are given in parentheses.

patric *D. mojavensis* should exhibit specific responsible behaviors that are measurably different from allopatric flies when paired with *D. arizonae* males. Furthermore, because the greatest increase in sexual isolation is observed with sympatric *D. mojavensis* females, these females should show the greatest behavioral difference when courted by heterospecific males.

Empirical results support these predictions. Males of both species preferentially court conspecific females, but this preference is more pronounced in *D. mojavensis* males from areas of sympatry with *D. arizonae* (Markow 1981). Furthermore, females of *D. arizonae* from all localities show extreme isolation from heterospecific males, while in *D. mojavensis*, only females from regions of sympatry behave differently toward *D. arizonae* males. Only half of the number of sympatric compared to allopatric *D. mojavensis* females exhibit the characteristic receptivity display to courting *D. arizonae* males, and of those ultimately indicating receptivity, effectively all attempted mountings fail when the female is from a sympatric strain (Markow 1981). The responsible male and female behaviors are under the control of different genetic factors (Markow 1981; Zouros 1981b).

If reinforcement is the explanation for the increased sexual isolation of sympatric *D. mojavensis* females, postmating isolation should be strongest in crosses in which the female parent is *D. mojavensis*, because this is where increased sexual isolation is strongest. Examining postmating isolation between these two species reveals, however, that testing this prediction is not simple, owing to the presence of postmating isolation at more than one level.

Postzygotic Isolation

Fertile females and sterile males are observed in the progeny of *D. arizonae* females mated to *D. mojavensis* males, consistent with Haldane's Rule. *Drosophila mojavensis* females crossed with *D. arizonae* males yield fertile female and male offspring, with the exception of *D. mojavensis* from one locality (Ruiz et al. 1990). Based solely upon hybrid sterility, the isolation can be summa-

rized as follows: AZ female \times MO male $>$ MO female \times AZ male.

The genetics both of hybrid male sterility and of viability have been investigated. Hybrid male sterility in the progeny of *D. arizonae* females and *D. mojavensis* males has been extensively studied by Zouros and his colleagues (Zouros 1981a; Vigneault and Zouros 1986; Zouros et al. 1988; Goulielmos and Zouros 1995), because fertile hybrid progeny can be backcrossed to obtain different karyotypic combinations from the two species. They found that all major chromosomes carry genetic information that cause male sterility and that the expression of this sterility depends on the genetic background with respect to the other chromosomes. Four chromosome combinations produce male sterility: Y_a/IV_{mm} , X_a/III_{ma} , X_a/V_{ma} , and Y_m/III_{ma} , where roman numerals correspond to different autosome pairs, m = chromosome derived from *D. mojavensis*, and a = chromosome derived from *D. arizonae*. Furthermore, they have identified a speciation gene, SMF (sperm motility factor), in chromosome IV that causes sterility in conjunction with the Y (Pantazidis and Zouros 1988).

The reciprocal cross, between *D. mojavensis* females and *D. arizonae* males, yields significantly lower numbers of offspring, despite the fertility of both sexes in the F_1 (Ruiz et al. 1990; Baker 1947). Thus, based on productivity, the direction of isolation is opposite that found for the reciprocal cross: MO female \times AZ male $>$ AZ female \times MO male. Possible explanations for the low progeny production from *D. mojavensis* females include asymmetrical sexual isolation (i.e., there are fewer progeny due to fewer interspecific matings involving *D. mojavensis* females), reproductive tract incompatibilities, postzygotic inviability, and selective sperm utilization by females. Behavioral isolation cannot account completely for the low productivity of these matings because it is observed even when the *D. mojavensis* females are from allopatric strains and the females are known to be inseminated. Remaining possible mechanisms involve either hybrid inviability or some sort of postmating but prezygotic phenomena in the female reproductive tract (Markow 1997).

Viability of hybrids (egg to adult mortality) has not been quantified, although larval to adult mortality does not appear to differ between the reciprocal crosses (Grant 1966). Postzygotic death, that is, dead larvae, from this cross (MO female \times AZ male) has been observed in our laboratory and others (E. Zouros, personal communication) but the number of eggs laid, the proportion of eggs fertilized, and the level of embryonic lethality have never been quantified.

Zouros (1981b) found that progeny of backcrosses showed no breakdown of viability and that there was no sex difference in viability in any of the categories of progeny. This could explain why Nagle and Mettler (1969) were able to create interspecific hybrid populations in which certain chromosome combinations exhibited heterosis compared to the parental species. These observations question whether the cost to *D. mojavensis* females mating with *D. arizonae* males is significant enough to favor character displacement through reinforcement. In fact, these observations suggest that inviability alone may not constitute strong enough selection against hybridization in regions of sympatry (Zouros 1981b).

Postmating Prezygotic Isolation

What other mechanisms might explain the lower number of offspring from *D. mojavensis* mothers? And, are they strong enough to impose sufficient costs to heterospecifically mated *D. mojavensis* females to select for increased sexual isolation in the region of sympatry? Below we present evidence bearing upon potential interference with oviposition or normal remating by female *D. mojavensis* concerning this question. Females of both *D. arizonae* and *D. mojavensis* normally exhibit a large mass in the uterus following intraspecific mating (Patterson 1947b), called the insemination reaction, lasting for 7–9 hours. The onset of oviposition begins shortly after that time, but it is unclear if oviposition is responsible for the disappearance of the mass, or if the mass must degenerate before oviposition can begin. Remating in females of these species also coincides with reduction in the mass (Patterson 1947b; Krebs 1989). In heterospecific matings, the mass tends to be larger and of longer duration: the increase in size and persistence of the mass appear to be positively correlated with the level of divergence between the two species (Patterson 1947b).

Patterson (1947b) observed that in crosses between mulleri complex species more distantly related than *D. arizonae* and *D. mojavensis*, the mass often lasted indefinitely or caused severe damage to the female reproductive tract. In interspecific crosses between other species, Patterson (1947b) also observed disintegrated eggs in the mass as well as evidence of sperm incapacitation or death. These observations suggested to Patterson that the mass is a potential reproductive isolating mechanism. When *D. mojavensis* females are mated to *D. arizonae* males, the mass is still fairly large after 72 hours, while

it disappears in the reciprocal cross after 9 hours. Because Baker (1947) observed that a large number of unproductive *D. mojavensis* females inseminated by *D. arizonae* males contained a large insemination reaction mass in their reproductive tracts, Ruiz et al. (1990) invoke the insemination reaction to explain the reduced productivity of *D. mojavensis* females mated to *D. arizonae* males, suggesting that the sperm of *D. arizonae* males are killed in the reproductive tracts of *D. mojavensis* females. Unfortunately, direct support for this hypothesis is lacking. Neither Baker (1947) nor Ruiz et al. (1990) reported motility of stored sperm, oviposition, or postzygotic mortality in these crosses.

We tested the hypothesis that the mass blocks oviposition in crosses between *D. mojavensis* females and *D. arizonae* males. Single females were confined with males until copulation was observed. Mated females were transferred daily for one week to fresh culture vials and the number of eggs was counted. Females then were dissected and the sperm storage organs were scored for the presence of sperm. Table 17.2 shows that oviposition is not prevented in *D. mojavensis* females inseminated by *D. arizonae* males, although in several combinations it is reduced. No reduction is observed in the reciprocal cross, and in fact, when the *D. mojavensis* males are from Sonora, there is an increase in oviposition by *D. arizonae* females. After one week, about three fourths of the females still had motile sperm and we observed no evidence of dead sperm in our dissections (table 17.3). While these motile sperm obviously are not killed in the female reproductive tract, their viability and recoverability are unknown. Obviously a large number are viable, since offspring are produced. About half of these females had a vestigial reaction mass in their uterine cavities, but it did not interfere with oviposition.

We also tested the hypotheses that sperm are inviable in the reproductive tract and that female remating is im-

Table 17.2. Mean number of eggs laid in interspecific crosses of *Drosophila arizonae* (Ariz) and *D. mojavensis* race A (MojA), race B_I (MojB_I), and race B_{II} (MojB_{II}).

Mating			Number of Eggs ($\bar{X} \pm SE$)
Females	Males	n	
MojA	MojA	16	157.6 \pm 15.5
MojA	Ariz	14	108.4 \pm 13.4
MojB _I	MojB _I	24	82.6 \pm 7.6
MojB _I	Ariz	No matings	—
MojB _{II}	MojB _{II}	23	85.6 \pm 9.1
MojB _{II}	Ariz	17	66.2 \pm 7.7
Ariz	Ariz	33	82.8 \pm 6.9
Ariz	MojA	24	81.7 \pm 7.4
Ariz	MojB _I	8	116.0 \pm 13.9
Ariz	MojB _{II}	10	76.2 \pm 15.5

Table 17.3. Insemination reaction mass and storage of motile sperm.

Female	×	Male	n(♀♀)	Mass	Sperm	Reference
<i>D. arizonae</i>		<i>D. mojavenis</i>	39	16	19	Patterson
<i>D. mojavenis</i>		<i>D. arizonae</i>	154	97	80	Patterson
<i>D. arizonae</i>		<i>D. mojavenis</i>	76	20	?	Baker
<i>D. mojavenis</i>		<i>D. arizonae</i>	90	74	?	Baker
<i>D. arizonae</i>		<i>D. mojavenis</i>	17	0	15	Present study
<i>D. mojavenis</i>		<i>D. arizonae</i>	22	8 ¹	17	Present study

Data are from present study and Baker (1947) assayed 7 days after mating, and from Patterson (1947b) assayed 96 hours after mating.

¹Degenerate mass.

paired by the insemination reaction. Individual females from each species were placed with single males of the other species until a copulation was observed. Pairs were separated and the females were held for 24 hours before being tested for remating by placing them individually with males of their own species for one hour. Of 15 heterospecifically mated females from each species, all remated within the first 20 minutes, as did females mated initially with their own males 24 hours earlier. Although the reaction mass persists for 72 hours in one of the hybridizations, it does not appear to cause any delay in remating.

We conclude that the low productivity from *D. mojavenis* females is not due to a reduction in oviposition or to oocytes being trapped in the reaction mass. The differences in oviposition rates are insufficient by themselves to account for the productivity differences between reciprocal crosses. The presence of motile sperm in the ventral receptacles of all mated *D. mojavenis* females demonstrates that *D. arizonae* sperm are transferred, stored and still alive after one week. Finally, females suffer no consequences in their ability to remate with conspecific males following a heterospecific mating. The unanswered question is the fertility versus viability of the oviposited eggs from *D. mojavenis* females mated to *D. arizonae* males. If a proportion of the eggs laid are not fertilized, it would suggest that either the motile sperm are not viable, or that females can exercise some choice over the sperm they allow to fertilize their eggs. Experiments are needed to determine if the low productivity is due to embryonic inviability or to the deposition of unfertilized eggs.

In summary, crosses between *D. arizonae* and *D. mojavenis* exhibit both prezygotic and postzygotic isolation in the following directions: behavioral, AZ female × MO male > MO female × AZ male; productivity, AZ female × MO male < MO female × AZ male; sterility, AZ female × MO male > MO female × AZ male. All three types of isolation exhibit asymmetry, but in different directions. Only behavioral asymmetry changes in regions of sympatry. With respect to the character displacement hypothesis for the behavioral isolation observed in sympatric *D. mojavenis*, it is unlikely, given the fertility and via-

bility of the F₁ hybrids from *D. mojavenis* females, that selection against hybridization could provide the sole explanation for the observed patterns.

Reinforcement of premating isolation due to existing postzygotic isolation may cause character displacement like that observed in sympatric *D. mojavenis*. However, modeling indicates that the parameters under which reinforcement may occur are quite restrictive (Maynard Smith 1966; Moore 1979, 1981; Spencer et al. 1986; Butlin 1989; Liou and Price 1994). In fact, substantial niche differentiation and hybrid fitnesses at or close to zero are usually invoked. Maynard Smith (1966) determined that hybrid fitness as low as 0.25 would result in populations blending into a single hybrid swarm within eight generations, causing selection for more viable hybrids as opposed to increased premating isolation. If we assume that hybridizing *D. arizonae* females and *D. mojavenis* males produce roughly as many offspring as with homospecific mates, then male sterility in the progeny of this cross would reduce fitnesses of parents and average offspring fitness to 0.50 at a minimum. In the reciprocal hybrid cross, Baker (1947) and Ruiz et al. (1990) reported sevenfold and threefold reductions in numbers of offspring, respectively. At most, then, the fitness of hybridizing *D. mojavenis* females and *D. arizonae* males would be reduced by a factor of 7. This apparent fitness disadvantage, however, may be offset by heterosis (Nagle and Mettler 1969). Furthermore, lifetime fitness reductions could be far less substantial because of the high frequency of remating in both species. These, admittedly rough, estimates of the reduced fitness resulting from hybridization question whether reinforcement alone could have driven the putative sympatric character displacement in *D. mojavenis*.

Intraspecific Isolation and Differentiation: *D. mojavenis*

Mettler (1963) first proposed the existence of two races of *D. mojavenis*: race A in the deserts of southern California and race B from the Sonoran desert of Arizona,

Sonora, northern Sinaloa, and the Baja peninsula. Allozyme differences led Zouros (1973) to propose a further subdivision of race B into B_I including populations from Arizona and mainland Mexico, and race B_{II} from Baja. The populations in these separate geographic areas utilize different host plants (figures 17.1 and 17.2). While the role of host adaptation in the evolution of genetic differences between races is unknown, Starmer et al. (1977) suggest a functional relationship between allelic variation for ADH and host chemistry. In order to be considered true host races, populations should exhibit the greatest fitness on their respective hosts. In those populations of *D. mojavensis* where this has been examined, the expected relationship was not found. Agria is the preferred host of all races of *D. mojavensis* and the one on which fitness is the greatest (Ruiz and Heed 1978). Host shifts, therefore, are more likely to reflect host availability in those desert regions invaded by *D. mojavensis*. The genetic differentiation between races of *D. mojavensis* (Zouros 1973; Heed 1978; Cleland et al. 1996) may be a reflection of differences in host ecology.

Zouros and d'Entremont (1980) showed the existence of significant sexual isolation between the B_I and B_{II} subraces. Populations from southern California and from Santa Catalina Island are not behaviorally isolated from each other or from B_I or B_{II} flies (Markow 1991). Despite suggestions that the B_I-B_{II} isolation might be influenced by rearing media (Brazner 1983; Etges 1992), a number of independent investigations have found this sexual isolation to be repeatable and significant (Zouros and d'Entremont 1980; Wasserman and Koepfer 1977; Markow 1991; Krebs and Markow 1989; Krebs 1990) even when flies were reared on tissue of alternate host cacti (Etges 1992; Fogleman and Markow, unpublished). Furthermore, reciprocal hybridization (Krebs 1990) and artificial selection experiments (Koepfer 1987a,b) have demonstrated a clear genetic basis for the observed sexual isolation, with different genetic mechanisms underlying male and female behavior.

The sexual isolation within *D. mojavensis* is asymmetrical: females from race B_I show reduced receptivity to B_{II} males. Zouros and d'Entremont (1980) attribute this to the pressure on B_I *D. mojavensis* from sympatry with *D. arizonae*, in that the selection for increased mating discrimination by *D. mojavensis* females simultaneously increased their discrimination against males from Baja as well. This interpretation assumes that the same genetic mechanism underlies discrimination against conspecifics as well as against *D. arizonae* males. Zouros (1981b) has localized the genes for female isolation from *D. arizonae* males to specific *D. mojavensis* chromosomes. Because the chromosomal locations of the genes for discrimination against *D. mojavensis* males from B_{II} are unknown, we cannot tell if they are the same. It is unlikely that the mechanism is a general one, however, since Sonora females do not always discriminate against *D. mojavensis*

males from other isolated geographic areas (Markow 1991).

Another assumption of the character displacement explanation is that the behavioral "characters" or cues used for discrimination are also the same. There is no information bearing on this question either. In an earlier study (Markow and Toolson 1990), differences were reported in cuticular hydrocarbon composition along with evidence that these differences influence mating behavior of *D. mojavensis*. Studies of mating pairs, however, showed no association between hydrocarbon composition and mating success (Markow and Toolson, unpublished).

No decrement in F₁ viability has been detected in crosses between any of the races of *D. mojavensis* in which it has been studied, but fertility and backcross fertility have not been investigated (Etges and Heed 1987; Etges 1990, 1993). However a number of studies of divergence for quantitative traits such as development time (Etges and Heed 1987; Etges 1990, 1993) and locomotor activity (Krebs 1991) suggest that genetic differences between races may, in fact, be extensive. Whether enough time has elapsed for the accumulation of mutations with postzygotic intraspecific hybrid effects is a question that begs investigation.

Rules and Patterns: Conformity or Endless Forms?

It is clear from the foregoing that *D. arizonae* and *D. mojavensis* both conform to and challenge some of the proposed rules. Haldane's Rule, that the heterogametic sex is the one most seriously compromised when sex differences exist in hybrid fitness, certainly is true for male sterility in the cross between *D. arizonae* females and *D. mojavensis* males. Recent models of the evolution of sterility and inviability (Orr 1993; Turelli and Orr 1995) predict that the genetics of hybrid sterility will be largely sex specific, but that the genes causing hybrid inviability should tend to be expressed equally in both sexes. We observe this pattern for the *D. arizonae*/*D. mojavensis* species pair. While the causes of the low productivity of the *D. mojavensis* female × *D. arizonae* male cross are unknown, there is an apparent lack of sex differences in the hybrids produced (Zouros 1981c; Grant 1966). The chromosomal incompatibilities in these species previously discussed support the prediction of sex-specific sterility genes.

Efforts to explain Haldane's Rule have led to another rule, attributing the responsible genetic factors to the X-chromosome (Coyne and Orr 1989a; Charlesworth et al. 1987). This interpretation, however, may be a function of the more limited types of analyses typically performed, that is, hybridization and first generation backcrosses. When specific chromosomes or parts of chromosomes can be combined, through introgression,

in either heterozygous or homozygous conditions, a broader array of chromosomal interactions can be examined for incompatibilities. The work of Zouros and his colleagues (Zouros 1981a; Vigneault and Zouros 1986; Zouros et al. 1988; Goulielmos and Zouros 1995) was the first to suggest that the importance of the X-chromosome in male sterility may not be as great as originally assumed. Although these investigators found that the X-chromosome plays a key role in some of the incompatibilities between *D. mojavensis* and *D. arizonae*, these same studies revealed that the Y-chromosome is involved in as many chromosomal incompatibilities causing sterility as the X. This result is unexpected given the proposed causes of the large X-effect: that the sex chromosomes experience more rapid evolutionary rates than the autosomes (Charlesworth et al. 1987; Coyne and Orr 1989a). Even assuming equal evolutionary rates between the X and Y chromosomes, if the role of each sex chromosome in determining fertility and viability is proportional to its size, we might expect the Y to be involved in far fewer incompatibilities than the X-chromosome. Instead, the Y-chromosome appears to play a disproportionately large role in determining male hybrid sterility in the *D. arizonae/D. mojavensis* system. Recent studies with other *Drosophila* species (Hollocher and Wu 1996; Lamnissou et al. 1996) also point to the importance of chromosomes other than the X-chromosome.

Wu and Davis (1993) have speculated that the highly specialized nature of spermatogenesis may cause this system to be less buffered against hybridization thus partially explaining Haldane's Rule in species with heterogametic males. Given the known importance of the Y-chromosome in determining *Drosophila* male fertility (Ashburner 1989) and the proposed heightened sensitivity of spermatogenesis to genetic perturbations, modeling and testing of the potential large Y effect of male hybrid sterility per Haldane's Rule may be a fruitful avenue for future investigation.

Prezygotic isolation also has been suggested to follow some rules. Coyne and Orr (1989a) have observed that species populations existing sympatrically with closely related species tend to express higher levels of premating isolation than do allopatric populations at comparable genetic distances. The *D. arizonae/D. mojavensis* species pair support this observation. Published genetic distances based on allozyme variability (Zouros 1973) indicate that each of the races of *D. mojavensis* are roughly genetically equidistant from *D. arizonae*, yet sympatric *D. mojavensis* Race B₁ exhibits significantly stronger premating reproductive isolation from the sister species than either of the allopatric races. On the surface, the pattern with *D. arizonae/D. mojavensis* is consistent with the higher level of isolation in sympatry, but the interpretation that reinforcement is the most likely explanation is questioned on the basis of the available observations.

Given the theoretical (Maynard Smith 1966; Moore 1979; Butlin 1989) difficulty in invoking reinforcement as a significant cause of increased premating isolation (but see Howard 1993), additional explanations for the observed increased isolation in sympatry must be sought. For this species pair, the wealth of information on their ecology and mating systems may be informative. The observed patterns of prezygotic isolation between *D. arizonae* and *D. mojavensis* and between populations of *D. mojavensis* could have originated as asymmetries having nothing to do with interspecific discrimination and mate recognition, instead reflecting coevolution between the sexes for different mating system components (West-Eberhard 1983). In the case of sexual behavior, male vigor and female receptivity coevolve in a population of the same species such that in strains where female receptivity is great, males are less vigorous. In strains where female receptivity thresholds are higher, males are more vigorous in their courtship. These differences may have nothing to do with discrimination, but are likely a function of the number of mating opportunities and the intensity of sexual selection in the recent evolutionary history of a population. For example, when the operational sex ratio is highly male biased, male vigor is favored. In less male-biased or in female-biased populations, female receptivity will be greater and males will not need to court as vigorously. Among *Drosophila* species the operational sex ratio varies widely due largely to enormous differences in female remating intervals and male maturation times (Markow 1996).

When two populations of the same species, having different male vigor and female receptivity, are placed together in a "choice test," asymmetries in the form of more matings between the vigorous males and receptive females are expected (Kence and Bryant 1978; Van den Berg et al. 1984). In the case of *D. mojavensis* from races B₁ and B_{II}, males and females differ significantly in their mating speeds (Krebs and Markow 1989). Males from B₁ court more quickly and vigorously than do B_{II} males, and females from the latter race are more receptive. Thus, the observed asymmetrical isolation between flies from these two races is expected on the basis of vigor/receptivity differences alone. It is clear that additional factors are responsible, however, since males do not initiate courtship of females from different strains or species at random, and it is possible to separate these components statistically (Zouros and d'Entremont, 1980).

How significant is this sort of observed sexual isolation to the development of biologically meaningful isolation? We do not mean to imply that deviations from random mating attributable to vigor/receptivity differences are unimportant. On the contrary, higher female receptivity thresholds may be a key prerequisite to the development of more specific, character-based behavioral divergence, such that females have more time to evaluate qualitative differences among suitors before mating. Thus, very early in the separation between two popula-

tions, asymmetrical sexual isolation may reflect vigor/receptivity differences, while later, additional, qualitative changes are expected. It is not clear, in the case of *D. arizonae* and *D. mojavensis*, exactly what the qualitative differences are.

Asymmetries in sperm utilization may also reflect differences in the coevolution of reproductive strategies. Male ejaculatory fluids contain proteins that stimulate oviposition, but females are known to exhibit differential responses to the proteins of different males (Markow 1982). It may not always be in the female's best interest to oviposit following mating, due either to lack of quality oviposition sites or to lack of quality sperm. Pairing males and females from strains that differ in this parameter should produce asymmetries in egg laying. Based on differences in resource availability for *D. mojavensis* from different host regions, we expect females of different populations to differ in the stimulation required to oviposit.

Generally, when multiple asymmetries exist at different levels or sites along the isolation continuum, certain combinations of them may facilitate the speciation process. These asymmetries can have their origins in the resource ecology of the populations in question without being a function of performance on particular hosts, per se. To what degree the observed isolation among *D. mojavensis* races is a reflection of nonspecific behavior versus behavior with the potential to significantly restrict gene flow is unclear.

These are just some suggested alternatives to reinforcement. The predictions of the reinforcement hypothesis, as it relates to both the inter- and intraspecific isolation, can be tested. One of these predictions, that the loci for sexual isolation within and between species are the same, ultimately can be tested by mapping the loci in both species. Second, if the responsible behaviors can be identified, they can be experimentally manipulated to see if they are the same in both species. Third, testing both species from the area of La Paz, Baja California, another region of sympatry, should give similar results to those observed in the sympatric flies from the mainland of Mexico. And finally, the degree to which both species share host cacti, and thus have the opportunity to hybridize, is something that can, contrary to many *Drosophila* species pairs whose hosts are less well known, actually be determined in this system.

The final pattern to which these two species appear to conform is the proposed earlier development of premating compared to postmating isolation in sympatry. Although we do not dispute the observed pattern in sympatry, we wonder how the general allopatric and sympatric patterns of pre- versus postmating isolation will hold up against future, more detailed scrutiny. We question whether these general patterns may be artifacts of the relative ease with which premating versus postmating isolation can be examined as opposed to a reflection of a true evolutionary pattern. Sexual isolation is far easier to test for and quan-

tify than is postmating isolation. A large number of flies can easily be placed together in a mating chamber and the number of matings of different types scored in a short time. Large sample sizes permit even subtle behavioral isolation to be detected. Postmating isolation, on the other hand, is more labor intensive to measure, and if it is subtle or incomplete, or only appears in segregating generations, it may go undetected. We suggest that tests for postmating isolation be more thorough to be certain it does not exist in some degree not easily revealed by the standard 0.0, 0.25, 0.50, and 1.00 scoring protocol. The lesson from studies of the genetics of sterility (Goulielmos and Zouros 1995; Hollocher and Wu 1996; Lamnissou et al. 1996) may apply to other rules and patterns as well.

Amid numerous and varied observations and specific results of experimentation, evolutionary biologists seek to impose order on the evolutionary process by proposing generalized mechanisms and patterns. The exhaustive surveys and insightful syntheses of Coyne and Orr (1989a,b) provide an invaluable framework for studies of speciation. Constructive analysis and experimentation have generated both support for and exceptions to these proposed rules, the relative weight of which will dictate their future acceptance, modification, or elimination. We hope that this brief analysis of the *D. arizonae/D. mojavensis* model system will inspire additional inquiry.

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