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# Local Ecology and Multiple Mating in a Natural Population of Drosophila melanogaster

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# LOCAL ECOLOGY AND MULTIPLE MATING IN A NATURAL POPULATION OF *DROSOPHILA MELANOGASTER*

The measurement of fitness components in natural populations is among the most important problems of population genetics. Much effort has been devoted to the measurement of one aspect of the reproductive component of fitness, the storage and use of sperm by females.

Reports of high frequencies (>50%) of multiple mating of *Drosophila* in nature are common (Anderson 1974; Milkman and Zeitler 1974; Stalker 1976; Cobbs 1977; Levine et al. 1980). Only two studies have reported low remating frequencies (<25%; Craddock and Johnson 1978; Griffiths et al. 1982). None of these studies has attempted to examine the effect of environmental differences on remating. Gromko et al. suggested that remating could be influenced by various ecological factors such as "temperature, population size and density, [and] distribution of resources" (1984, p. 389).

In this note, we report the results of a small study of multiple mating that takes advantage of a population of *Drosophila melanogaster* in a unique ecological situation in California's Sonoma Valley. In the course of estimating remating frequencies, we have developed new procedures for detecting multiple mating in individual females and have made an observation about the pattern of sperm use in wild-caught multiply mated *D. melanogaster* females.

The area we studied is the environment in and around the Gundlach-Bundschu winery. In this area, *D. melanogaster* flies are found in two distinct habitats: inside and outside the winery building. This building is surrounded by vineyards to the west and by a scrub-oak woodland around the rest. There is no resident *Drosophila* population inside the building. During the day, flies are attracted into the winery and accumulate. By sunset, densities inside the winery are high by normal field standards: thousands of flies can be collected with a sweep net in a matter of minutes. However, these flies are killed every evening by an overnight pyrethrin fogging. In the morning no flies can be found in the winery, and we have been unable to find any larvae or pupae inside (Marks et al. 1980). The winery building, therefore, functions as a large trap, collecting flies from the surrounding woodland habitat during the day.

Flies were collected simultaneously from inside and outside the winery, shortly after completion of the fall harvest around sunset. The weather was clear and dry. Inside the winery we collected with sweep nets; outside, at traps baited with actively fermenting banana mash set among the oaks. Flies were collected from the baited traps every 10 min. Since *D. melanogaster* flies require more than 10 min to complete copulation (Spieth 1952), this practice minimizes the possibility

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TABLE 1

COMBINED ALLELE FREQUENCIES, $p_i$, FOR EACH OF THE SAMPLED LOCI, $\chi^2$ TESTS OF GOODNESS OF FIT TO HARDY-WEINBERG FOR SAMPLES FROM INSIDE AND OUTSIDE THE WINERY, AND CONTINGENCY $\chi^2$ TESTS FOR DIFFERENCES IN ALLELE FREQUENCY INSIDE AND OUTSIDE

| | *Pgm* | | *Adh* | | *αGpdh* | | *Est-6* | |
|---|---|---|---|---|---|---|---|---|
| | Allele | $p_i$ | Allele | $p_i$ | Allele | $p_i$ | Allele | $p_i$ |
| | 0.55 | 0.011 | *F* | 0.673 | *F* | 0.699 | *F* | 0.310 |
| | 0.70 | 0.120 | *S* | 0.327 | *S* | 0.301 | *S* | 0.690 |
| | 1.00 | 0.835 | | | | | | |
| | 1.20 | 0.019 | | | | | | |
| | 1.50 | 0.015 | | | | | | |
| Hardy-Weinberg | | | | | | | | |
| Inside | $\chi^2_6 = 8.16$ | | $\chi^2_1 = 0.10$ | | $\chi^2_1 = 3.18$ | | $\chi^2_1 = 1.27$ | |
| Outside | $\chi^2_6 = 7.24$ | | $\chi^2_1 = 0.05$ | | $\chi^2_1 = 0.14$ | | $\chi^2_1 = 1.20$ | |
| Contingency test | $\chi^2_1 = 2.14$ | | $\chi^2_1 = 0.47$ | | $\chi^2_1 = 1.95$ | | $\chi^2_1 = 0.34$ | |

NOTE.—Sample sizes: 133 individuals inside the winery; 114 individuals outside. None of the individual $\chi^2$'s is significant.

that flies will mate in the traps. Flies from each collection were anesthetized immediately, and single females were placed into individual vials. The collection scheme was designed to allow the measurement of the natural remating frequencies at these two sites, uncomplicated by any remating induced by the collection methods.

In the lab these females were transferred to a fresh culture vial every day for 6 more days. This keeps larval densities low, allows collection of many progeny, and permits examination of temporal variation in sperm use. All the original females and their adult progeny were collected and frozen. Electrophoretic genotypes of these females and their progeny were determined on starch gels for four polymorphic enzyme loci: alcohol dehydrogenase (*Adh*; II-50.1), α-glycerophosphate dehydrogenase (*αGpdh*; II-17.8), phosphoglucomutase (*Pgm*; III-43.4), and esterase-6 (*Est-6*; III-36.8).

The total number of progeny of females from inside the winery did not differ significantly from that of females outside the winery (inside, $\bar{N} = 82.43$, $s = 17.25$; outside, $\bar{N} = 79.06$, $s = 18.72$; $t = 1.49$, NS). Electrophoresis was also performed on a sample of 133 flies from inside the winery and 114 flies from outside. The results are shown in table 1. No deviations from Hardy-Weinberg expectations were detected at any locus, including *Adh*. There were no differences in allele frequency inside and outside the winery at any locus. These results are consistent with previous studies at this site (Marks et al. 1980).

Genetic analyses of progeny broods were carried out on all females from which we obtained a sample of more than 30 progeny over the 7-day collection period: 31 females from inside the winery, and 33 females from outside. An initial screening of up to 10 progeny per day was performed on these females. A total of 3608 progeny from 64 females were examined, an average of 56 progeny per female.

We have used a variety of techniques to detect multiple mating of individual females. We elected to maximize the probability of detection by using several

analytical techniques, including two not previously used. A priori, we would infer a multiple mating under the following conditions:

1. too many alleles in the progeny brood (Birdsall and Nash 1973; Anderson 1974; Craddock and Johnson 1978; Darling et al. 1980; Levine et al. 1980);
2. too many genotypes at linked loci (here extended to singly or doubly heterozygous females; Milkman and Zeitler 1974);
3. too few genotypes at unlinked loci (new method);
4. single- or multiple-locus lack of fit to Mendelian expectations (Zouros and Krimbas 1970; Sassaman 1978); or
5. temporal variation in the frequency of progeny genotypes (new method).

The rationale of condition 3 is as follows. Males doubly heterozygous at two unlinked loci produce four types of gametes. If a large progeny array has only three of the predicted four types, then a simple exact test of the hypothesis of a single father can be made as the probability of getting a sample of size $n$, in which one expected class is missing, independent of the distribution of individuals in all other classes. For example, consider a doubly homozygous female, *aabb*, who has produced a large number of progeny including the three genotypes *AaBb*, *Aabb*, and *aaBb*. The absence of *aabb* progeny has two possible simple explanations, not equally probable. The first is that the progeny were sired by two different males, say *AABb* and *AaBB*. The second is that one male was involved (*AaBb*) and that the absence of *aabb* progeny is due to sampling error. The simple exact test for a single male in this example is given by $(\frac{3}{4})^n$, in which $n$ is the number of progeny scored. Clearly, if a large number of progeny from a single female is scored, very strong probability statements can be made. From our data, in the 10 cases for which this method was applicable, the probabilities of unipaternal broods ranged from $4.6 \times 10^{-4}$ to $7.6 \times 10^{-10}$.

The rationale behind condition 5, temporal variation in the frequency of progeny genotypes, comes from laboratory work on sperm use in multiply mated flies. These studies have revealed a brooding effect, in which the female more or less rapidly switches over to using the second male's sperm (Prout and Bundgaard 1977; Pyle and Gromko 1978; Gromko et al. 1984). In general, we would infer multiple mating if we observe a change in genotype frequency through time in a single female's progeny.

In this study, each test was applied to each brood. The proportion of multiply mated females was estimated as the number of females determined to be carrying sperm from more than one male by any of the methods of detection. These data are presented in table 2.

By any of criteria 2–4 singly and by all criteria jointly, the proportions of multiply mated females inside and outside the winery are significantly different. It is not surprising that we did not find any progeny broods with too many alleles (criterion 1). Criterion 1 requires loci with many alleles (never fewer than 4), and its power is proportional to the evenness of the allele frequency distribution. Though there were five *Pgm* alleles in our samples, one allele dominates the frequency distribution (table 1).

The failure to find temporal variation in the frequency of progeny genotypes (criterion 5) was somewhat surprising. Several different analyses were attempted to discover trends in genotype frequencies among the progeny, all of which failed.

TABLE 2

NUMBER OF FEMALES DETERMINED TO BE MULTIPLY MATED, LISTED BY CRITERIA FOR DETECTION AND CONTINGENCY TESTS FOR DIFFERENCES BETWEEN THE TWO HABITATS

| CRITERION | $\chi^2$ | HABITAT | |
|---|---|---|---|
| | | Inside | Outside |
| 1. Too many alleles | | 0 | 0 |
| 2. Too many genotypes (linked loci) | 4.37* | 6 | 1 |
| 3. Too few genotypes[a] | 8.20** | 9 (8) | 1 (1) |
| 4. Goodness of fit[b] | | | |
| Multiple loci | 15.57*** | 17 (3) | 3 (2) |
| Single locus | 24.01*** | 20 (3) | 2 (0) |
| 5. Temporal sperm use | | 0 | 0 |
| TOTAL | 18.72*** | 20 | 4 |
| Total number of broods examined | | 31 | 33 |

NOTE.—The number in parentheses gives the number of females found to be multiply mated among those not so classified by a lower-numbered method.

[a] A multiple mating is inferred if the probability of the sample, assuming a single male, is less than 0.001.

[b] A multiple mating is inferred if the $\chi^2$ is significant at $P < 0.01$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

We further examined the 16 broods for which multiple mating was indicated by conditions 2 or 3. If the transition to the last male's sperm in as dramatic as that found by many other workers (e.g., Prout and Bundgaard 1977) and if females from inside the winery have remated within the last 12 h, as appears possible from our data, then we should see a trend in allele frequency. First, we carried out electrophoresis on any progeny from these 16 broods that had not been run in the initial screening. Inspection of the combined raw data revealed that the frequency of the progeny from the two males showed no trends through time. For example, from the 10 females known to be multiply mated by conditions 2 or 3, the average regression coefficient of allele frequencies through time was 0.021, and none of the individual regressions was significant. Furthermore, assuming that the least frequent progeny type is that of the first male, the average frequency of progeny from the second male, $P_2$, was 0.714, substantially smaller than that reported for category I (no brooding effect over time) by Gromko et al. (1984). Perhaps this experiment has missed the transition to predominant use of the last male's sperm, either because second matings have occurred long before our sample or because of the small sample sizes typical of the daily progeny production by one individual female. It is also possible that multiply mated females in nature (involving natural genotypes) may not show the brooding effect that is seen in experiments involving laboratory markers (Prout and Bundgaard 1977; Pyle and Gromko 1978; Gilbert and Richmond 1981; Gromko et al. 1984). The interaction between wholly wild-type sperm may be different from the interaction of sperm from laboratory stocks (Turner and Anderson 1984).

To the best of our knowledge, ours is the first demonstration that local ecolog-

ical differences can affect the frequency of multiple mating. We cannot rule out the possibility that recently remated females are preferentially attracted inside the winery. Obviously, many environmental differences exist between the winery and the surrounding woodland. Further studies of the effects of density, food cues, a variety of volatile compounds, and other factors on multiple mating are indicated by our observation.

The relationship between density and frequency of multiple mating has been examined experimentally by Gromko and Gerhart (1984). They found that increased densities actually decreased the frequency of multiple matings. However, even the lowest densities they used are probably much higher than those found in nature. At considerably lower densities, Harshman et al. (1988) found no evidence for an effect of density when several wild-type stocks were used for the first mating. Even so, densities in nature may be sufficiently sparse and the environment sufficiently complicated that the actual frequency of contacts differs drastically from that in a laboratory setting.

This report documents the conjecture that the frequency of multiple mating observed in the wild depends strongly on ecological circumstances.

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