Notes and Comments

The Effects of Selection and Bottlenecks on Male Mating Success in Peripheral Isolates

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Mayr (1954, 1963) suggested that new biological barriers to gene flow often require genetic revolutions to break up the well-integrated genetic systems that he felt characterized species (see Barton and Charlesworth 1984 for a discussion). Influenced by Wright (1940), Mayr suggested a prominent role for small founder populations located at the edge of a species' range (peripheral isolates) where isolation and subsequent drift could lead to incipient speciation. Mayr's intuitive peripheral isolate model proved influential (Bush 1975; Lynch 1989) and was expanded and refined (Carson 1968, 1997; Kaneshiro 1980; Templeton 1980, 1996; Carson and Templeton 1984).

Peripheral isolates formed from single founder events may be expected to show decreased levels of additive genetic variance, altered allele frequencies, and increased homozygosity relative to the preisolation population (Falconer and Mackay 1996). Though these effects are transient in populations that subsequently expand (Barton 1989), they can have varied evolutionary consequences (Barton and Charlesworth 1984; Charlesworth and Charlesworth 1987; Barton 1989; Templeton 1996; Wade et al. 1996). For example, increased homozygosity is often associated with a marked decrease in fitness (Falconer and Mackay 1996; Keller 1998), while changes in the genetic background can alter the amount and direction of response to selection (Goodnight 1988; Meffert 1995; Wade et al. 1996). Importantly, peripheral isolates may often arise in novel environments (such as might be expected at the edge of a range), where they will experience novel selection pressures. Selection is considered an important force promoting speciation (see reviews by Rice and Hostert 1993; Schluter 1996).

Some versions of Mayr's peripheral isolate theory have been successfully tested in the laboratory, particularly Carson's (1968) founder-flush-crash model (Powell 1978; Dodd and Powell 1985; Ringo et al. 1985; Meffert and Bryant 1991; but see Rice and Hostert 1993; Moya et al. 1995; Templeton 1996; Rundle et al. 1998). Surprisingly, we do not know of a test of peripheral isolate speciation that includes both single founder events and selection. Using *Drosophila melanogaster*, we investigate how single founder events and a novel environment affect the likelihood of nonrandom mating between recent peripheral isolates and ancestral populations. We focus on two different components of mating that can alter gene flow: male mating success and assortative mating, both based on a female choice design.

Material and Methods

Stocks and Derivation of Lines

We used *Drosophila melanogaster* taken from a large, randomly mating stock of flies originally collected in Dahomey in 1970. This stock was kept at a large population size in population cages in Edinburgh and London at 25°C until 1995, when a large sample of these flies were brought to Vancouver to begin a stock population maintained in half-pint bottles, at population sizes in the thousands. The Dahomey stock has substantial genetic variation for both quantitative and life-history characters (Wilkinson et al.

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1990; Whitlock and Fowler 1996; D. Bourguet, personal communication) and displays substantial inbreeding depression after full-sib mating (K. Fowler and M. C. Whitlock, unpublished observations).

From this ancestral stock, 68 new lines were founded in February 1997, 17 assigned to each of four experimental treatments arranged in a simple two-factor design (shown in fig. 1). Lines were established either by a single pair or by 200 males and 200 females (the "bottlenecked" and "unbottlenecked" treatment lines, respectively). The resulting bottlenecked and unbottlenecked lines were then raised on eight vials of either a "selected" or "nonselected" environment. The nonselected environment consists of vials with 15 mL normal cornmeal media. The selected environment is a low-pH treatment, with 0.1 mL of 2.7 M sulfuric acid added to the surface of normal food 2–3 d before use. The resulting food in the selected lines had a surface pH of approximately 2.5.

Subsequent generations of flies were transferred to new food vials every 17 or 18 d. Each selected line was paired with a nonselected line of the same bottlenecking type. The lines were flushed to, and then maintained at, a population size of 400 individuals and divided equally among eight vials, which were mixed at each transfer. In generations where the total output of a line was less than 400, its paired line was also maintained at this smaller size, to control for the added genetic drift. The mean census population size for unbottlenecked lines was 250 flies ($N = 20$ generations, SD = 54). The mean inbreeding coefficient after 20 generations was 0.32 ($N = 17$, SD = 0.035) for the bottlenecked treatment and 0.05 ($N = 17$, SD = 0.005) for the unbottlenecked treatment.

For the last six generations, two vials of normal food were added as backups for each selected line. After a normal set of transfers, flies from two vials were serially transferred to these vials to lay eggs. These backup vials were used in the subsequent generation to top up the line to 200 flies if fewer then 200 emerged from the eight selection lines. Only one pair of lines in one generation required this backup.

Response to Selection

After 24 generations, lines were tested for their response to the selection treatment. In order to control for maternal effects, all lines (selected and nonselected) were serially transferred to low-pH medium in generation 22. In generation 23, eight replicate (low-pH medium) vials per line were established, each replicate consisting of five mated females. Females were allowed to lay eggs for 24.5 h, with selected and nonselected lines paired during transfers to control for time-of-day effects. Fourteen days later, total production per vial was measured.

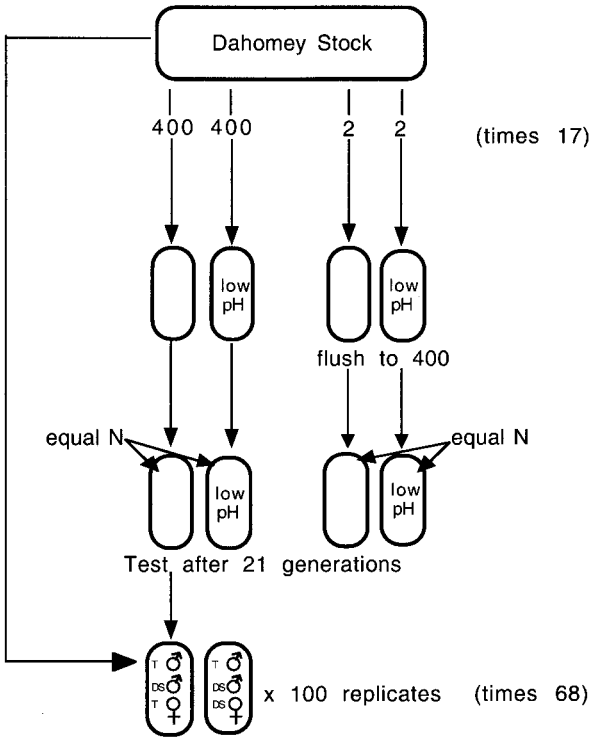

Figure 1: Experimental design. Four treatments are shown: unbottlenecked (200 males and 200 females) or bottlenecked (1 pair) starting populations and selected (low-pH) or not selected (normal-pH) growth media. Seventeen replicates were set up for each treatment. Subsequent generations were maintained at a maximum of 400 flies. Throughout the experiment, population sizes of each selected and corresponding nonselected line were matched. After 21 or 22 generations, single males from each of the treatment (*T*) lines were placed with single males from the Dahomey stock (*DS*) in competition for mating with a single female: 100 replicates using Dahomey stock females and 100 replicates using experimental treatment females (a balanced female choice design).

Mating Trials

After 21 or 22 generations of the experiment (February and March 1998), flies from the 68 lines were tested for premating isolation from the Dahomey stock population using a simple female choice design in which a single female is placed in a vial with two males (Partridge et al. 1985; Meffert and Bryant 1991; Rundle et al. 1998). All females and the majority of males were collected as virgins using CO_2 anesthesia at eclosion; subsequent handling to put flies into mating vials was done using cold anesthesia, 2–6 d posteclosion.

For each line, 200 independent female choice trials were performed in vials. One group of 100 vials each contained a virgin female from the experimental line and a male from her line and a male from the stock line. The other

100 vials each contained a virgin stock female, a stock male, and a male from the experimental line. Males were marked by feeding them overnight in vials with abundant yeast saturated in red or blue food coloring (Food-Club, Scott-Bathgate). All males were marked, and treatment and stock males were reciprocally marked within each line to balance any possible effects of color. No such color effects were seen; of the 9,184 matings observed, 49.2% involved blue males, no different from random expectation (*G*-test with $\hat{p} = 0.5$: $P = .12$).

Trials were conducted throughout the day. Flies were observed for up to 2 h and were only scored as having mated if they stayed *in copula* during the disturbance of being moved to the microscope for identification. Over 67% of trials resulted in matings within 2 h, and 50.7% of the matings were by treatment females (*G*-test with $\hat{p} = 0.5$: $P = .15$). Nonrandom mating between experimental lines and the stock population may result from male-male interactions, male propensity to mate, true female choice, or their interaction.

Analysis

Response to selection was tested with ANOVA, using the line means (mean of eight vials) as our replicate measure. We also performed a paired *t*-test on the temporally matched selected and nonselected lines.

We tested for the effects of bottlenecking and selection on male mating success and assortative mating using a general linear modeling approach. Male mating success for an experimental line was measured as the proportion of the total matings that are by experimental males. As there was no difference in female propensity to mate between experimental and stock populations, this measure was pooled across females. The measure was arcsin square-root transformed before statistical analysis.

We evaluated assortative mating using the index Y (Bishop et al. 1975) based on the cross-product ratio (α) of a 2 × 2 contingency table containing the number of matings of each male and female combination. The index is defined as

$$Y = \frac{\sqrt{\alpha} - 1}{\sqrt{\alpha} + 1},$$

where α is the number of stock by stock matings times the number of treatment by treatment matings divided by the number of stock by treatment matings times the number of treatment by stock matings. The index varies from -1 for perfect negative assortative mating to $+1$ for perfect assortative mating, with 0 indicating nonassortative mating. The index Y is a margin-free index (Bishop et al. 1975) and thus is not biased by varying propensities to mate (cf. Casares et al. 1998). Individual values of Y for a line may be tested for significance using the statistic $X^2(Y)$ (Spieth and Ringo 1983), which is χ^2 distributed with 1 df (Fienberg 1977). We were primarily interested in average effects of our treatments and treated Y as a simple measure of premating isolation. The Y measures were not transformed.

Results

Response to Selection

It took many generations to flush the bottlenecked lines to 400 individuals, and the majority of unbottlenecked lines produced fewer than 400 adults early on in the experiment. The selected and nonselected lines were paired, and these patterns were due, in the great majority of cases, to the lower production in the selected treatments. After 24 generations, however, on low-pH medium, nonselected lines produced an average of 21.6 (bottlenecked; SD = 9.5) or 24.1 (unbottlenecked; SD = 6.1) flies per five females, and selected lines 22.4 (bottlenecked; SD = 7.3) or 26.2 (unbottlenecked; SD = 6.7) flies per five females. Though in the predicted direction, this is not evidence for adaptation to low-pH medium (ANOVA: $F = 0.76$, df = 1, 66, $P = .39$; paired *t*-test: $t = 1.04$, P [one-tailed] = .15).

Premating Isolation

Assortative mating scores (Y) were more or less normally distributed, with a mean isolation of -0.011 (fig. 2*A*). Raw mating numbers may be obtained from the authors. Six of the 68 lines showed significant nonrandom mating at $\alpha = 0.05$ (uncorrected for multiple comparisons). Four exhibited an excess of heterotypic mating (two unbottlenecked, unselected lines, $P = .003$, $P = .04$; one unbottlenecked, selected line, $P = .04$; and one bottlenecked, unselected line, $P = .007$), while two lines showed an excess of homotypic mating, consistent with premating isolation (both selected, one bottlenecked, $P = .04$, and one unbottlenecked, $P = .05$). Six out of 68 is no more than what might be expected due to Type I error (*G*-test with William's correction [Sokal and Rohlf 1981], with $\hat{p} = 0.5$: $P = .19$).

Neither selection (partial $F = 0.035$, df = 1, 64, $P = .85$) nor bottlenecking (partial $F = 0.0026$, df = 1, 64, $P = .96$) had any measurable effect on mean Y. The interaction term was equally nonsignificant (partial $F = 0.027$, df = 1, 64, $P = .87$). We paired the selected and nonselected lines to control for changes in N_e so that we can perform a more powerful paired *t*-test to look for a

mean effect of selection on *Y*. There is no such effect (paired *t*-test: $t = 0.05$; $P = .96$).

Male Mating Success

Treatment males were much less successful at securing matings than were males from the stock population, both overall (40.7% of the matings were by treatment males: *G*-test with $\hat{p} = 0.5$, $P < .0001$) and when examined separately (mean proportion mating: unbottlenecked, not selected = 0.45; unbottlenecked, selected = 0.44; bottlenecked, not selected = 0.39; bottlenecked, selected = 0.34; all with $P < .01$ on the basis of a *G*-test with $\hat{p} = 0.5$). Mating success was, however, differently affected by the treatments (fig. 2*B*; table 1): bottlenecking had a highly significant effect ($P = .0002$), while selection had none, neither alone ($P = .18$) nor in interaction with bottlenecking ($P = .21$). Because population sizes varied among lines throughout the experiment, long-term effective population size (N_e) can be substituted for initial treatment as a continuous measure of inbreeding. If we substitute the continuous variable N_e, estimated as the harmonic mean of the population size throughout the experiment (Falconer and Mackay 1996) for the bottlenecked and unbottlenecked treatments, the effects of bottlenecking remain significant ($F = 14.72$, df = 1, 64, $P = .0003$). In this fully described model, selection does show a marginally significant effect (partial $F = 3.76$, df = 1, 64, $P = .06$), with selection associated with a drop in mean male mating success, but the interaction term does not (partial $F = 1.95$, df = 1, 64, $P = .17$). Selection ceases to explain significant variation in mating success when this interaction term is dropped (partial $F = 1.83$, df = 1,65, $P = .18$).

Table 1: ANOVA of the effects of bottlenecking and selection on proportion of treatment males mating (arcsine square-root transformed)

Source	df	Corrected SS	*F*	*P*
Bottlenecking	1	.08457	15.08	.0002
Selection	1	.01031	1.84	.180
Error	65	.36447[a]	…	…

[a] Interaction variance included in error term (interaction SS = 0.00881, $F = 1.585$, $P = .21$). SS = sum of squares.

Discussion

Genetic Drift and Premating Isolation

Bottlenecks, with or without selection, did not lead to significant premating isolation in our experiment. There

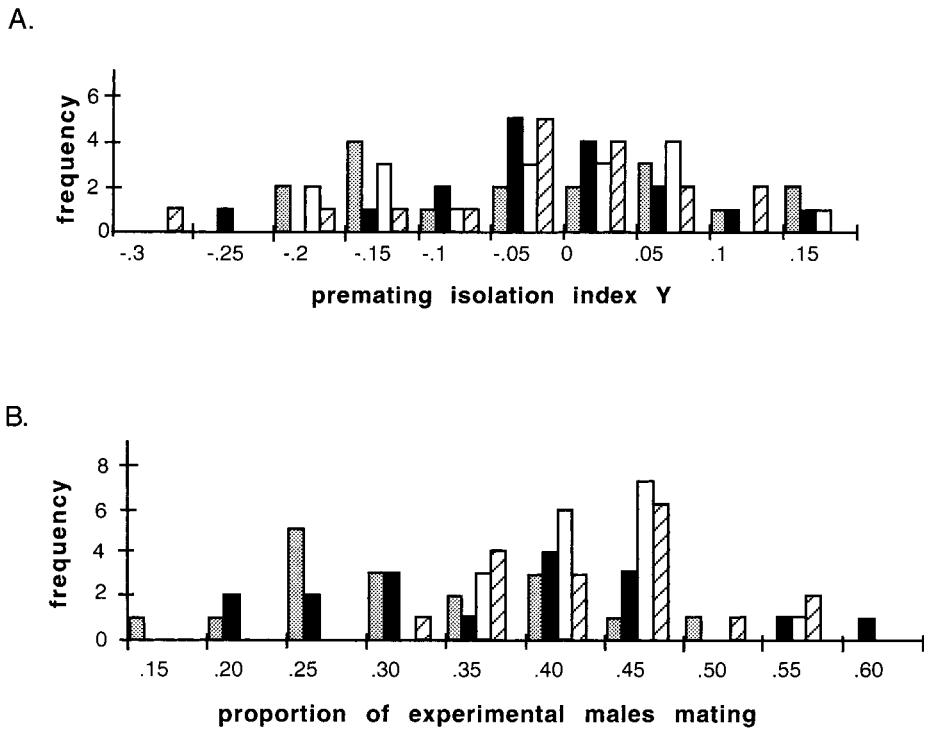

Figure 2: *A*, Distribution of *Y*, the measure of nonrandom mating, across lines. *B*, Distribution of the proportion of treatment males mating, across lines. Legend for treatments: *gray* = bottlenecked, selected; *black* = bottlenecked, not selected; *white* = unbottlenecked, selected; *diagonal lines* = unbottlenecked, not selected.

are three possible explanations. First, the experiment may not have been run long enough for drift-induced changes to produce significant premating isolation. However, the flush cycle in our experiment (21–22 generations) is comparable with other investigations that have found some evidence of premating isolation (e.g., the first set of experiments of Powell 1978 and Galiana et al. 1993). Experiments such as that by Moya et al. (1995), which ran much longer, actually used repeated flush cycles of comparable duration.

A second possibility is that *Drosophila melanogaster* may be in some way resistant to bottleneck-induced speciation, and Templeton (1980, 1996) cites reasons why this might be so. While success of experiments using species with similar genetic characteristics mitigate Templeton's specific objections (Rundle et al. 1998), *D. melanogaster* is a cosmopolitan species that shows little evidence of behavioral isolation (Templeton 1996; for an exception, see Wu et al. 1995). In ecological terms, this species may be "weedy." Weedy species may be adapted to withstand the genetic perturbations caused by founder events, though such a notion has yet to be formally tested (Rundle et al. 1998).

We favor the third explanation, that single founder events do not commonly lead to premating isolation (Rice and Hostert 1993; Moya et al. 1995; Rundle et al. 1998). Other experiments testing for premating isolation included aspects that, though favorable to the hypothesis, may be unrealistic in nature. Previous studies have made use of hybrid parental populations or populations new to the lab environment (Powell 1978; Dodd and Powell 1985; Ringo et al. 1985; Meffert and Bryant 1991; Galiana et al. 1993). Further, the great majority of tests were performed after repeated bottlenecks and among different isolates, rather than between isolates and their parental populations. Indeed, the 34 bottlenecked lines tested here represent a large proportion of the total number of well-replicated, single-bottleneck peripheral isolates ever tested. Being ignorant of the frequency with which isolates form in the wild, we have no yardstick with which to evaluate the importance of bottleneck-mediated speciation (Templeton 1996), but experimental evidence suggests that it is not common.

Novel Environments and Premating Isolation

Selection also did not lead to premating isolation in our experiments, and there was no interaction between selection and bottlenecking on premating isolation (cf. Meffert 1995 and references therein). One possible reason that we did not see a change in premating isolation due to selection was that there was very little response to that selection in terms of pH tolerance itself. Though the trend is in the predicted direction, it is surprising that we did not find a stronger adaptive response to low pH. Without evidence for adaptation to the novel environment, there is little evidence for genetic change as a result of selection, and the correlated changes expected in reproductive behavior, therefore, may not have occurred.

As in any experiment of this sort, it is possible that reproductive isolation may have evolved if the experiment had run longer. The duration of selection in our experiment is comparable in length to other experiments that have found evidence for the evolution of reproductive isolation. Hurd and Eisenberg (1975) found premating isolation after only 16 generations of selection on geotaxis in the housefly, while Grant and Mettler (1969) were able to document premating isolation in *D. melanogaster* after only 14–20 generations of selection on a complex escape reaction. De Oliviera and Cordeiro (1980) were able to induce assortative mating by selection in low pH in *Drosophila willistoni* after 26 generations of selection. Where de Oliviera and Cordeiro (1980) produced a growth medium of uniformly low pH, we added acid on top of the food; this may have produced a more complex environment with refuge areas of normal pH. Nevertheless, in this experiment, isolation in a novel environment did not lead to reproductive isolation. This is an unexpected result and augments the relatively few published examples where isolation in a novel environment did not lead to increased premating isolation (cf. Rice and Hostert 1993). Because the relative importance of a process in nature is often measured by ease of its production in the lab, this negative result is noteworthy.

Male Mating Success

All the experimental treatments showed decreased mating success, and the most likely explanation is inbreeding. Unbottlenecked lines were slightly inbred (mean $F = 0.05$) and showed a small (5%–6%) decrease in male mating success, while the bottlenecked lines had a mean inbreeding coefficient of 0.32 and did almost three times as poorly (11%–16% decrease).

The amount of inbreeding depression for male mating success that we measured is comparable with that found both in short term sib-mating experiments (Sharp 1984) and single chromosome substitution studies (cited in Hughes 1995; K. Hughes, personal communication). This is remarkable because, although these previous results found significant declines immediately following inbreeding, we found equally significant declines in this major fitness component after 20 generations at relatively large population sizes. The effects of a small population size during colonization can have long-term effects on the dynamics of the resulting population.

The Fate of Peripheral Isolates

Our results have important implications for the fate of peripheral isolates in nature. In the simplest case, recent peripheral isolates formed from bottlenecks may be even less likely than previously suggested to form incipient species. If founder-flush events produce males that compete poorly with ancestral males for mates, then, in the face of renewed contact with the ancestral species, even low levels of migration of individuals from the parental population may be accompanied by high levels of gene flow. The high relative mating success of incoming ancestral males would tend to decrease the power of drift, lessening the possibility of genetic revolutions and bottleneck-mediated speciation (Carson and Templeton 1984). Such a process might be further exacerbated if females prefer migrant males generally (see, e.g., Wallace 1970). Incipient drift-mediated phenotypic changes that might have led to speciation could be lost. Increased gene flow will also affect the process of natural selection. If genes from the center are maladapted to the periphery, increased gene flow might diminish the rate of adaptation of peripheral isolates to their environment (cf. Burt 1995). If, however, the rate of adaptation of the isolates is dependent on new mutations, then moderate gene flow might increase that rate (Grant and Grant 1996, 1997; Holt 1996).

To investigate the plausibility of these scenarios, components governing male mating success must be evaluated in more natural conditions. Gromko and Markow (1993) conducted one of few field studies on *D. melanogaster* mating behavior. Their results suggest that male-male interactions are not as important as female behavior in determining male mating success. Over 85% of the females in the field carried sperm from previous matings, and females that mated carried much less sperm than females that did not. Because of the refractory period exhibited by mated females, the operational sex ratio in nature is heavily male biased. According to Gromko and Markow (1993), the most important reproductive task facing a male in nature is simply to find the rare receptive females. If male mating success includes search behavior that is affected by inbreeding, then we may have underestimated the effects of inbreeding in our experiment; inbred males did poorly even when offered a receptive female in close quarters.

Since the modern synthesis, genetic drift has been considered a powerful force in evolution (Wright 1978). It may indeed govern the fate of many neutral or nearly neutral point mutations (Kimura 1983; Coyne et al. 1997). Its putative role in spurring adaptive change, however, has recently been called into question (Coyne et al. 1997). Our results are consistent with the idea that bottlenecking and subsequent drift may do more to hinder than to help the speciation process. Measuring the importance of selection versus drift in real-life examples of incipient speciation remains paramount.

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