CONCURRENT MULTIPLE PATERNITY IN NATURAL AND LABORATORY POPULATIONS OF DROSOPHILA MELANOGASTER

ROGER MILKMAN AND RODNEY R. ZEITLER

Department of Zoology, The University of Iowa, Iowa City, Iowa 52242

Manuscript received March 11, 1974

ABSTRACT

Electrophoretic analysis of two or three linked enzyme loci in progenies of homozygous females from cage and natural populations demonstrates frequent concurrent multiple paternity in both cases.

ELECTROPHORETIC analysis of progenies shows that a significant proportion of D. melanogaster females, both from cage populations and from nature, carried sperm from more than one male at a time. This observation indicates that an iso-female line may contain more than four haploid genomes and that uneven distributions of alleles among offspring need not reflect unequal segregation.

MATERIALS AND METHODS

Female flies were permitted individually to lay eggs in standard food vials, then genotyped at two or three loci on the second chromosome. The progenies of homozygous females were examined, and those containing more than two genotypes were considered to stem from more than one father, there being no crossing over in the males.

The loci studied were α-glycerophosphate dehydrogenase, malate dehydrogenase, and alcohol dehydrogenase. Cellulose acetate electrophoresis was performed using Gelman supplies and equipment, standard staining techniques, and the Adamkewicz multiple applicator (ADAMKIEWICZ and MILKMAN 1970).

RESULTS AND DISCUSSION

Of the cage females, 9 of 45 double homozygotes produced progeny with more than 2 genotypes; 5 of 13 triple homozygotes did also. Additional double inseminations can be inferred: the observed certain cases are those where the two male parents have a total of more than two chromosomal types. The observed frequency, then, is equal to the true frequency of multiple mating, multiplied by the frequency of fulfillment of the above conditions. This frequency is calculated by computing and adding the probabilities of having four and three chromosomal types, respectively, among four chromosomes, given the appropriate allele frequencies and assuming linkage equilibrium. (In both cage and wild populations no significant linkage disequilibrium was detected between α-GDH and ADH.)

\[ P = 24p_i p_j p_k p_l + 12 \sum p_i^2 p_j p_k \]

1 This work was supported in part by NIH Grant GM 18967.
2 Present address: College of Medicine, University of Iowa, Iowa City, Iowa 52242.

$P$ is the frequency of detectability, $p$ is frequency of chromosomal type, and the coefficients represent the number of permutations. For doubly homozygous cage females ($\alpha$-GDH and $ADH$), $P = 0.40$, and so the observed value 0.20 $(9/45)$ is corrected to 0.50. For the triple homozygotes, 0.38 $(5/13)$ is corrected to 0.48 by a similar method, the correction being less because the proportion of cases with more than two chromosomal types is greater when more loci are involved.

The 204 wild females, collected at the Coral Fruit Market, Coralville, Iowa, included 81 double homozygotes. Of the 37 progenies in which 20 flies have been tested, 7 contained more than 2 genotypes. The estimated frequency of multiple paternity in the wild sample is 0.47. All the results are presented in Table 1.

It will be seen that some genotype distributions are quite uneven. This may be due to sampling, differential "viability" of chromosomes, meiotic drive, or the participation of two different homozygous male parents. (These cases are not included in the calculations, since the correction makes them redundant.) It is also recognized that when one of two concurrent male parents makes the larger contribution by far, the multiplicity may go undetected in a small progeny sample. For example, the first 20 flies of one sample contained two genotypes and were so reported; when this sample was increased to 60, three flies of a third genotype were observed. Thus the estimates are minimal estimates of the frequency of concurrent multiple paternity.

DOBZHANSKY and PAVLOVSKY (1967) exploited chromosomal arrangements to demonstrate multiple paternity in the laboratory cultures of $D$. pseudoobscura. ANDERSON (1974) has just extended this observation to a natural population. FUERST, PENDLEBURY and KIDWELL (1973) detected multiple paternity in bottle cultures of $D$. melanogaster using two unlinked visible markers. RICHMOND and EHRMAN (1974) have drawn similar conclusions for $D$. paulistorum in vial cultures and in population cages on the basis of genotypes at a single locus, ($tetrazolium$ oxidase in two experiments, *white-eyed* in a third). ZOROS and KRIMBAS (1970) exploited the existence of a large number of alleles at two esterase loci to demonstrate multiple paternity in a natural population of the olive fruit fly.

### TABLE 1

<table>
<thead>
<tr>
<th>Genotypes (N)</th>
<th>Number of loci at which mother is homozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Cage population*</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2A‡</td>
<td>27</td>
</tr>
<tr>
<td>2B‡</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>

* Frequencies of fast allele: $\alpha$ Gdh, 0.33; Mdh 0.39; Adh 0.26.
‡ Frequencies of fast allele: $\alpha$ Gdh, 0.82; Adh 0.40.
‡ 2A: more common genotype < 14 out of 20. 2B: more common genotype > 14 out of 20.
MULTIPLE PATERNITY IN DROSOPHILA

Drosophila. The present observations appear to be the second demonstration of frequent concurrent multiple paternity in natural populations of Drosophila. BIRDSALL and NASH (1973) have demonstrated multiple paternity in Peromyscus; certain of their calculations are in error and have been corrected by MERRITT and WU (1975).

Estimation of genic heterozygosity, chromosomal heterozygosity, and asymmetrical segregation via progeny analysis should allow for the effects of concurrent multiple paternity in this and doubtless other Drosophila species. For example, RICHMOND and POWELL (1970) concluded that heterosis was operating at the sex-linked tetrazolium oxidase locus in D. paulistorum, since they found that well over 50% of the females each gave rise to F₁ females of two common genotypes. This observation could be accounted for by multiple paternity involving males carrying different To alleles. When direct genotyping is impractical and sex-linked genes are involved, the examination of several male offspring as well as females would be an appropriate means of distinguishing heterozygous mothers from multiply inseminated ones.

We are grateful for the contributions of LAWRENCE LAYFER to this study, and for the reviewers’ constructive comments.

LITERATURE CITED


