

POSTMATING ISOLATION ANALYSIS IN FOUNDER-FLUSH
EXPERIMENTAL POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

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In this paper, we report a detailed analysis intended to detect postmating barriers in experimental populations of *Drosophila pseudoobscura* obtained through nine founder-flush cycles. The number of offspring produced was determined in three consecutive generations of hybridization. It is found that the evolution of pre-mating barriers, as shown by two of these populations, is not necessarily accompanied by the evolution of postmating ones.

Under the founder-flush model of speciation proposed by Carson (1971), the first step in the speciation process is usually thought to be an incidental genetic change through founder effect affecting mating behavior so that sexual pre-mating isolation evolves. Postmating barriers are unnecessary under this theory, although they may evolve incidentally afterwards. This order of events is exactly opposite to that predicted by the reinforcement speciation model (Dobzhansky 1951; Ayala 1991), in which allopatric populations develop postmating barriers (cross-sterility, inviability of hybrids, or hybrid breakdown) and, after secondary contact, pre-mating barriers may evolve adaptively.

Founder-flush experiments performed to date have concentrated on pre-mating barriers among experimentally bottlenecked populations, mainly searching for sexual isolation with multiple choice mating tests (Powell 1989; Ringo et al. 1985; Meffert and Bryant 1991; Galiana et al. 1993). With respect to postmating barriers, Powell (1989) did not detect sterility nor morphologic anomalies of genitalia in his hybrids, and Ringo et al. (1985) found a slight but progressive reduction in average fertility over the six studied founder-flush-crash cycles. Here we report an analysis of fertility with four populations of *D. pseudoobscura* from another founder-flush experiment (Galiana et al. 1989, 1993). This study was performed for a small number of populations and only during one founder-flush cycle. We concentrated our effort on a detailed analysis of fertility, over three consecutive generations of hybridization.

MATERIAL AND METHODS

Experimental Populations.—In December 1984, 27 populations were derived from an ancestral population of *D. pseudoobscura* (BCA) sampled in Bryce Canyon (Utah) and 18 from a different ancestral population (MA) sampled in Lago Zirahuén (Mexico). These populations were maintained according to a founder-flush-crash protocol (see Galiana et al. 1993 for a full description of the protocol). Each population

was founded with n virgin pairs, with $n = 1, 3, 5, 7,$ or 9 , and then allowed to grow “exponentially,” under noncompetition conditions for a few generations (flush phase). Next, a competitive crash was induced, and from the emerging progeny of each crash generation culture, n virgin pairs were randomly chosen in order to start a new founder-flush-crash cycle. Four of these populations were chosen for this experiment: M3, a derived population founded cyclically by one pair of flies; BC7, a derived population founded by three pairs; M25, a derived population founded by nine pairs; and BCA, one of the two ancestral populations kept under high, constant population size during the protocol. At the beginning of the experiment (June 1989), all three derived populations were at their ninth founder-flush-crash cycle (after 5 yr in the lab). M3 and BC7 were chosen as previous experiments (Galiana et al. 1989) had shown evidence of pre-mating isolation, whereas M25 and BCA were chosen as controls because they showed no evidence of this kind. Estimates of the fraction of heterozygosity lost at the beginning of these experiments were obtained as in Galiana et al. (1993). The values obtained are 0.88 for M3, 0.53 for BC7, 0.24 for M25, and 0.14 for BCA.

Postmating Experiments.—Postmating analysis consisted of the study of the number of offspring produced by pairs from the same or from different populations. First, it was recorded whether the couple had any offspring, and if they did, how many. Single couples were taken instead of a group of males and females (as in Moya and Ayala 1989); thus, if an individual had no offspring its sterility was not masked by other's fecundity. Also, interference from pre-mating isolation is reduced to a minimum as all individuals are forced to mate. Fifteen couples of all 16 pairwise male-female combinations among the four populations were tested in each of five temporally separate replicates of tests. Five- to seven-day-old pairs were placed in a fresh culture vial for seven days. After 10 more days, the offspring started to emerge. Every second day, new F_1 individuals were counted and removed. Counting was finished 16 days later, when all individuals had emerged. Among the first emergents, virgin individuals were taken from each vial, and then males and females were kept separated 5–7 days more until used to establish the second generation. Every second-generation pair was formed by a brother-sister pair derived from the same vial. If a vial had no offspring, the corresponding pair was taken from another one to complete 15 second-generation pairs. This was repeated again to obtain a third generation.

TABLE 1. Mean with standard error of the number of offspring (fert) as well as the number of families (out of 75) without any offspring either because of parents' death or culture contamination (x) or due to other unknown causes (y) for each generation (gen). Results for the five replicates of experiments have been pooled in this summary.

Male	Gen	Female											
		M3			BC7			M25			BCA		
		Fert	x	y	Fert	x	y	Fert	x	y	Fert	x	y
M3	1	68.1 ± 3.2	2	4	79.2 ± 3.0	5	3	67.3 ± 3.0	8	8	64.5 ± 3.2	4	3
	2	73.7 ± 3.8	1	3	84.0 ± 3.0	0	1	85.1 ± 3.2	5	2	77.0 ± 3.0	2	0
	3	61.3 ± 4.8	1	11	80.6 ± 3.5	0	2	83.8 ± 4.3	0	2	73.9 ± 4.0	1	8
BC7	1	66.3 ± 3.1	3	3	58.4 ± 2.5	3	2	61.1 ± 3.0	6	4	55.6 ± 3.1	1	4
	2	84.5 ± 3.6	1	1	60.9 ± 4.2	2	7	68.5 ± 2.9	0	3	54.1 ± 3.8	5	7
	3	73.6 ± 4.0	0	5	43.2 ± 3.5	2	6	59.0 ± 3.8	1	2	52.9 ± 4.7	5	12
M25	1	60.3 ± 3.1	3	5	65.8 ± 3.6	2	7	65.1 ± 3.2	2	3	64.6 ± 3.5	1	3
	2	82.4 ± 3.1	1	1	65.3 ± 3.9	2	2	84.5 ± 4.4	1	2	74.2 ± 4.6	1	3
	3	81.2 ± 3.4	0	4	63.6 ± 4.6	5	4	85.5 ± 4.7	3	2	68.4 ± 4.4	4	4
BCA	1	59.8 ± 2.7	2	6	67.9 ± 4.0	5	2	67.2 ± 3.1	2	5	65.9 ± 2.8	1	1
	2	78.0 ± 3.9	0	4	52.6 ± 4.4	6	11	77.1 ± 3.8	4	3	67.2 ± 4.2	4	2
	3	75.9 ± 3.6	0	4	59.0 ± 4.0	0	12	73.0 ± 4.1	0	2	64.7 ± 5.9	6	22

Five replicates of tests repeating this three-generation protocol were performed, each one delayed with respect to the preceding one (spanning from June 1989 to February 1990). All tests in a replicate were synchronous. Thirty-six hundred families were used (16 pair combinations, 15 pairs, five replicates and three generations), 900 for every cross between two given populations.

Statistical Analyses.—Analyses of variance (ANOVA) and a posteriori *t*-tests (Sokal and Rohlf 1981) were performed to detect differences in number of offspring attributable to the following factors: four different types of mating for every cross (for instance, males BC7 or M3 and females BC7 or M3); three generations, and five different replicates. Fertility is defined here as the number of offspring produced by one fertile pair. There was a maximum of 75 repeats (15 per replicate, 5 replicates) for every type of mating. Pairs without offspring have been analyzed separately, as the fraction of sterile families. Independent ANOVA have been applied to every cross. In every set of 15 families, some of them (*x*)

had no offspring due to early death of parent(s) or culture contamination, others (*y*) had no descent due to other causes, and the rest (*z*) had some offspring; thus, for every set of families $x + y + z = 15$. Fertility was measured over the *z* families in every replicate. We have also studied the variables $x/15$ (experimental error), and $y/(y + z)$ (sterility fraction). The transformations used were square root for number of offspring and angular for the other two.

RESULTS

Table 1 shows the average fertility and the number of pairs without offspring due to experimental error (*x*) or other causes (*y*). Data have been grouped in Table 1 although they are not homogeneous among replicates, but the factor has been taken into account in further discussions. Table 2 shows a summary of the ANOVA tests for the transformed fertility.

With respect to generations, in almost every case we have found by means of a posteriori *t*-tests that fertility in the

TABLE 2. Summary of ANOVA results for fertility (root-squared transformed) in the six analyzed crosses. Mean squares values and their significance are given for each main factor and interactions. *P*, pair type; *G*, hybridization generation; and *R*, replicate. Main factor *P* has been partitioned into three simple orthogonal comparisons (Sokal and Rohlf 1981): Hm-Ht, homogamic versus heterogamic pairs; Hm, between homogamic pairs; and Ht, between heterogamic pairs. The same partitioning has been performed with the $P \times G$ first order interaction.

Source of variation	Cross					
	M3 × BC7	M3 × M25	M3 × BCA	BC7 × M25	BC7 × BCA	M25 × BCA
<i>P</i>	126.93***	28.37***	12.31***	88.34***	23.98***	27.18***
Hm-Ht	287.56***	21.96*	35.34**	4.69	7.87	3.08
Hm	71.74***	59.05***	1.15	260.68***	50.90***	71.87***
Ht	21.51***	6.19	0.04	0.30	12.30	8.32
<i>G</i>	43.95***	61.72***	20.63**	25.40**	37.50***	16.15*
<i>R</i>	22.65***	46.22***	56.35***	45.82***	66.56***	75.33***
$P \times G$	11.18**	13.62**	13.01**	20.10***	9.06	10.32*
Hm-Ht	28.11***	9.92	7.81***	2.57	20.04**	2.10
Hm	1.37	29.75***	0.70	52.19***	2.98	20.17**
Ht	4.89**	1.35	0.50	4.44	7.73	0.38
<i>P-R</i>	4.71	8.92**	4.14	6.92*	7.49	6.63
<i>G-R</i>	32.05***	26.16***	19.67***	37.71***	39.10***	46.57***
<i>P-G-R</i>	5.30	5.38*	7.30*	4.65	6.51	6.30
Residual	3.84	3.50	4.03	3.81	4.53	4.20

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

second generation was higher than in the first and, often, than in the third ones. The only significant exception corresponds to the cross BC7 × BCA, where fertility declined along generations, with a significant difference between the first and the second generation.

Mating type also had a significant effect on the number of offspring when the four classes of matings were considered, but further partitioning of this factor into individual tests revealed important differences. When only two types (homogamic versus heterogamic pairs) were taken into account, the factor was significant only in the three crosses involving population M3 (Table 2) and always in the direction of higher offspring fertility in heterogamic pairs (see Table 1).

Most first-order interactions involving the type of pair are due to pair × generation. Among these, there are different reasons for the appearance of interactions in each cross. So, there are three cases where interaction is mainly due to homogamic versus heterogamic matings behaving differently in each generation, whereas in others differences arise between either homogamic (crosses M3 × M25 and BC7 × M25) or heterogamic pairs (BC7 × M3 and M25 × BCA). All the remaining interaction terms involve the factor replicate and can be assigned to error fluctuation among the different replicates. For the six crosses, the sum of squares explained by the main factors lies between 40% and 50% of the total explained by the linear model.

With respect to y , sterility due to unidentified causes, there was a strong effect of factor generation in cross BC7 × BCA ($P < 0.001$, analyses not shown); in fact, in both heterogamic matings between these two populations there was a high fraction of families without offspring in generations 2 and 3 (Table 1). Intrapopulation fractions of sterile pairs are high in M3 ($y_3 = 11$), BC7 ($y_2 + y_3 = 13$) and BCA ($y_3 = 22$) but not in M25 ($y_2 + y_3 = 4$). The causes of sterility could be diverse: pairs could have not mated (and hence we would be measuring a premating barrier), or the parental individuals could be sterile, or the offspring could be inviable in a early phase of development. We have not analyzed which one of these possibilities was correct.

There are no significant differences with respect to experimental error ($x/15$); hence, conditions can be considered homogeneous throughout the experiment.

DISCUSSION

We have not detected postmating reproductive barriers. On the contrary, our data show a moderate hybrid vigor effect for hybridizations involving the population with the narrowest bottleneck (M3). This population was affected by inbreeding depression at the moment of the analysis, after nine cycles, and an estimated variability loss around 90% (Galiana et al. 1993), as observed in an interspecific competition experiment performed independently with this population during the same founder-flush cycle (López-Bueno et al. 1992).

The lowest values of fertility for heterogamic pairs are those between BC7 and BCA for generations 2 and 3. These results might suggest the presence of an incipient postmating isolation for the cross BC7 × BCA. However, these results can be partially explained by the decline in fertility in the third generation of the homogamic cross BC7 × BC7, which

may represent also inbreeding depression rather than postmating isolation from BCA.

In Carson's (1975) model of speciation, a premating barrier is assumed to evolve first during the speciation process. Afterwards, given that gene flow no longer exists between ancestral and derived populations, postmating barriers may evolve incidentally through the processes of pleiotropic divergence (first envisioned by Muller 1939) and/or hitchhiking (Rice and Hostert 1993). In Dobzhansky's (1951), adaptive-divergence model of speciation populations that will later become different species evolve postmating barriers (cross-sterility, inviability of hybrids, or hybrid breakdown) in allopatry and, after secondary contact, premating barriers evolve adaptively. Recent versions of the adaptive-divergence model consider that this second phase is not essential so that postmating barriers might evolve also in allopatry through the same processes (Ayala 1991; Rice and Hostert 1993). What seems clear is that populations can evolve in different ways, as crosses between taxa can be found that exhibit either mainly premating barriers (e.g., several Hawaiian *Drosophila*, see Templeton 1977) or mainly postmating barriers (see Ayala 1991).

We have found that some assortative mating evolved without any previous postmating barrier: populations BC7 and M3 showed partial positive assortative mating in cycles 4, 5, and 7, lasting for more than 40 generations (Galiana et al. 1993), although significance was lost in further multiple choice analyses at cycles 11, 12, and 13 (Galiana et al. 1995). And these two populations have shown a clear hybrid vigor effect in the fertility analysis. Bottlenecks followed by population expansions could elicit some ethological changes in a short period of time, but this period of time was not enough to produce any measurable postmating incompatibility through the process of pleiotropic divergence.

Our results are compatible with the order of events envisioned by Carson (1971), but of course we recognize that this is only a weak evidence in support of Carson's theory as the assortative mating we detected was far from being complete and stable.

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MULTIVARIATE PATTERNS OF GENETIC DIFFERENTIATION SUPPORT COMPLEX COLONIZATION SCHEMES IN *BUFO MARINUS* POPULATIONS

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A fundamental hypothesis in population genetics is that the history of populations is expressed in the spatial distribution of genetic data such as allelic frequencies (Sokal and Wartenberg 1979; Felsenstein 1982; Endler 1982, 1983; Sokal 1986; Thorpe et al. 1991). Spatial patterns of genetic variation have been used to infer the occurrence of specific temporal events in the history of some human (Menozzi et al. 1978; Piazza et al. 1981; Sokal and Menozzi 1982; Sokal et al. 1990; Cavalli-Sforza et al. 1993, 1994) and *Drosophila* populations (Menozzi and Krimbas 1992), and in some systems of host/parasitoid interaction (Feder et al. 1988; McPheron et al. 1988; Frank 1991). Slatkin (1985) also reviewed the topic of gene flow in natural populations and cited numerous studies that measured population “history” (e.g., dispersal of organisms) by observation and/or experimental manipulation. He compared these measurements to patterns in genetic data and found concordance between the two.

Species with known histories of colonization or range expansion are particularly useful in this context, because it is possible to determine from independent data sets—a genetic one and, generally, an observational one—whether historic information can be recovered from population genetic data. One such case is the introduction of *Bufo marinus* to different regions of Australia including the Moreton Bay region, south-east Queensland, Australia. The range expansion

of *B. marinus* in this region has been well documented independently of genetic data, and consists of three spatially complex phases (Easteal and Floyd 1986). Easteal (1985) did not find good concordance between the colonization events and patterns revealed by the genetic analysis of 10 polymorphic loci. However, are population genetic tools really well suited for detecting different events of range expansion?

Endler (1983) explicitly wrote that genetic data were the sum of ecological information, of historic information and of “noise” (e.g., sampling bias). Independently, multivariate ordination techniques were used according to an identical equation that assumes data are a sum of information and “noise” (for instance, see Jackson 1993). There is an obvious concordance between these two “equations”, but ordination techniques have been very rarely used in population genetics except for principal components analysis (generally in human genetics, e.g., Menozzi et al. 1978; Piazza et al. 1981; Rendine et al. 1986; Cavalli-Sforza et al. 1993, 1994) and multidimensional scaling (e.g., Lessa 1990), despite their ability to consider all loci simultaneously rather than separately in some cases (e.g., autocorrelation methods, but see Wartenberg 1985a,b).

Principal components analysis and other ordination methods have the disadvantage of being unrelated to any classic population genetic parameter such as *F*-statistics. Some or-