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Premating Isolation Is Determined by Larval Rearing Substrates in Cactophilic *Drosophila mojavensis.* IV. Correlated Responses in Behavioral Isolation to Artificial Selection on a Life-History Trait

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ABSTRACT: Studies of behavioral isolation among geographically isolated populations of *Drosophila mojavensis* have provided an understanding of incipient speciation wherein phylogeny and ecology play a prominent role. Populations of *D. mojavensis* in mainland Mexico and southern Arizona exhibit low but significant premating isolation from Baja California populations in laboratory mate choice tests. These same populations have undergone considerable life-history evolution in response to use of different host plants, suggesting that behavioral isolation between populations is a pleiotropic consequence of adaptation to different environments, or Mayr's geographic speciation hypothesis. This hypothesis was tested using bidirectional artificial selection on egg-to-adult development time in replicate lines of a mainland and Baja population cultured on two host cacti for 13 generations. Response to selection was greatest in the slow lines cultured on one host, yet there was uneven response in some lines due to variation in cactus tissue quality. Realized heritabilities for development time ranged from 0.04 to 0.16, which is consistent with previous estimates from half-sib/full-sib analyses of genetic variation. In most lines that responded to selection, premating isolation decreased to near zero. Correlated responses in behavioral isolation suggest that adaptation to contrasting environments can cause secondary responses in mate recognition systems that can influence the formation of new species.

Keywords: speciation, premating isolation, rearing substrates, *Drosophila,* cactus.

The origins of reproductive isolation in sexually reproducing organisms that share a common fertilization system remain an unresolved problem in studies of speciation. Multiple mechanisms may cause reproductive isolation among groups such as drift and/or selection in allopatry (Dobzhansky 1940; Mayr 1963) or parapatry (Slatkin 1982), but modifications in breeding systems that give rise to disassortative mating upon secondary contact are poorly understood. Paterson (1978, 1980, 1993) emphasized the role of intrademic "mate recognition systems" as primary determinants of speciation, when and if it occurs. Carson (1987, 1995) also pointed out that the evolution of common mate recognition systems via coadaptation of male-female signaling systems and sexual selection may be a major cause of speciation in animals. He suggested that interactions between potential mates within demes must be the driving force of sexual selection with only secondary consequences for reproductive isolation, consistent with Paterson's recognition model. Variation in mate recognition systems among isolated demes may also respond to local ecological conditions, leading to the evolution of modified equilibrium signaling systems (Butlin 1995). The effects of local conditions leading to divergence in mating systems may lead to premating isolation should such demes ever come into secondary contact. Identifying these causes for divergence are essential to the understanding of species formation.

The evolution of premating isolation among populations of cactophilic *Drosophila mojavensis* has been a model system for understanding how such signaling systems respond to local environmental conditions since Zouros and D'Entremont (1974) first observed behavioral isolation among geographically isolated populations. A number of laboratory studies have since confirmed the existence of premating isolation between Baja California populations and those from mainland Mexico and Arizona, isolated by the Gulf of California (Zouros and D'Entremont 1980; Markow et al. 1983; Koepfer 1987*a*,

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1987*b;* Krebs and Markow 1989; Markow 1991). In male choice, female choice, and multiple choice mating trials (see Fraser and Boake 1997 for a comparison of these techniques), a common observation is increased intensity of mainland female discrimination against mating with Baja males, leading to "one-way" premating isolation. Wasserman and Koepfer (1977) hypothesized that the distribution of mainland *D. mojavensis* populations overlapped with that of a sibling species, *Drosophila arizonae,* leading to the evolution of altered mate signaling systems in *D. mojavensis* from the ancestral condition in Baja California where *D. arizonae* is absent. Together with studies of variation in the degree of premating isolation between species, Wasserman and Koepfer (1977, 1980) concluded that reproductive character displacement had occurred between mainland *D. mojavensis* and *D. arizonae.*

However, other potential ecological factors responsible for behavioral isolation between peninsular and mainland populations of *D. mojavensis* have not been ruled out. Markow et al. (1983) suggested possible pleiotropic effects due to use of different host cacti could also explain the observed premating isolation between populations of *D. mojavensis* because peninsular and mainland populations use different host plants. However, they concluded that since premating isolation was not evident between populations of other Sonoran Desert *Drosophila* species distributed on both sides of the Gulf of California, factors other than reproductive character displacement, such as the use of different host cacti, were probably unimportant for *D. mojavensis.*

Brazner (1983) demonstrated that rearing *D. mojavensis* on laboratory media significantly increased premating isolation over that with flies reared on fermenting cactus tissues. Other studies (Etges 1992; Brazner and Etges 1993) extended these results and showed that lab food–reared flies used in all previous analyses of premating isolation exhibited significantly greater premating isolation, higher levels of mainland female discrimination against mating with Baja males, and longer male and female times to copulation as compared with that observed for flies reared on fermenting cactus tissues, particularly the cactus host used widely in Baja California, pitaya agria cactus, *Stenocereus gummosus.* Rearing flies on another major host, *Stenocereus thurberi,* organ pipe cactus, caused low but significant premating isolation between populations. Thus, use of different host plants in nature can affect patterns of mate choice, making it unclear how employing lab food–reared flies is relevant to understanding sexual selection and isolation in the wild.

The present study was designed to test the pleiotropy hypothesis for the evolution of premating isolation between Baja California and mainland populations of *D. mojavensis* because considerable life-history evolution has occurred since *D. mojavensis* colonized mainland Sonora from Baja California in response to switching from agria to organ pipe cactus hosts (Etges and Heed 1987; Etges 1989*a,* 1989*b,* 1993; Etges and Klassen 1989). The presence of these host races (Etges 1990) makes possible a test of the pleiotropy hypothesis for the origins of premating isolation (Muller 1940; Mayr 1963): if behavioral isolation between mainland and Baja California populations of *D. mojavensis* originated as a correlated response to adaptation to different environments, then altering gene frequencies influencing one or more life-history traits should reveal correlated responses in adult mating behaviors. Since Koepfer (1987*a*) demonstrated a rapid response to artificial selection on premating isolation between mainland and Baja California strains, I predicted there was sufficient genetic variation underlying interdemic mate choice behaviors in *D. mojavensis* that levels of premating isolation might respond to selection on a correlated trait. In this study, I used bidirectional artificial selection on egg-to-adult development time in both mainland and Baja California populations cultured on both agria and organ pipe tissues. Changes in behavioral isolation between populations were assessed each generation. The null hypothesis was that intensity of premating isolation is unrelated to any response to selection on development time.

Material and Methods

Husbandry

All *Drosophila mojavensis* were collected from naturally occurring agria cactus rots in March 1991. Sample sizes, locations, and techniques are described elsewhere (Etges 1992), and premating isolation in these original stocks is also summarized. A mainland population was derived from 502 adults collected at Punta Onah, Sonora, where both agria and organ pipe cactus are sympatric. The Baja stock originated from Punta Prieta, Baja California Norte, from 275 adults aspirated from agria rots and from 1,913 adults that emerged from eight agria rots returned to the laboratory. All flies were cultured on banana food (Brazner and Etges 1993) in 8-dram shell vials for six to seven generations before artificial selection began. Several thousand adults from each population were then introduced into separate 12,720-cm^3 population cages, and after 2-wk, eggs were collected and transferred to banana food in ½-pint milk bottles. These cultures were grown in an incubator at moderate densities exposed to a 14L:10D photoperiod that cycled from 27° to 17°C. Adults from at least 12 bottles per population were separated by sex, aged for 2 wk, and 200 adults of each

sex were combined into individual oviposition chambers. Twelve replicate chambers were established for each population. Six chambers were randomly assigned to agria and the other six to organ pipe cactus treatments. Each set of six was divided into two replicate control lines, two fast lines, and two slow lines. Therefore, there were 4,800 adults used as parents each generation, divided into 24 selection and control lines cultured throughout the experiment.

From each oviposition chamber, eggs were collected over 10-h intervals (usually 0800 hours to 1800 hours) in petri dishes containing 1% agar-fermented cactus juice and held overnight in the incubator described above. The following day, eggs from each chamber were washed in deionized water, 70% ethanol, and again in sterile deionized water. Eggs were counted out onto a 1-cm^2 piece of sterilized filter paper in groups of 250 and placed on fermenting cactus. Cactus cultures were set up in plugged $^1/_2$-pint bottles with 75 g of aquarium gravel at the bottom covered with a 5.5 cm diameter piece of filter paper. Bottles were then autoclaved, allowed to cool, and after 60 g of either agria or organ pipe tissue were in place, autoclaved again for 10 min. After cooling to room temperature, each culture was inoculated with 0.1 cm^3 of a pectolytic bacterium, *Erwinia cacticida* (Alcorn et al. 1991), and 0.1 cm^3 of a mixture of seven species of yeasts common in natural agria and organ pipe rots (Starmer 1982; Fogleman and Starmer 1985), *Pichia cactophila, Pichia mexicana, Pichia amethionina* var. *amethionina, Cryptococcus cereanus, Candida valida, Candida ingens,* and *Candida sonorensis.* Four subreplicate cactus cultures were started from each of the 24 oviposition chambers, so there were 96 cultures (19,200 flies) maintained each generation (2 populations $\times$ 2 cacti $\times$ 3 selection lines $\times$ 2 replicates $\times$ 4 cultures). All cultures were grown in an incubator programmed as described above and were rotated to different shelves in the incubator every few days to avoid the effects of temperature stratification. Due to the large volume of fresh cactus tissue required each generation (5.76 kg), it was not possible to remove tissue quality variation as a potential source of environmental variation (Etges 1993) during selection.

Selection Procedures

All emerging adults from each bottle were collected daily, separated by sex, and aged for at least 12–14 d in vials containing banana food at room temperature (20°–24°C) because male *D. mojavensis* reach sexual maturity after 8–10 d at 25°C (Markow 1982). Egg-to-adult development time was measured in days, and viability was calculated as the number of eclosed adults divided by the number of counted eggs that hatched. For the fast lines, the first 50 females and 50 males to eclose from each of the four cultures for each replicate line (200 females and 200 males total) were combined into an oviposition chamber and were the source of eggs for the next generation. The last 50 females and 50 males to eclose from each of the four cultures for each replicate slow line were similarly used as parents for each succeeding generation. For the control lines, all adults of each sex from each of the four cultures were pooled without regard to development time. Groups of 50 females and 50 males were counted from each of the four replicates and combined to serve as the parents for each control line every generation. Thus, effective population sizes (N_e) were approximately 400 for each of the 24 selection and control lines throughout the 13 generations of the experiment.

Behavioral Isolation Tests

Premating isolation was measured for the first 12 generations between aged, mainland, and Baja adults grouped by cactus, selection line, and replicate. Thus, estimates of premating isolation between mainland and Baja adults were made using the same replicate lines selected in the same direction and grown on the same substrate. I recorded the number of copulations in groups of 15 pairs of virgin Baja males and females combined with 15 pairs of virgin mainland males and females in a plastic petri dish containing filter paper moistened with fermenting cactus juice. Two such mating chambers were observed simultaneously for 1 h in a darkened room. Adults from each population were lightly dusted with fluorescent powder (Radiant Color, Richmond, Calif.) of different colors 24 h prior to observation allowing identification of copulating pairs. Dust color was alternated between tests. All copulating pairs were followed for several minutes to avoid including any pseudocopulations in the data set (Markow et al. 1983).

Flies cultured in generation 13 were used in behavioral isolation tests designed to directly observe any altered behaviors caused by selection on development time. The number of copulations were recorded in mating trials as described earlier, but the two groups used were replicate lines of mainland and Baja flies cultured on the same cactus and selected in opposite directions for development time. For example, mating trials were performed with mainland and Baja lines grown on agria, but one population was a fast selection line and the other population had been selected for slow development time, and so on.

Statistical Procedures

Variation in egg-to-adult development time and viability over the entire experiment were assessed by ANOVA

(PROC GLM; SAS Institute 1985). Linear regression analyses (PROC REG) were used to measure selection responses in development time and correlated responses in viability and premating isolation. All development time data were tested for normality: $\log_{10}$ transformations of the data significantly improved normality. All viability data were arcsin transformed. The slopes of all pairs of replicate lines were tested for heterogeneity by performing ANCOVAs in PROC GLM.

Both Hill's (1972) and Muir's (1986) procedures for adjusting selection responses for uncontrolled variation in the control lines were used. The latter method employs multiple regressions to estimate the degree to which the selection lines should be corrected (Cohan and Hoffman 1989). Only results using the latter method are shown; all other results are available from me. Possible nonlinear responses to selection caused by changes in genetic variance (Heath et al. 1995) or depletion of genetic variation during the experiment were assessed: regression analyses of only the first nine and 10 generations of data were performed because of the absence of continued increases in development time in the slow lines after generation 9, and polynomial regression analyses were used to test for higher-order effects in the model. All of these reanalyses failed to explain any more of the variation than simple linear regression analysis. Some of the erratic generation-to-generation variation caused by organ pipe tissues were still apparent after control line corrections, so an outlier analysis was performed (Freund and Littell 1991, pp. 59–70). In several instances, generation means were found to bias significantly the estimation of the regression results, and so in these cases, the regressions were recalculated with those outliers eliminated from the analysis.

Realized heritabilities, h^2, were calculated using the corrected data,

$$h^2 = R(i\sigma_p)^{-1}, \tag{1}$$

where R is the slope of the response to selection, i is the intensity of artificial selection, and σ_p is the phenotypic standard deviation of each replicate line (Falconer 1981, p. 175). Because the number of adults selected each generation was held constant to maximize N_e, but the total number of adults per bottle varied due to variation in viability, selection intensities varied somewhat (mean i = 1.258, SD = 0.033). Estimating realized heritabilities in this way also assumed that phenotypic standard deviations were constant so I used Hartley's F_{max} test (Hartley 1950) to detect changes in variance over the 13 generations of artificial selection (Sokal and Rohlf 1981).

Premating isolation was estimated by calculating Yule's V index (Yule 1912) because it provides a more unbiased estimate and results in more accurate hypothesis testing than several other isolation indices (Gilbert and Starmer 1985). This index and its variance are given by:

$$V = [(n_{11} \times n_{22}) - (n_{12} \times n_{21})]/\sqrt{(\mathrm{F1} \times \mathrm{F2} \times \mathrm{M1} \times \mathrm{M2})}, \tag{2}$$

and

$$\begin{aligned}\mathrm{var}(V) = V^2\Big(&-4/N) + [n_{11} \times n_{22}(n_{11} + n_{22}) \\ &+ n_{12} \times n_{21}(n_{12} \times n_{21})]/ \\ &[(n_{11} \times n_{22}) - (n_{12} \times n_{21})]^2 \\ &- 0.75\,\{[\mathrm{F1} - \mathrm{F2})^2/(N \times \mathrm{F1} \times \mathrm{F2})] \\ &+ [(\mathrm{M1} - \mathrm{M2})^2/(N \times \mathrm{M1} \times \mathrm{M2})]\} \\ &+ 0.50\,\{[(n_{11} \times n_{22}) \\ &- (n_{12} \times n_{21})]\,(\mathrm{F1} - \mathrm{F2})(\mathrm{M1} - \mathrm{M2})\}/ \\ &(N \times \mathrm{F1} \times \mathrm{F2} \times \mathrm{M1} \times \mathrm{M2})\Big),\end{aligned} \tag{3}$$

where F1, F2, M1, and M2 are total numbers of females and males of the first and second populations, respectively, N is the total number of matings observed, and n_{11} is the number of homogamic matings between females and males from one population, n_{12} is the number of heterogamic matings between females of the first population and males from the second, and so on. The t-tests were performed as:

$$t = V/\sqrt{\mathrm{var}(V)}. \tag{4}$$

Indices of female-based assortative mating were calculated following Zouros and D'Entremont (1980) and Malagolowkin-Cohen et al. (1965), where

$$I_1 = (n_{11} - n_{12})/(n_{11} + n_{12}), \tag{5}$$

and

$$I_2 = (n_{22} - n_{21})/(n_{21} + n_{22}). \tag{6}$$

The variable I_1 estimates the degree of female-based assortative mating for females of strain one and I_2 for strain two. Both I_1 and I_2 are expected to be close to 0 under the null hypothesis of no female choice. The standard error of $I_i (i = 1, 2)$ is

$$S_i = [(1 - I_i^2)/(n_{i1} + n_{i2})]^{1/2}. \tag{7}$$

Zouros and D'Entremont (1980) provided an index of mating propensity where

$$\hat{k}(n_{12} + n_{22})/\,(n_{11} + n_{21}) \tag{8}$$

and the variance of $\hat{k}$ is

$$V(\hat{k}) = \hat{k}(1 + \hat{k})^2/N. \tag{9}$$

If male mating propensity is equivalent between populations, $\hat{k} = 1$. Variation in Yule's V, I_1, I_2, and $\hat{k}$ over the experiment was assessed by ANOVA, linear regression, and analysis of correlations with development time. These behavioral indices were arcsin transformed prior to analysis.

Results

Responses to artificial selection on egg-to-adult development time were greatest in the slow lines cultured on organ pipe tissues, but after correcting for control line variation, response was also apparent in the fast lines (fig. 1, table 1). In all cases, males and females responded similarly to selection, but male development time was significantly greater than that for females (Etges 1993). There were no differences in the slopes of the selection responses of pairs of replicate lines as revealed by ANCOVA. The erratic changes in development time in some of the lines resulted from uncontrolled variation in cactus tissue quality known to affect the expression of these life-history characters (Etges 1989*b,* 1993). Since only older, yellow-brown tissues were used like those found fermenting in nature with flies (Etges 1989*b*), this type of nutritional variation must be experienced by *Drosophila mojavensis* in the wild.

The response of these populations to growth on agria and organ pipe tissues was consistent with previous studies (Etges 1989*b,* 1990, 1993): mainland populations expressed longer egg-to-adult development times than did Baja populations, particularly on organ pipe causing a significant population-by-cactus interaction term in the ANOVA (table 2). The overall mean development times of the slow lines (19.38 d) exceeded the controls (18.47 d), which in turn were significantly longer than the fast lines (18.00 d; Tukey's Studentized Range test, $P < .05$).

Increases in development time caused by changes in organ pipe tissues during the experiment caused less apparent response in selection in the fast lines than was actually achieved. Response to selection was apparent in three of four of the fast lines cultured on organ pipe (table 1). The apparent lack of consistency in response to selection among replicate lines was largely due to variation in organ pipe tissue quality that affected replicate lines differently. Responses to selection on agria for faster development time was marginal in just the two mainland lines (table 1). With the sexes analyzed separately, only four of 24 organ pipe and three of 24 agria contrasts yielded evidence for heterogeneous variances. Thus, the majority of heritability estimates were unbiased, ranging from about 0.11 for the fast lines to 0.08 for the slow lines (table 1). Therefore, both mainland and Baja populations responded to selection for shorter development times on organ pipe and to a far lesser extent on agria showing that additive genetic variance for this trait is expressed in an environment-specific manner (Etges 1993; see below).

Egg-to-adult viability remained high throughout the experiment (fig. 2). In three of 24 of the selection and control lines, viability increased over the course of the experiment, and slopes for all lines were positive (results not shown). Overall, flies reared on organ pipe had higher viabilities than those reared on agria ($F = 37.21$, $P < .0001$). There were no viability differences among populations or selection lines, suggesting that these viability increases were largely environmental, that is, due to variation in cactus tissue quality.

Correlated Responses in Premating Behaviors

Premating isolation between Baja and mainland replicate lines decreased in both sets of slow lines cultured on organ pipe, one set of fast lines on organ pipe, and one set on agria (fig. 3, table 3). There were no detectable changes in any of the control lines over the 13 generations of selection. It is striking that premating isolation decreased to nearly zero in three of four selection lines cultured on organ pipe cactus (although the S2 line's regression was marginally significant, $P = .07$) and the only lines cultured on agria for which there was a significant response to selection for development time, the AGS2 lines (table 3). There were no differences in the number of copulations recorded among the replicate control, fast, and slow lines or between agria and organ pipe treatments (overall mean number of copulations per trial = 91.6, SD = 11.74), so the sample sizes across lines were comparable. Over all lines, organ pipe cactus induced higher premating isolation than did agria (Yule's $V = 0.226$ vs. 0.147, respectively; $P < .05$; table 4), consistent with previous studies (Etges 1992; Brazner and Etges 1993). Further, the selection and control lines differed markedly in levels of premating isolation when substrates were considered: a significant cactus-by-line interaction term in the ANOVA showed that the significant difference in premating isolation induced by these cactus substrates decreased in both the fast and slow lines relative to the controls (data not shown).

These results suggest that levels of premating isolation between populations of *D. mojavensis* responded to selection on development time. This correlated response, like the direct response to selection, was more pronounced in the organ pipe selection lines because behavioral isolation was higher in organ pipe–reared flies. For the agria-

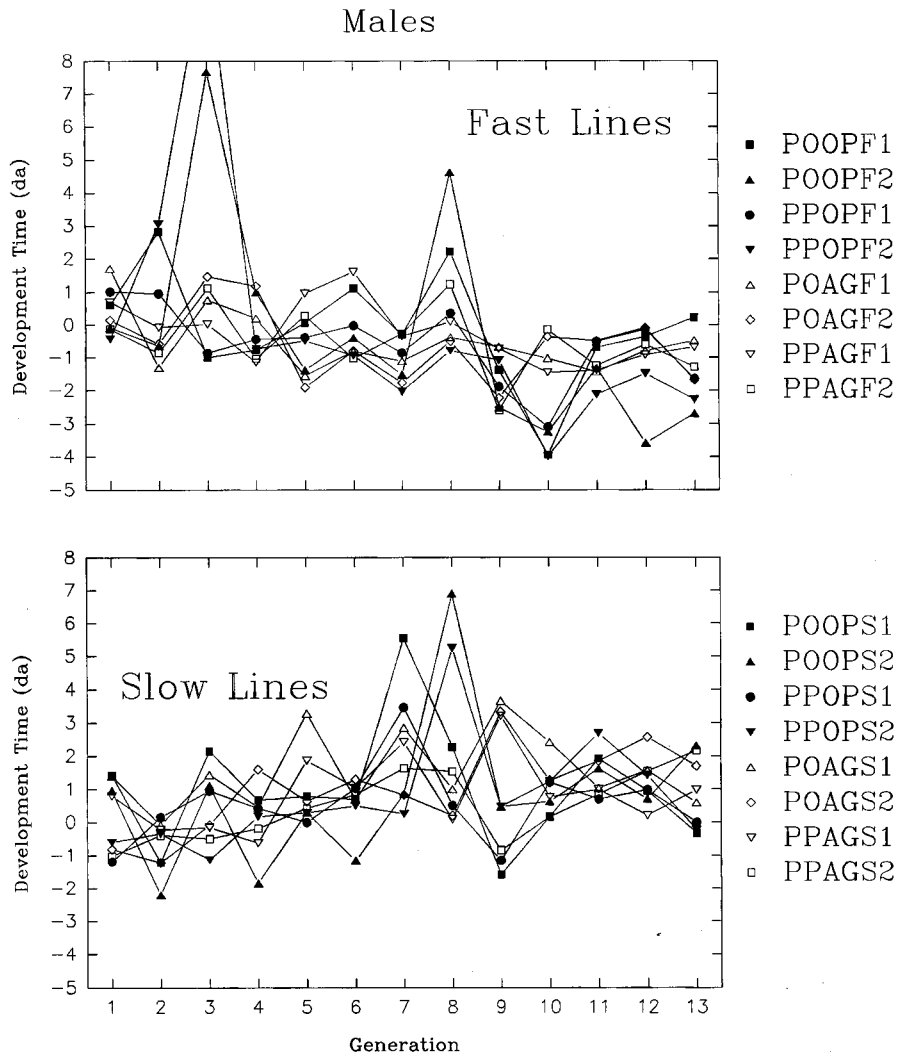

Figure 1: Changes in male and female egg-to-adult development time over the course of 13 generations of artificial selection in the fast (*F*) and slow (*S*) lines after correcting the data for changes in the control lines using the procedures in Muir (1986). Individual line designations refer to mainland (*PO,* Punta Onah, Sonora) and Baja California (*PP,* Punta Prieta) populations reared on organ pipe (*OP*) or agria (*AG*) cactus. Numbers (*1, 2*) refer to replicate lines. For example, POAGS1 refers to the mainland population reared on agria, replicate line 1 artificially selected for slow development time. See text for details.

reared lines, there were responses to selection for fast development time (table 1) consistent with predictions based on higher rates of tissue fermentation (Etges 1989*b*), but there was less apparent change in premating isolation over the course of the experiment (fig. 3). Thus, increases in egg-to-adult development time had a larger effect on premating isolation than did decreases in development time.

Because premating isolation is a composite trait, one or both of the populations involved may have been responsible for changes in components of mating behavior over the course of the experiment. There was no a priori way of predicting which components of mating behavior might be affected by selection on development time. Further, the four behavioral isolation statistics (table 3) are not independent because all are based on the observed numbers of copulations in a given mating trial (as discussed by Marin 1991). One or both indices of female-based assortative mating ("female choice") changed in concert with premating isolation (table 3), so selection on development time decreased levels of female choice in both populations. For the fast lines cultured on organ pipe (OPF2), only mainland female-based assortative mating responded along with Yule's *V*. Overall, mainland

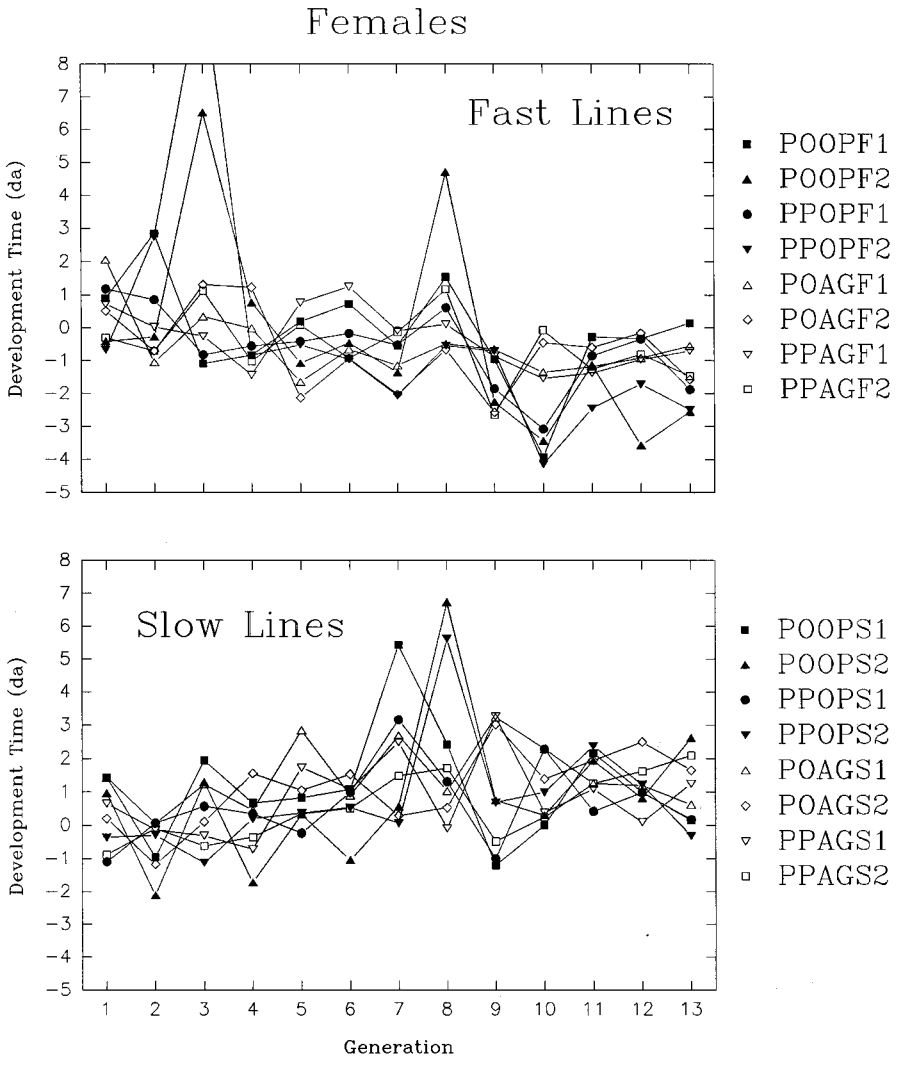

Figure 1 (*Continued*)

female-based assortative mating was significantly greater when cultured on organ pipe cactus than on agria (for organ pipe, $I_1 = 0.370$, and for agria, $I_1 = 0.216$, $P < .05$, Tukey's Multiple Range Test), but there was no main effect of rearing substrates on Baja female-based assortative mating (table 4). Baja females mated more often with Baja males when reared on agria than on organ pipe in the selection lines. However, Baja flies expressed greater female-based assortative mating on organ pipe cactus, leading to a significant cactus-by-selection line interaction in the control lines (table 4). The direction of this interaction was opposite to that for Yule's V where premating isolation was greater for flies reared on organ pipe cactus. Here, I_2 was greater in the fast and slow selection lines reared on agria, demonstrating that mate choice by Baja females was significantly influenced by agria cactus, their natural host (data not shown). Therefore, in those cases where development time changed, artificial selection for long or short development time reduced premating isolation between populations, diminished the differences in premating isolation due to host cacti, and reversed the influence of each cactus on Baja female-based assortative mating.

There were no significant responses in male mating propensity differences due to selection on development time (table 3), but organ pipe tissues significantly increased differences in male mating propensity, $\hat{k}$, over that found for agria-reared flies (table 4; for organ pipe–reared flies, $\hat{k} = 0.78$, and for agria-reared flies, $\hat{k} = 0.90$, Tukey's Studentized Range test, $P < .05$; greater deviations from unity are caused by differences in mating propensity). Differences between Baja and mainland male

Table 1: Response to selection and realized heritabilities (h^2) for egg-to-adult development time after 13 generations in the replicate fast and slow lines corrected for uncontrolled variation in the control lines (Muir 1986)

	Females					*Males*				
Population and host	*Slope*	*SE*	r	t	h^2	*Slope*	*SE*	r	t	h^2
Fast lines:										
Mainland:										
Organ pipe:										
Fast 1[a]	−.141	.122	.472	−.74	.097	−.152	.122	.593	−1.23	.097
Fast 2[a]	−.226	.064	.680	−3.60**	.154	−.253	.067	.696	−3.81**	.156
Agria:										
Fast 1	−.124	.064	.711	−1.91†	.108	−.115	.063	.692	−1.76	.097
Fast 2	−.117	.061	.708	−1.90†	.081	−.115	.058	.719	−1.99†	.080
Baja:										
Organ pipe:										
Fast 1	−.131	.069	.703	−1.88†	.120	−.113	.071	.658	−1.58	.110
Fast 2[a]	−.193	.099	.694	−1.96†	.125	−.195	.099	.692	−1.98†	.126
Agria:										
Fast 1	−.105	.064	.668	−1.68	.116	−.116	.059	.713	−2.00†	.119
Fast 2	−.003	.057	.044	−.01	.076	−.024	.053	.367	−.44	.068
Slow lines:										
Mainland:										
Organ pipe:										
Slow 1	.188	.115	.665	1.66	.012	.183	.124	.637	1.50	.020
Slow 2[a]	.242	.083	.676	2.91*	.106	.198	.087	.631	2.27*	.104
Agria:										
Slow 1	.033	.062	.395	.54	.035	.047	.080	.416	.60	.035
Slow 2	.216	.061	.839	3.70**	.155	.247	.066	.866	3.94**	.159
Baja:										
Organ pipe:										
Slow 1[a]	.038	.087	.363	.46	.056	.110	.087	.598	1.31	.036
Slow 2[a]	.004	.066	.276	.19	.116	.025	.105	.266	.12	.126
Agria:										
Slow 1	.065	.084	.477	.80	.059	.054	.080	.446	.71	.055
Slow 2	.220	.047	.904	4.68***	.151	.201	.056	.857	3.58**	.139

	Mean $h^2 \pm SE$[b]	
	Females	*Males*
Fast lines:		
Mainland	.110 ± .031	.108 ± .033
Baja	.109 ± .022	.106 ± .026
All	.109 ± .025	.107 ± .028
Slow lines:		
Mainland	.077 ± .046	.080 ± .064
Baja	.096 ± .046	.089 ± .051
All	.086 ± .053	.084 ± .054

Note: The two populations of *Drosophila mojavensis* are mainland and Baja, which are cultured on two host cacti (organ pipe and agria); see text for details. *P* values are based on log-transformed data.

[a]Regression is based on data with outliers removed.

[b]Standard errors of the heritabilities are based on the four or eight heritability estimates.

†$.10 < P < .05$.

*$P < .05$.

**$P < .01$.

***$P < .001$.

Table 2: ANOVA results for egg-to-adult development time and viability over all generations of artificial selection

	Egg-to-adult development time				*Viability*			
Source	*df*	*Type IV SS*	F	P	*df*	*Type IV SS*	F	P
Population	1	.959	253.79	.0001	1	.001	.02	NS
Cactus	1	.482	62.46	.0001	1	5.093	109.98	.0001
Sex	1	.036	4.63	.0318	. . .	. . .	. . .	. . .
Selection line	2	.500	32.41	.0001	2	.171	1.84	NS
Line number[a]	3	.011	.47	NS	3	.148	1.06	NS
Population by cactus	1	.058	7.52	.0063	1	.106	2.28	NS
Population by sex	1	.007	.92	NS	. . .	. . .	. . .	. . .
Population by selection line	2	.011	.72	NS	2	.230	2.48	.0838
Cactus by selection line	2	.009	.61	NS	2	.017	.18	NS
Population by cactus by selection line	2	.007	.45	NS	2	.019	.20	NS
Error	606	4.678			1,229	56.913		

Note: "Line number" refers to the two replicate lines for each of the selection and control lines. Development time data were $\log_{10}$ transformed, and viability data were arcsin transformed prior to the analysis. SS = sums of squares.

[a]Replicate lines were nested within selection lines.

mating success were reduced when reared on agria tissues.

Spearman rank correlations between female and male development time with each of the four indices of mating behavior (Yule's V, I_1, I_2 and $\hat{k}$) were also calculated to assess correlated responses in behavior directly in each of the selection lines (results available from me). For the majority of slow lines, development time was negatively correlated with premating isolation, but few of the correlations were statistically significant. In those cases where female-based assortative mating was correlated with changes in development time, it was the mainland female index, I_1, that changed in concert with premating isolation. Thus, when premating isolation decreased in this experiment, it was most often accompanied by decreases in the degree of mainland female choice.

Behavioral Isolation between Lines Selected in Opposite Directions for Development Time

Replicate fast and slow lines of mainland and Baja flies reared on the same host were used to assess premating isolation in generation 13 to explore the nature of the decreases in premating isolation in the selection lines (table 5). Premating isolation was not significantly different from 0 in any of these mating tests except for those in the control lines reared on organ pipe. In both cases, Baja female-based assortative mating, I_2, was significantly greater than 0, the same pattern that was observed in the initial characterization of behavioral isolation in these populations (Etges 1992). Thus, the behavioral isolation expressed by the control lines at generation 13 was unchanged from that initially observed in the original stocks removed four to five generations from nature.

Discussion

Male and female mating behaviors in *Drosophila mojavensis* are influenced by both ecological and genetic causes. Preadult rearing environments alter the intensity of premating isolation, levels of female discrimination, and mating speed (Etges 1992; Brazner and Etges 1993). Because premating isolation declined in the selection lines in which there was the greatest selection response and remained unchanged in all the control lines, changes in gene frequencies of loci that influenced development time caused decreases in premating isolation and levels of female discrimination, an example of behavioral pleiotropy (fig. 3, table 3). Behavioral isolation among Baja and mainland populations of *D. mojavensis* is therefore, in part, a correlated response to changes in development time, a life-history character that has evolved since *D. mojavensis* colonized mainland Mexico from Baja California by switching host plants (Heed 1982; Etges and Heed 1987; Etges 1990). Life-history trajectories may be dynamic because of the existence of additive genetic variance in development time in these populations (table 1). Additive genetic variability accounted for 10%–15% of the total phenotypic variation in development time based on a two environment full-sib/half-sib breeding design (Etges 1993), so the realized heritabilities based on responses to selection here (table 1) are concordant with these previous estimates. This implies that adult mating behaviors may continue to evolve in concert with devel-

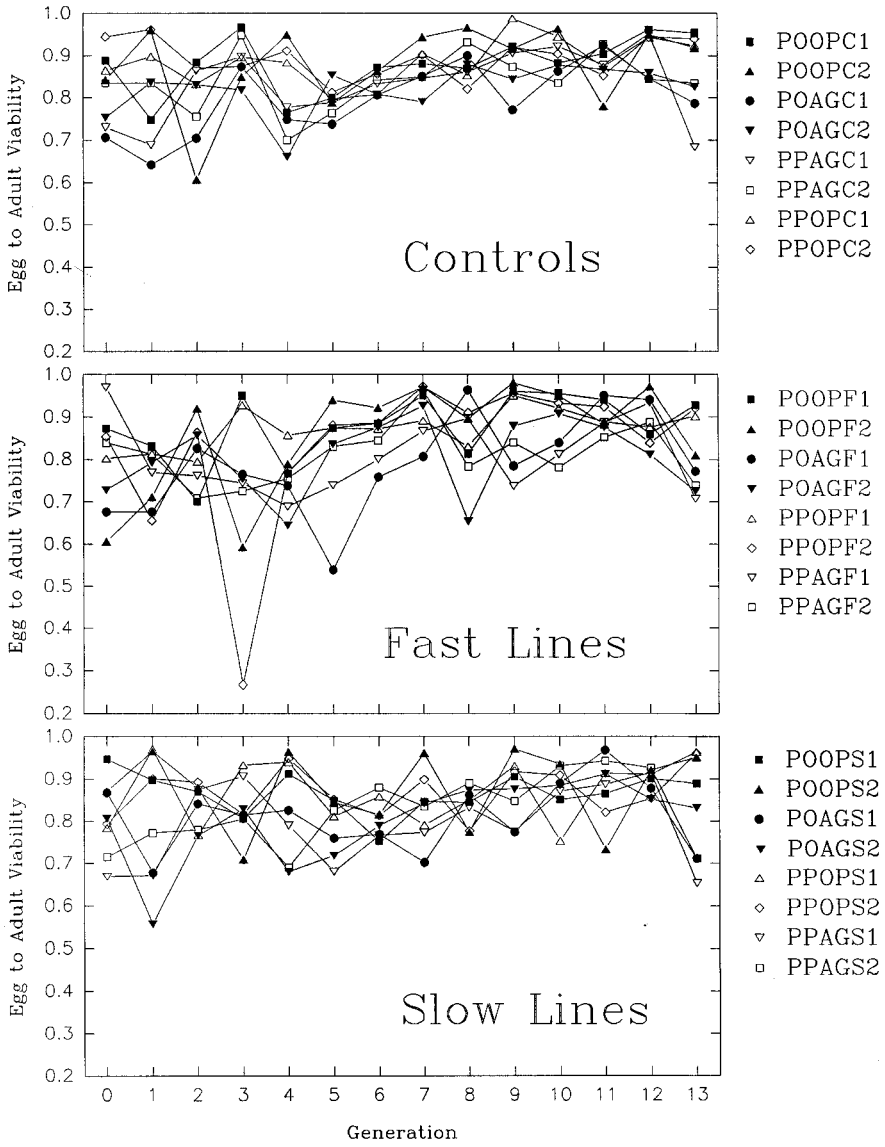

Figure 2: Changes in egg-to-adult viability over the course of 13 generations of artificial selection in the control (*C*), fast (*F*), and slow (*S*) lines. Generation 0 refers to the base populations described elsewhere (Etges 1992). Individual line designations are described in figure 1.

opment time, particularly in mainland organ pipe cactus–using populations.

Premating isolation within and between demes of *D. mojavensis* is therefore not only phenotypically plastic (Etges 1992) but can also evolve quite rapidly. Host-rearing effects extend to adult epicuticular hydrocarbons (Stennett and Etges 1997), putative contact pheromones in this species (Markow and Toolson 1990; Toolson et al. 1990), and the degree of behavioral isolation between *D. mojavensis* and *Drosophila arizonae* (W. J. Etges, unpublished data). Koepfer (1987*a*) performed artificial selection for increased sexual isolation between a mainland population derived from a single pair-mating and a Baja stock derived by intercrossing six geographically isolated populations from Baja California. Response to selection was apparent after three generations caused by increased isolation between mainland females and Baja males, although multiple choice tests were not used and isolation between Koepfer's control lines significantly increased. Thus, sufficient genetic variability exists in mate signaling systems in *D. mojavensis* to allow rapid evolution, consistent with many such laboratory studies of selection on mating systems (Koopman 1950; Ehrman 1965; Kessler 1966, 1969; Dobzhansky et al. 1976) and correlated re-

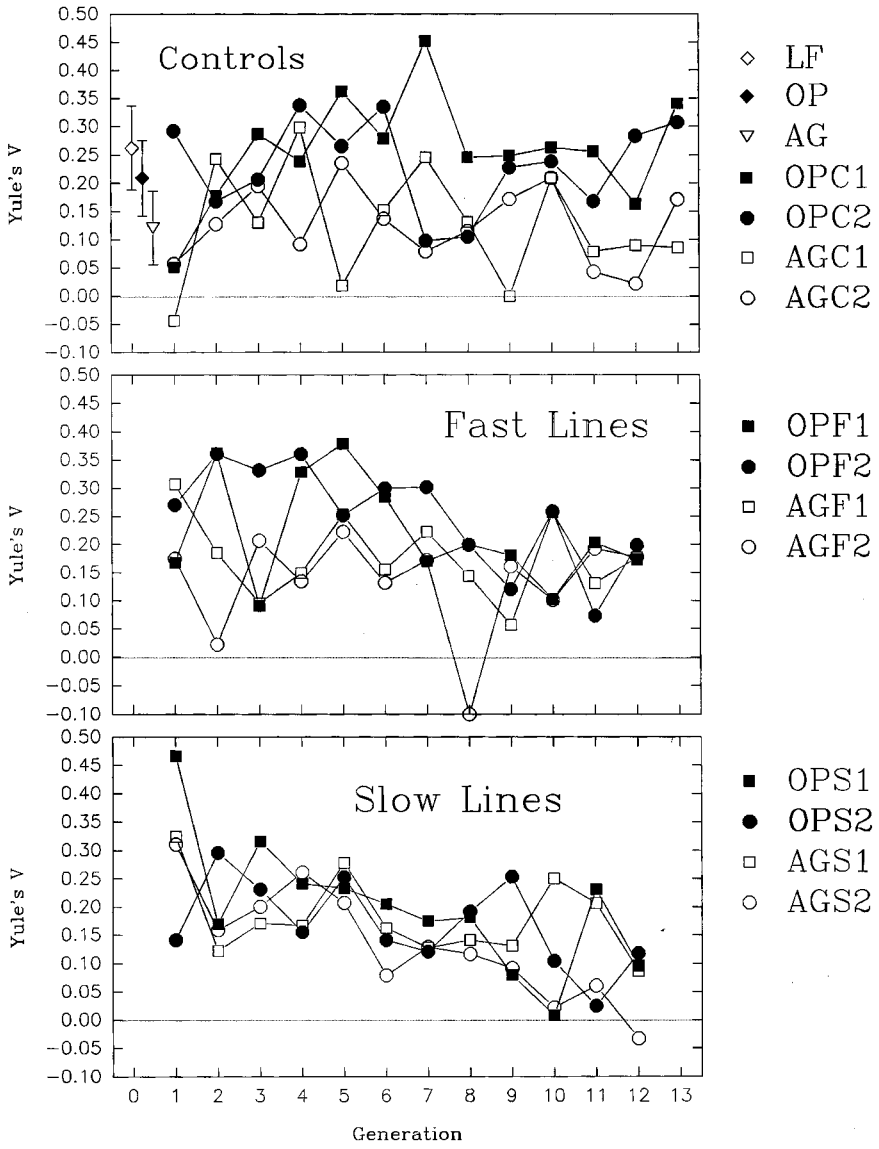

Figure 3: Changes in premating isolation, using estimates of Yule's *V*, over the course of this selection experiment. Line designations are as described in figure 1, except for the three estimates of Yule's *V* (±1 SE) indicated by *LF* (lab food), *OP* (organ pipe), and *AG* (agria) in the upper panel. These data refer to the estimates of premating isolation for these two populations described elsewhere (Etges 1992).

sponses to selection on adult longevity (Pletcher et al. 1997). The lack of any significant premating isolation between each of the fast and slow replicate lines (table 5) suggests that the decreases in premating isolation were due to genes with similar effects in the fast and slow lines. Koepfer's results provided significant insight into understanding the evolution of behavioral isolation in *Drosophila.* The present study extends these results to cactus-reared *D. mojavensis,* showing how adult mating behaviors are genetically tied to ecologically relevant components of fitness.

Responses in behavioral isolation due to artificial selection on development time is strong evidence that incipient speciation in *D. mojavensis* is occurring because of adaptive divergence and geographic isolation, a classic example of the geographic model of speciation (Mayr 1963). All available evidence suggests that populations of *D. mojavensis* in Baja California are ancestral to those in mainland Sonora and Sinaloa in Mexico and to those in Arizona (Heed 1982; Heed and Mangan 1986). Phylogenetic analysis of inversion polymorphisms in *D. mojavensis* clearly showed that southern Baja populations,

Table 3: Linear regression results for correlated responses in behavioral isolation over 12 generations of artificial selection (13 generations for the control lines) on egg-to-adult development time in a mainland and Baja population of *Drosophila mojavensis* cultured on two host cacti (OP or AG)

	Mating statistic											
	Yule's V			I_1			I_2			$\hat{k}$		
Line	*Slope*	*SE*	t	*Slope*	*SE*	t	*Slope*	*SE*	t	*Slope*	*SE*	t
OPC1	.008	.008	1.00	.013	.017	.78	.002	.014	.15	−.018	.025	.03
OPC2	−.001	.006	−.08	−.005	.009	−.57	.005	.009	.54	.018	.013	1.37
AGC1	−.003	.008	−.37	.013	.017	.78	.002	.014	.15	−.018	.025	−.73
AGC2	−.001	.005	−.27	.001	.014	.08	−.004	.009	−.43	.004	.016	−.26
OPF1	−.010	.008	−1.20	−.018	.012	−1.54	−.001	.015	−.04	.013	.018	.71
OPF2	−.019	.006	−3.40**	−.028	.010	−2.94*	−.009	.011	−.79	.009	.011	.79
AGF1	−.005	.006	−.88	−.008	.016	−.50	−.002	.010	−.22	.012	.021	.58
AGF2	.001	.008	.01	.016	.015	1.07	−.015	.011	−1.34	−.029	.020	−1.47
OPS1	−.024	.008	−3.22**	−.024	.013	−1.87†	−.222	.011	−2.06†	.002	.014	.15
OPS2	−.012	.006	−2.00†	.001	.015	.01	−.024	.012	−1.98†	−.023	.023	.98
AGS1	−.007	.006	−1.15	−.003	.015	−.19	−.010	.014	−.72	−.011	.023	−.48
AGS2	−.248	.004	−6.01***	−.021	.008	−2.34*	−.028	.009	−3.27**	−.005	.015	−.36

Note: Replicate lines (1 or 2) are grouped into control, fast, and slow (C, F, and S, respectively). Yule's V is an estimate of premating isolation, I_1 is an index of mainland female-based assortative mating, I_2 is an index of Baja female-based assortative mating, and $\hat{k}$ is an estimate of difference in mating propensity between mainland and Baja males. See the text for details. P values are based on arcsin-transformed data.

†$.10 < P < .05$.

*$P < .05$.

**$P < .01$.

***$P < .001$.

north of the Cape region, are ancestral to all others (Etges et al. 1998). Agria cactus is widespread in Baja California and on the islands in the Gulf of California, is present in a small coastal area in Sonora, and is thought to be the preferred host by *D. mojavensis* in areas where both agria and organ pipe are sympatric and where agria is absent (Fellows and Heed 1972; Downing 1985; Newby 1996). Thus, when *D. mojavensis* colonized the mainland, it shifted to organ pipe cactus, a secondary host, causing life-history evolution (Etges 1990). A secondary consequence to the process of adapting to organ pipe were shifts in patterns of mate choice in mainland populations.

The evolution of premating isolation among populations of *D. mojavensis* is therefore another example of the origin of behavioral isolation due to divergent selection in allopatry, a frequently observed result in laboratory studies designed to initiate reproductive isolation (Rice

Table 4: ANOVA results for variation in premating isolation, female-based assortative mating, and male mating propensity over the 13 generations of artificial selection on egg-to-adult development time in two populations of *Drosophila mojavensis*

		Dependent variable											
		Yule's V			I_1			I_2			$\hat{k}$		
Source	*df*	*Type IV SS*	F	P	*Type IV SS*	F	P	*Type IV SS*	F	P	*Type IV SS*	F	P
Cactus	1	.224	26.58	.0001	.884	30.86	.0001	.002	.07	NS	.603	12.01	.0007
Selection line	2	.016	.95	NS	.061	1.06	NS	.004	.08	NS	.016	.16	NS
Replicate line[a]	3	.022	.87	NS	.100	1.16	NS	.049	.77	NS	.182	1.21	NS
Cactus by selection line	2	.055	3.28	.0405	.040	.70	NS	.176	4.14	.0180	.158	1.57	NS
Error	139	1.170			3.981			2.965			6.980		

Note: All data were arcsin transformed prior to analysis. SS = sums of squares.

[a]Replicates nested within selection lines.

Table 5: Results of mating tests with generation 13 flies

	Number of observed copulations[a]							
Host and line cross	*A*	*B*	*C*	*D*	*Yule's* V ± *SE*	I_1 ± *SE*	I_2 ± *SE*	$\hat{k}$ ± *SE*
Organ pipe:								
Control 1 × control 1	30	35	18	14	.341***	.250	.429*	1.205
					±.096	±.140	±.129	±.246
Control 2 × control 2	25	41	14	20	.307**	.282	.344*	1.222
					±.093	±.154	±.120	±.246
Fast 1 × slow 1	26	30	18	22	.167	.182	.154	1.000
					±.100	±.148	±.137	±.204
Fast 2 × slow 2	25	28	22	14	.200†	.064	.333*	1.282
					±.104	±.146	±.145	±.274
Slow 1 × fast 1	28	23	11	34	.125	.436**	−.193	.548**
					±.101	±.144	±.130	±.117
Slow 2 × fast 2	24	29	22	28	.030	.043	.018	.981
					±.099	±.147	±.132	±.193
Agria:								
Control 1 × control 1	26	30	24	23	.086	.040	.132	1.102
					±.010	±.141	±.136	±.217
Control 2 × control 2	30	35	21	25	.171	.176	.167	1.018
					±.094	±.138	±.127	±.193
Fast 1 × slow 1	29	27	21	24	.110	.160	.059	.906
					±.099	±.140	±.140	±.180
Fast 2 × slow 2	37	23	18	28	.127	.345**	−.098	.631**
					±.096	±.127	±.139	±.126
Slow 1 × fast 1	30	20	23	33	−.058	.132	−.245	.683**
					±.097	±.136	±.133	±.135
Slow 2 × fast 2	31	29	23	24	.121	.148	.094	.945
					±.096	±.135	±.137	±.183

Note: Line crosses refer to behavioral isolation tests made with the experimental lines after 13 generations of artificial selection. Each line cross involved a mainland and Baja California population of *Drosophila mojavensis* cultured on two host cacti, organ pipe and agria. Replicate lines (1, 2) from each of the fast, slow, and control lines are described in table 1.

[a]A is the number of observed copulations between mainland females and males; B is the number of observed copulations between Baja females and males; C is the number of observed copulations between mainland females and Baja males; and D is the number of observed copulations between Baja females and mainland males.

†$.10 < P < .05$.

*$P < .05$.

**$P < .01$.

***$P < .001$.

and Hostert 1993). Previous hypotheses concerning reproductive character displacement between *D. arizonae* and mainland *D. mojavensis* as the primary mechanism for behavior isolation between Baja California and mainland populations of *D. mojavensis* (Zouros and D'Entremont 1980; Markow et al. 1983; Koepfer 1987*a*, 1987*b*) may not necessarily be rejected but are certainly weakened as primary causes. Reinforcement of premating isolation in sympatry has been suggested to be the cause of reproductive character displacement in this case (Wasserman and Koepfer 1977; Zouros and D'Entremont 1980), despite the fact that the hybrid fitnesses are high but variable (Ruiz et al. 1990) and that hybrids from nature have never been observed (W. B. Heed, personal communication). Butlin (1989) made clear that reinforcement should be considered only in cases where increased isolation is due to selection against hybrids, a point demonstrated experimentally by Hostert (1997). Butlin suggested that differentiation in mate recognition systems due to interactions between species should be called *reproductive character displacement.* Current sympatry is low given the few cases of host plant sharing (Markow et al. 1983; Ruiz and Heed 1988; W. J. Etges, unpublished data) versus the range sizes of both species

(Heed 1982), suggesting that the degree of interaction between species facilitating potential reproductive character displacement in nature is low. Historical patterns of species interactions may have been higher, but this is unknowable. Advances in our understanding of the diversification of mating systems leading to incipient speciation, that aspect of species divergence about which we know so little, will be facilitated by in-depth analysis of ecological interactions between populations and adaptation to local environmental conditions. More data are also needed concerning sexual selection within demes so that the effects of sexual selection can be evaluated in relation to sexual isolation between populations.

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