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SINGLE FOUNDER-FLUSH EVENTS AND THE EVOLUTION OF REPRODUCTIVE ISOLATION

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Abstract.—By demonstrating the evolution of significant premating isolation, previous laboratory experiments have provided some evidence for the founder-flush model of speciation. However, these experiments are subject to a number of criticisms, including the use of hybrid populations recently collected from the wild and the use of multiple bottlenecks. Here we present the results of a test of founder-flush speciation using a single, well-adapted laboratory stock of *Drosophila melanogaster* subjected to one founder-flush event. The experiment was replicated at larger scale than previous studies, and results indicate that none of 50 independent founder-flush lines evolved significant assortative mating relative to the control (base) population. This suggests a diminished emphasis on population bottlenecks in speciation of *D. melanogaster* and perhaps in general.

Key words.—Bottleneck, *Drosophila*, founder-flush, premating isolation, speciation.

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The founder-flush model of speciation, as developed by Carson (Carson and Templeton 1984 and references therein), envisions a specific role for population bottlenecks in speciation. Genetic drift acts in a founder population to disrupt ancestrally coadapted gene complexes. As the founder population establishes itself in its new environment, a period of rapid population growth immediately follows (the "flush" phase), minimizing the loss of ancestral genetic variation.

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Relaxed selection during the flush enables recombination to produce variation that normally would be selected out of the population. Continued population growth eventually results in selection increasing again as the growing population saturates its environment. The high levels of genetic variation produced by recombination, in association with the disrupted (previously coadapted) gene complexes, cause the population to respond rapidly to the selective pressures in potentially novel ways (due to the new gene combinations present). The population is free to shift, via selection, to an alternative adaptive peak, which leads to speciation.

A second model of bottleneck-induced speciation, called genetic transilience (Templeton 1980), differs from founder-flush in certain details (e.g., the dynamics of selection and the nature of the genetic variation responding to selection; see Carson and Templeton 1984; Templeton 1996). However, genetic transilience requires similar genetic and demographic conditions (Carson and Templeton 1984; Templeton 1996). Thus founder-flush and genetic transilience models of speciation can co-occur (Carson and Templeton 1984) and have not been distinguished in past experimental work on the role of bottlenecks in speciation. We therefore make no distinction between the models and use the term founder-flush broadly, referring to both models of bottleneck-induced speciation.

The strength of the experimental evidence supporting the founder-flush model of speciation has been a topic of recent debate (Barton 1989; Rice and Hostert 1993; Templeton 1996). Much of this debate centers on how to synthesize the results of several studies. However, other issues regarding the strength of evidence for founder-flush have received less attention.

For instance, conditions under which past experiments were done have been criticized (Charlesworth et al. 1982; Rice and Hostert 1993). These critics argue, for example, that Templeton's (1979, 1989) use of parthenogenesis in *D. mercatorum* to create extreme bottlenecks is irrelevant to speciation in diploid, sexual organisms (but see Templeton 1996). Many of the previous studies of founder-flush speciation, although not as extreme as this, employed experimental designs that were, in some way, unrepresentative of conditions that would likely ever occur in natural, sexual populations. These studies were designed to investigate the plausibility of founder-flush speciation and therefore used conditions that maximized the probability of reproductive isolation evolving. Although this was appropriate for the early studies, it is now necessary to ask whether founder-flush speciation can arise under more natural conditions.

A summary of some of the conditions used in past studies is presented in Table 1; two of these stand out. First, in some studies (e.g., Powell 1978; Ringo et al. 1985) strains of different populations from diverse geographic locations are pooled to create artificially high genetic variance and linkage disequilibrium in the base population from which the founder lines are derived. Second, multiple (serial) bottlenecks are used before testing for reproductive isolation in many studies (e.g., Meffert and Bryant 1991; Galiana et al. 1993). Both of these conditions increase the likelihood of speciation via the founder-flush model, and all of the studies of which we are aware suffer from one or both of these problems. (Although they provide results consistent with the founder-flush

TABLE 1. Summary of characteristics of past laboratory studies providing evidence consistent with founder-flush speciation, contrasted to the characteristics of the current study.

		Powell (1978) Dodd and Powell (1985)	Ringo et al. (1985)	Meffert and Bryant (1991)	Galiana et al. (1993) Moya et al. (1995)	This study
Unnatural conditions	Hybrid base population (# of source populations)	yes (4)	yes (69)	no	no	no
	Number of bottlenecks	1 then 4	1–6	5	4, 5, 7	1
Other concerns	New to lab	yes	yes (0–2 yrs.)	yes	6 months	no
	Selected most homokaryotypic lines	yes	no[2]	no	1 yes 1 no	no
	Experimental line × experimental line mating trials	yes	yes, but not relied upon	yes	yes	no
Statistical concerns	Mating cages	yes	yes	no	yes	no
	# of replicate isolation trials within a line[1]	~4 (Dodd and Powell 1985)	8–10	~60	at least 4	100
	# of independent bottleneck lines	12 (1 bottleneck) 8 (4 bottlenecks)	8	6 (2 each of 1-, 4-, and 16-pair bottlenecks)	45 (27 and 18 lines of 1-, 3-, 5-, 7-, 9-pair bottlenecks)[3]	50
Results	# of lines showing positive assortative mating	3 of 8 (Powell 1978) 1 of 8 (Dodd and Powell 1985)	1	2	1 of 15[4] 7 of 10[5] retests: 0[6]	0

[1] In studies employing mating cages to measure premating isolation, although replicate mating cage tests were performed, the data from these separate trials were pooled for the analysis, thus losing this information.

[2] Attempted, but base population was chromosomally monomorphic, so it was unnecessary.

[3] Two different base populations were used with 27 founder-flush lines derived from one and 18 from the other. Various numbers of replicates of 1-, 3-, 5-, 7-, and 9-pair bottlenecks were used. Only seven 1-pair bottleneck lines (four from one base population and three from the other) were used.

[4] In the original experiment (Galiana et al. 1993), only 15 lines were tested three times; of these, one showed consistent positive assortative mating.

[5] Templeton's (1996) interpretation of Galiana et al. (1993).

[6] Moya et al. (1995) retested all lines from Galiana et al. (1993) that showed significant positive assortative mating at least once. Of 51 combinations tested multiple times, 11 exhibited assortative mating the second time, one the third time, and none the fourth time.

model without suffering from these criticisms, the results of Arita and Kaneshiro [1979] and Ahearn [1980] are not considered because they lack any replication and have no control for the effects of selection in allopatry.) We find it unlikely that these conditions are representative of what occurs in nature.

A number of the past studies of founder-flush speciation used populations that were new to laboratory conditions (Table 1). Such a design may be more representative of what occurs in natural founder events (colonization of an "island" by a few founding individuals likely imposes novel selective pressures). Nonetheless, using populations in novel environments is problematic experimentally. Founder-flush speciation models do not require altered environments (see Templeton 1980; Carson and Templeton 1984), and the selection due to novel environments even without bottlenecks is known to cause the evolution of reproduction isolation (Mayr 1963; Rice and Hostert 1993). By using populations new to the laboratory, these past studies have no control for the effects of divergence due to strong, uniform selection in allopatry. It is therefore impossible to separate the effect of the founder-flush event from that of the "unintentional" selection due to the new environment. To determine whether founder-flush events cause speciation, their effect must be disentangled from that of selection, and therefore tests must be done in the environment in which the species is already adapted.

Finally, with the exception of Templeton's (1979, 1989) work using parthenogenetic strains of *Drosophila mercatorum*, past studies of founder-flush speciation have produced only partial reproductive isolation in the form of incomplete assortative mating, not speciation (e.g., Powell 1978; Dodd and Powell 1985; Ringo et al. 1985; Meffert and Bryant 1991; Galiana et al. 1993; for reviews, see Templeton 1996; Rice and Hostert; 1993; Barton 1989). If placed in sympatry, the founder-flush lines would almost certainly fuse. It is also noteworthy that in the cases in which multiple mate preference tests were performed at different times, some of the positive assortative mating from initial trials was revealed to be unstable and vanished in later tests (e.g., see Dodd and Powell 1985; Ringo et al. 1985; Moya et al. 1995). Nevertheless, some stable assortative mating has been detected; this suggests that founder-flush processes can lead to the evolution of some reproductive isolation. However, given the concerns outlined above, it would be premature to generalize about founder-flush speciation in the wild from these few studies.

We would like to know the probability that founder-flush population dynamics cause reproductive isolation more generally. We therefore used *Drosophila melanogaster* as a model system to mimic the kind of population size changes that might occur in a founding species. We wanted to address the concerns and correct the problems outlined above to determine whether evidence for founder-flush speciation would still be found. Thus, our experimental design differed from past studies in a number of ways. Our control (base) population was derived from a single, large population of *D. melanogaster* that was long adapted to laboratory conditions. Founder-flush lines underwent one single-pair bottleneck and were then flushed to a large population size. These founder-flush lines were then tested for reproductive isolation. In addition, unlike previous studies we did not bias our selection of lines from the base population to minimize chromosomal (inversion) polymorphisms (i.e., to maximize recombination and thus the likelihood of founder-flush speciation arising), and we did not test founder-flush lines for reproductive isolation against one another but rather tested each line against the control population (see Table 1).

Past studies also suffer from a lack of replication of independent founder-flush lines (Table 1; Galiana et al. [1993] is an exception although many of the replicate lines used bottlenecks of various sizes). If founder-flush speciation is rare, replication at the scale of independent lines is crucial to detecting this mode of speciation. For this reason, we tested 50 replicate founder-flush lines for premating isolation from the single base population from which they were all derived.

Methods

Stock and Culture.—A stock of *D. melanogaster* (the Dahomey population) has been maintained at large population size since its collection in Benin in 1970. This stock was initially kept in Edinburgh, moved to London in 1993, and in late 1995 a large (> 1000) sample of flies was moved to Vancouver to establish a stock population. These flies express a large amount of genetic variation for various morphological (Whitlock and Fowler 1996; unpubl. data) and life-history (Wilkinson et al. 1990) traits. They also show substantial declines in fitness upon inbreeding (Fowler and Whitlock, unpubl.; Mooers et al. 1999). The stock is long adapted to a laboratory environment. All flies were kept at approximately 25°C. Handling was performed at room temperature with CO_2 or cold anaesthesia.

Demography.—In late July 1996, 218 virgin pairs of flies (1 male and 1 female) collected from this laboratory population were placed into vials as pairs, left for six days to mate and lay eggs, and then removed and discarded. These constitute the founder-flush lines. Nonvirgin flies from the stock were put into 25 half-pint bottles to create a large ($N > 2000$), outbred stock population (the control population). At day 14, the progeny of 100 of the pairs were chosen at random and put individually into bottles as founders of 100 founder-flush lines; the other vials were discarded (two lines that were chosen had not produced offspring by day 14 and were replaced by two other randomly chosen lines). After the first generation, each line was given two bottles for population expansion (the flush); this was increased to four bottles per line for generations 3–8. Following this population flush, each line was maintained in five vials by transferring approximately 250 flies every generation. All bottles/vials for each line were mixed every generation when transferred to maintain panmictic populations. The large, panmictic control population was maintained at the same schedule in 25 bottles for the first eight generations and then in vials for subsequent generations. To prevent larval overcrowding during the flush (to achieve the relaxed selection phase posited by the founder-flush model), adults were allowed one day for egg laying in the bottles or four hours in vials. Adults were left longer (judged by visual comparison) in lines with low fecundity to allow the populations to flush.

Test for Premating Isolation.—We performed premating

isolation tests between each founder-flush line and the control. Tests were performed in late March and April 1997, 12–14 generations after the single founder event that established each line. Males and females used in these tests were collected and separated as virgins (within eight hours after emergence) using CO_2 anaesthesia and held in vials with food for two to four days before their trial. Tests were performed using 50 randomly chosen founder-flush lines of the 80 that survived to this point (20 lines were lost due to low population size or experimenter error).

Female-choice mating tests were conducted by placing one virgin female and two males together in a 40-ml vial with approximately 7 ml of medium, using cold anaesthesia. For each founder-flush line, 100 trials were performed, a set of 50 replicates using single founder-flush females and another set of 50 replicates using single control females. In each trial a female was placed in a vial with a control and a founder-flush male. For identification purposes, the tip of the wings of one of the males was clipped immediately prior to every trial. An equal number of trials had founder-flush or control males with their wings clipped in each replicate set. This created a balanced design that removed bias due to effects of clipping on the probability of mating.

Each test lasted a maximum of 90 min, with an observer checking once every 15 minutes to determine whether a mating was occurring. When the female was observed to mate with one of the two males, the time was recorded and the trial was ended. The trial was ended if no mating was observed in the first 90 min.

When measuring premating isolation, there is always the concern that differences between the laboratory and natural environments could potentially affect mating behavior (e.g., see Gromko and Markow 1993). The use of cages as opposed to vials to test for premating isolation may be more representative of conditions in nature. Our experimental design was chosen to deal with a central issue, the lack of appropriate replication of mating trials in past studies. When using mating cages, the only truly independent replicate is the cage itself because multiple matings within a cage are not independent of one another (the relative frequencies of the different types of flies changes as the trial proceeds and flies are mated). When using vials, however, each separate vial represents an independent replicate. Similar types of premating isolation tests to ours have been used successfully by others to detect significant premating isolation (e.g., Arita and Kaneshiro [1979] and Ahearn [1980] used one male and two females in a vial; Meffert and Bryant [1991] used one female with two males in testing premating isolation in the housefly).

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 in each male and female combination. Y is defined as:

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

where α is the number of control-by-control matings times the number of experimental-by-experimental matings divided by the number of control-by-experimental matings times the number of experimental-by-control matings. Y is a margin-free index (Bishop et al. 1975) and is thus not influenced by varying propensities to mate. Y varies from -1 for perfect negative assortative mating to $+1$ for perfect assortative mating, with zero indicating nonassortative mating. The significance of Y is evaluated using the statistic $X^2(Y)$, which is χ^2 distributed with one degree of freedom (Fienberg 1977). To avoid the loss of statistical power due to multiple comparisons, a preliminary test of each of the 50 founder-flush lines was used to identify those displaying potentially significant positive assortative mating. The top three lines (highest $X^2[Y]$ values) were then retested, using 200 mating trials per line for increased statistical power, to determine whether the observed positive assortative mating was statistically significant.

To test for discrimination at a finer scale, we also tested for differences in the time taken to mate. Mann-Whitney U-tests were used to determine for each of the 50 founder-flush lines whether there were any differences in how quickly homotypic versus heterotypic matings occurred.

Of a total of 3904 observed matings, 2181 (56%) were with unclipped males and 1723 (44%) were with clipped males, suggesting a small but significant clipping effect. Three lines (42, 75, 89) had clipping effects significantly more extreme than expected by chance (28%, 19%, 26% clipped matings, respectively), so the results from these three lines may have less power to test for isolation than the other lines.

Because of the overall clipping effect (44% clipped matings), the retest of lines with potentially significant positive assortative mating was performed twice, once using the previous clipping design and once using food coloring. Food coloring (red or blue; Food Club™ brand, Scott-Bathgate Ltd.) was added to the food the night before the trials in a balanced design (half of the trials with experimental males blue and control males red and half of the trials with the colors reversed). Male color (red or blue) had little effect on mating frequencies (48% matings with blue males).

Results

The distribution of assortative mating scores (Y) was approximately normal (Fig. 1) with a mean isolation of -0.03. Only one of the 50 lines showed significant positive assortative mating (uncorrected for multiple comparisons) under the first round of testing (Table 2). This line (28) and the two next most extreme lines showing positive assortative mating (124 and 83) were retested. All three of these lines showed near-zero and nonsignificant assortative mating scores in the retests using both clipping and food coloring (Table 2). These results indicate that the distribution of assortative mating scores (Fig. 1) does not represent variation in biologically meaningful assortative mating, but rather sampling noise in randomly mating lines.

The test for differences in time to mating revealed two lines of the 50 in which homotypic matings occurred significantly faster than heterotypic matings (line 92: $U = 2.46$, $P = 0.007$; line 38, $U = 1.66$, $P = 0.049$). These lines, however, showed no tendency towards positive assortative mating (line 92: $Y = -0.13$, $P = 0.28$; line 38: $Y = 0.12$, P

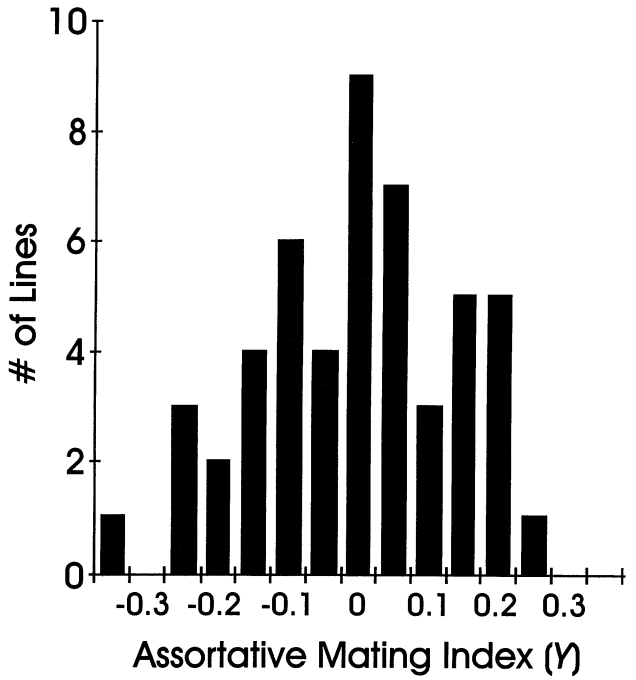

FIG. 1. Frequency distribution of assortative mating scores (Y) from initial trials. Positive Y values represent positive assortative mating.

= 0.25), and two lines of 50 in the tail of the distribution is no more than is expected from a Type I error rate of 5%.

DISCUSSION

The lack of detectable assortative mating in any of the 50 independent founder-flush lines suggests that the role of population bottlenecks in the speciation process should be deemphasized. Our results stand in contrast to the relative ease with which past studies (see Table 1) have found statistically significant premating isolation. The best explanation for this discrepancy is the experimental differences (see Table 1) between our study and past studies, in particular that we used a single population of laboratory-adapted flies and subjected them to only a single bottleneck. Of course, the influence of founder events in the speciation process may vary among taxa (due to genetic, life-history, ecological, or other differences), and the results of this study do not rule out a role for founder-flush in all cases. However, our results do suggest that the evolution of premating isolation and speciation do not necessarily accompany bottlenecks at the frequencies suggested by past studies.

An alternative interpretation for our results stems from a prediction made by Templeton (1980) and Carson and Templeton (1984). Templeton (1980) cites certain traits of *D. melanogaster* that, relative to other *Drosophila* species, reduce the likelihood of speciation via his genetic transilience model. These include fewer chromosomes, a shorter total map distance in *D. melanogaster* than in *D. pseudoobscura* (the species used by Powell 1978 and Galiana et al. 1993), and the observation that inbreeding induces negative assortative mating in *D. melanogaster* (Averhof and Richardson 1974) but not in *D. pseudoobscura* (Powell and Morton 1979). Carson and Templeton (1984) also suggest that *D. melanogaster* is a "weedy," colonizing species and such species have attributes that make them resistant to founder-flush speciation. If one accepts the grounds on which these predictions are based, then a negative result in this case can be interpreted as showing some support for the founder-flush model (i.e., finding a negative result where one was predicted). Without further study, we do not accept such an interpretation for the reasons outlined below.

It is interesting to note that Ringo et al. (1985), using *D. simulans*, found a much higher frequency of premating isolation than we did (one of eight founder-flush lines evolved sexual isolation from the ancestral line and two other lines displayed sporadic isolation over time). *Drosophila simulans* has the same number of chromosomes as *D. melanogaster* (four) and a total map length intermediate between *D. melanogaster* and *D. pseudoobscura* (*D. simulans* total map length is about 1.3 times that of *D. melanogaster* compared with about 1.6 times for *D. pseudoobscura*; True et al. 1996). In contrast to Ringo et al.'s (1985) results, the lack of any detectable premating isolation evolving in any of our 50 replicate lines suggests that these differences do not explain the discrepancy between our results and those of past studies. The predictions of Templeton (1980) have not been made quantitatively. We have no idea what influence, for example, a 23% decrease in recombination would have on the likelihood of speciation under this model, so therefore we must view the predictions as speculative.

The idea that weedy species may have genotypes that are resistant to founder-induced change is an interesting, but insufficiently documented, possibility. The observation that inbreeding causes negative assortative mating in *D. melanogaster* (Averhof and Richardson 1974, 1976), but does not appear to do so in *D. pseudoobscura* (Powell and Morton 1979), could be a symptom of this "general purpose genotype." However, Carson and Templeton (1984) acknowledges that the idea of a general purpose genotype (a term introduced by Baker [1965] in reference to certain characteristics of weedy plants) is incompletely documented, and we lack suf-

TABLE 2. Assortative mating index scores (Y) and significance for top three lines displaying positive assortative mating in the original trial.

	Original test			Retest with clipping			Retest with food coloring		
Line	Y	$X^2(Y)$	P	Y	$X^2(Y)$	P	Y	$X^2(Y)$	P
28	0.218	3.733	0.053	0.086	0.915	0.339	−0.041	0.226	0.634
124	0.180	2.511	0.113	<0.001	<0.001	0.996	−0.063	0.399	0.528
83	0.155	2.119	0.146	0.142	2.171	0.141	0.009	0.009	0.926

ficient comparative studies to identify the features that cause species to be weedy.

Although the founder-flush model makes no specific predictions concerning the time frame for speciation, it is a model that invokes selection, and selection requires time. The founder-flush model envisions the founder event acting as a trigger, but the genetic changes causing speciation accrue over the generations following the bottleneck (Carson and Templeton 1984; Templeton 1989). It is possible that in our experiment selection was taking a founder-flush population in a new direction, but that the response of the population was slow. Although no detectable premating isolation evolved in the time course of our experiment (12–14 generations), we cannot rule out the possibility that given more time, some may have evolved. Nevertheless, populations kept in allopatry will eventually evolve some reproductive isolation, regardless of their population size history, and attributing such an outcome to the effects of the founder-flush becomes increasingly difficult.

Thus, with a single population bottleneck, reproductive isolation did not evolve in a large sample of replicate founder-flush lines. This suggests that, at least in *D. melanogaster*, single population bottlenecks do not often lead to the evolution of reproductive isolation. Other mechanisms of speciation should therefore be given more credence.

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