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Evidence for selection by male mating success in natural populations of *Drosophila pseudoobscura**

(components of selection/evolution/population genetics)

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ABSTRACT **Gene arrangement frequencies were determined at two stages in the life history of *Drosophila pseudoobscura* taken from nature. Three populations in the central highlands of Mexico were each sampled twice during 1976. Gene arrangement frequencies were measured in adult males and in larvae that were the offspring of females collected at the same time. The adult males were in all likelihood a representative sample of those who fathered the larvae produced by the wild females. Differences in gene arrangement frequency between these two life stages should indicate the operation of natural selection. One-third of our comparisons of common gene arrangement frequencies in males and in larvae from the next generation were statistically significant, as were one-third of our comparisons of total frequency arrays in the two life stages. We consider the components of selection that could produce such frequency changes and reason that male mating success must be the major one. Gene arrangement frequencies in the Mexican populations fluctuate within wide bounds. Selection must act to retain the polymorphism in the face of this flux in gene arrangement frequencies, and we suggest that male mating success plays an important role.**

One of the early triumphs of ecological genetics was the demonstration that selection in nature could be intense—in fact, one or several orders of magnitude more powerful than the founders of population genetics imagined. No experimental system played a more important role in the analysis of selection than the chromosomal polymorphism for gene arrangements in *Drosophila pseudoobscura*. In this species a series of inversions on the third chromosome binds large blocks of genes together as units, just as though they were alleles of a single "supergene." Natural selection was first implicated when Dobzhansky (2) showed that the frequencies of certain gene arrangements went through seasonal cycles in two of three populations on Mt. San Jacinto in California; subsequent studies showed that these cycles were repeated in years scattered over a span of 2 decades (3, 4). The frequencies of gene arrangements in the third population on Mt. San Jacinto did not cycle, but between 1939 and 1946 they underwent a directional change that Dobzhansky (5) also ascribed to natural selection. Dobzhansky and Levene (6) then showed that karyotypic frequencies in eggs laid by wild females were generally in accord with Hardy–Weinberg expectations, but that frequencies in wild males were not. They concluded that the karyotypes suffered differential mortality during the transition from fertilized egg to adult fly. That selection on the *D. pseudoobscura* inversions occurred in nature seemed to be settled, and it was generally taken for granted that viability differences accounted for the major part of it.

Some 20 years later, a series of papers by Prout (7–10) stimulated evolutionary biologists to pay greater attention to the various components to fitness. These components determine the separate bits of selection that operate at specific stages in the organism's life history, often tied to some important activity such as mating or competition for food. By measuring them it is possible to understand considerably more about the causes of selection than is possible from measurements made at intervals of one or more generations. One result of such analyses has been a realization that selection occurs as much through differential fertility as through differential viability (9–14). Fertility may, in fact, be the most important component of fitness in many cases, including the inversion polymorphism in *D. pseudoobscura* (15). Included in fertility are all those attributes that determine the number of offspring produced by an adult who reaches reproductive age, most important among them being female fecundity and male mating success.

For the *D. pseudoobscura* karyotypes, large differences in male mating success have been shown in mating chambers, where individual copulations can be observed (16, 17). In large experimental populations, fertility differences among both male and female karyotypes have been found (15, 18), and they are at least as large as the viability differences reported previously (19). It would seem reasonable, then, to expect a major role for fertility differences in nature. Despite the attention given to the *D. pseudoobscura* inversions, however, the matter of fertility differences in nature has been nearly untouched. Only a single population has been studied in this regard (20). It is the purpose of this report to present data from three natural populations that bear on this question of fertility differences among the *D. pseudoobscura* karyotypes, data that provide evidence for selection by male mating success.

MATERIALS AND METHODS

Rationale of Our Analysis. Adults were captured in nature, and gene arrangement frequencies in males and in the offspring of females were determined. Nearly all females were inseminated and carried sufficient sperm to produce a sizable number of offspring. It has been known for many years that *Drosophila* males mate repeatedly, and recently evidence has been advanced that females do so as well (21–25). A large fraction of *D. pseudoobscura* females carry the sperm of two or more males (21, 23, 25). Whether sperm from the last copulation displace those from earlier matings is not known for this species, although such a sperm displacement has been shown for *D. melanogaster* in the laboratory (26). Repeated matings, coupled with a high fecundity, make it likely that the turnover of sperm in *D. pseudoobscura* females is rapid. And this rapid turnover

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of sperm in turn makes it likely that the males in each of our collections were a representative sample of those that mated with the females of the same collection. The mating success of the male genotypes determined the frequencies of gene arrangements in the sperm carried by females. *Hence, one test for selective differences in male mating success is to compare gene arrangement frequencies in males with those in larvae produced by females collected at the same time.*

This reasoning is based on the assumption that other components of fitness do not confound our analysis, and in particular that karyotypic frequencies in adult males and females are alike, or nearly so. We must examine this assumption. Selection by differences in female fecundity may be ruled out in our experiment, because each female contributed equally to the larval frequencies we calculated. And since almost all females from nature were inseminated, there was little possibility of selection among females by differential mating success. Viability differences between male and female karyotypes could lead to different frequencies of the inversion types in reproducing adults of the two sexes, and hence to differences between males and their larval offspring. This differential viability in the sexes seems to be the most important factor that could complicate our analysis for selection by male mating success, and therefore we have tested for it in one collection. As detailed below, we find no evidence for it.

At least two other possible forms of selection could influence the test we propose, and thus deserve mention. Viabilities might differ in sexually immature and mature flies of either sex, so that gene arrangement frequencies in the males taken in a collection might differ from those in reproducing males. We think it unlikely, however, that viabilities differ with age to the extent required to account for our data. Another possibility is that brood mixtures occur among males, and perhaps females as well. That is, adults from different demes in the collecting area, with different gene arrangement frequencies, might be brought together by the baits. The males in a collection, then, might not represent those that mated with the females taken at the same baits. This explanation requires considerably more genetic microdifferentiation than is known for *D. pseudoobscura*. Neither of these two mechanisms can be excluded at present, although we feel they are unlikely to play more than minor roles in the populations we have studied.

Thus we reason that significant differences in gene arrangement frequency between males and larvae of the next generation are indeed evidence for selection by male mating success, although this evidence must be considered preliminary until additional experimentation allows a direct comparison of inversion frequencies between males and the sperm they have deposited in females.

Experimental Procedure. Adult males and females were collected over baits of fermenting bananas. They were separated by sex shortly after capture. Each wild male was placed in a bottle with several virgin females homozygous for the Treeline gene arrangement. Each wild female was placed in a separate bottle without males, so that any offspring would be the result of a mating between the female and a male from the natural population. The resulting larvae were reared in near-optimal conditions of temperature, nutrition, and density. Chromosomes were studied in squash preparations of salivary glands dissected from third instar larvae and stained with aceto-lactic orcein. Usually a single larva from each culture was studied, allowing identification of one chromosome in male lines and two in female lines. Sometimes, when samples were small, we studied as many as five larvae from male cultures and eight from female cultures in order to increase the number of chromosomes analyzed. Gene arrangements of the third chromosome were identified according to the descriptions in Dobzhansky (27), Kastritsis and Crumpacker (28, 29), and Olvera *et al.* (30). They are designated by abbreviations explained in the legends to Tables 1 and 2.

Collections were taken in the Central Highlands of Mexico at three localities: (1) a forested area near Tulancingo, state of Hidalgo; (2) a park near Amecameca, state of Mexico; and (3) a forested hillside above Lake Zirahuen, near Patzcuaro, state of Michoacan. These sites were sampled on a rotating basis, one per month, so that each was sampled at 3-month intervals. We chose for analysis all data from 1976. Our only restriction was that a minimum of 40 chromosomes be diagnosed, both in males and in larvae, so that statistical comparisons would be meaningful. Two collections from each locality met this criterion.

To test for possible sex differences in viability, we compared gene arrangement frequencies in adult males and females collected at Amecameca in January 1977. Females were cleared of sperm by storage at 5°C for a month and then mated to males from a laboratory strain homozygous for the Standard gene arrangement. Adult males were mated to virgin females from this same Standard strain. Salivary chromosomes of 10 larvae from each culture were identified, and thus the frequencies of gene arrangements in adult males and females were determined.

RESULTS

Gene arrangement frequencies in adult males and females from Amecameca are given in Table 1. A test for homogeneity between the sexes revealed no significant difference ($\chi^2 = 2.3$, degrees of freedom = 3, $0.5 < P < 0.75$). Karyotypic frequencies in the sexes were also compared, and, as might be expected, the difference between male and female arrays was not statistically significant ($\chi^2 = 6.1$, degrees of freedom = 5, $0.25 < P < 0.5$). These data provide no evidence for sex differences in karyotypic viabilities.

Chromosomal frequencies in males and in larvae are given in Table 2. The three populations differed appreciably in the frequencies of gene arrangements and in the extent of chromosomal polymorphism. Amecameca contained predominantly Treeline and Cuernavaca, with Santa Cruz moderately frequent in the June sample. The common gene arrangements at Tulancingo were also Cuernavaca and Treeline, but in April there were moderate levels of Santa Cruz, Estes Park, and Hidalgo. Zirahuen is the most chromosomally polymorphic population of *D. pseudoobscura* ever studied; Treeline, Cuernavaca, and Santa Cruz were frequent, while Oaxaca, Estes Park, and Olympic were moderately well represented.

The differences in gene arrangement frequency between males and larvae are labeled Δp in Table 2, and these values are the basis of our test for selection. Under the null hypothesis that there was no selection, the gene arrangement frequencies in males and larvae should be the same, and Δp should be zero. To test this hypothesis in any one sample, we added the frequencies in males and larvae to obtain an average frequency

Table 1. Percent frequencies of gene arrangements in adult males and females collected at Amecameca, Mexico, in January 1977

	TL	CU	EP	Other	*n*
Females	26.3	65.9	6.3	1.5	650
Males	30.5	60.9	7.0	1.6	312
Difference	−4.2	5.0	−0.7	−0.1	

n is the number of chromosomes examined. The common gene arrangements are Treeline (TL), Cuernavaca (CU), and Estes Park (EP); Other includes rare endemics.

Table 2. Percent frequencies of gene arrangements in adult males (p_M) and in larvae (p_L) produced by females collected simultaneously with these males

		TL	CU	SC	OA	EP	OL	HI	Other	*n*
	p_M	35.4	55.7	5.1	0.0	2.5	0.6	0.6	0.0	71
Amecameca	p_L	35.2	57.7	2.8	0.0	4.2	0.0	0.0	0.0	158
June 1976	Δp	−0.2	2.0	−2.3	0.0	1.7	−0.6	−0.6	0.0	
	p_M	63.6	36.4	0.0	0.0	0.0	0.0	0.0	0.0	66
Amecameca	p_L	30.8	66.7	0.5	0.0	0.5	0.0	1.0	0.5	204
Sept. 1976	Δp	−32.8**	30.3*	0.5	0.0	0.5	0.0	1.0	0.5	
	p_M	10.8	30.1	43.0	6.5	1.1	7.5	0.0	1.1	93
Zirahuen	p_L	23.8	22.4	40.2	7.5	3.3	0.9	0.5	1.4	214
Jan. 1976	Δp	13.0**	−7.7	−2.8	1.0	2.2	−6.6**	0.5	0.3	
	p_M	23.3	26.7	40.0	2.2	3.3	1.1	2.2	1.1	90
Zirahuen	p_L	25.4	19.8	36.7	7.6	4.2	5.4	0.3	0.6	354
April 1976	Δp	2.1	−6.9	−3.3	5.4	0.9	4.3	−1.9	−0.5	
	p_M	52.4	30.2	4.8	1.6	4.8	1.6	4.8	0.0	63
Tulancingo	p_L	50.8	38.5	6.2	0.8	2.3	0.0	1.5	0.0	130
May 1976	Δp	−1.6	8.3	1.4	−0.8	−2.5	−1.6	−3.3	0.0	
	p_M	34.2	60.8	0.0	0.0	2.5	1.3	0.0	1.3	79
Tulancingo	p_L	47.2	47.3	0.0	0.0	3.2	1.4	0.9	0.0	218
Aug. 1976	Δp	13.0*	−13.5*	0.0	0.0	0.7	0.1	0.9	−1.3	

Data are given for three localities from the central highlands of Mexico. Differences in gene arrangement frequency between larvae and males ($p_L - p_M$) are listed as Δp and their statistical significance at 0.05 (*) and 0.01 (**) levels are indicated. *n* is the number of chromosomes examined. Gene arrangements not already named in Table 1 are Santa Cruz (SC), Oaxaca (OA), Olympic (OL), and Hidalgo (HI).

$\bar{p}$. Under our null hypothesis the variance of Δp is $\bar{p}(1-\bar{p})(1/n_M + 1/n_L)$, in which n_M and n_L are the number of chromosomes identified in males and larvae; $\Delta p / \sqrt{\text{var } \Delta p}$ will be distributed as a normal deviate. This test is equivalent to a 2 × 2 contingency χ^2 on the gene arrangement frequencies in males and larvae. Changes in gene arrangement frequency significant at the 0.05 level or better are indicated in Table 2. Since frequency changes under selection are directly proportional to the chromosomal frequency before selection, we expect progressively smaller changes for less and less frequent chromosomes. We are reasonably assured of detecting real changes only for the common inversions. There was a statistically significant change in gene arrangement frequency in one of the two samples from each of our three collecting stations. Of the 14 possible comparisons of common gene arrangements, 5 were statistically significant. These demonstrable differences in chromosome frequency between males and their offspring are convincing evidence for differences among male karyotypes in mating success.

It is one of the distressing "facts of life" for population geneticists that the effect of natural selection on gene frequencies is hard to demonstrate. Most experiments are structured, by virtue of the mechanics of Mendelian genetics, so that large—even huge—sample sizes are required to be reasonably assured of detecting selection of weak or moderate intensity. Our experiments are no exception, and there is a high probability that we have failed to detect selection of small to moderate intensity. It is all the more remarkable, then, that we have found as many as one-third of our comparisons involving common gene arrangements to be statistically significant.

We have also compared the complete arrays of frequencies in males with those in larvae by means of χ^2 contingency tests. Many of the rare gene arrangements had to be grouped to assure validity of the statistical test. This comparison was statistically highly significant in the collections at Amecameca in September ($P < 0.001$) and at Zirahuen in January ($P = 0.003$). It was not significant at the conventional 5% level in the collection at Tulcancingo in August ($P = 0.11$). It is, of course, possible for one or two frequencies to differ significantly, but for the whole arrays not to do so. The differences between gene arrangement frequencies in males and larvae from wild females are not as strongly established for the August collection at Tulancingo as for Amecameca in September or Zirahuen in January. We feel the tests indicate biologically meaningful differences at Tulancingo, but we note that our evidence for selection does not hinge upon this locality. Two of six population samples showed significant differences between total arrays of chromosomal morphs in males and the larvae of wild females, and these differences are in themselves compelling evidence of selection.

DISCUSSION

The selection that has operated to produce the Δps in Table 2 is more powerful than a first glance at the data may indicate. Let p_M and p_F be the frequencies of some gene arrangement in the sperm and eggs that participate in fertilization. The frequencies in the resulting zygotes, which we have measured in the larvae produced by wild females, will be $p_L = (p_M + p_F)/2$. The quantity we have calculated as Δp is $(p_L - p_M)$, which on substituting for p_L becomes $\Delta p = (p_F - p_M)/2$. Any Δp in Table 2, then, is only *half* the difference in frequency between male and female gametes. Male and female gametes must have differed by more than 60% for the Treeline and Cuernavaca chromosomes at Amecameca in September, by 25% for Cuernavaca at Zirahuen in January, and by 25% for both Treeline and Cuernavaca at Tulancingo in August.

Male mating success seems to be the only component of fitness that may play a major role in bringing about such large frequency differences between eggs and sperm. As a kind of selection, it has several features that deserve mention. First, unlike some other components of fitness such as viability, mating success is expected to operate unequally on the two

sexes. For males the frequency of mating determines the genes and chromosomes contributed to the next generation. The fecundity of females, on the other hand, is not dominated by success in mating but by other physiological factors associated with egg production.

Second, male mating success imposes little if any load on a population. Differences among genotypes in viability or in female fecundity can lower a population's fitness in terms of growth rate or other ecologically relevant measures, and the size of this genetic load is a problem for any explanation of our data in terms of viability selection. Under some circumstances such a load could be disadvantageous. So long as there are enough sperm to fertilize all the eggs a female can lay, which seems to be the case for *D. pseudoobscura*, selection by male mating success does not lower the reproductive potential of the population. It does not change the relative frequencies of genotypes within any one generation, but rather involves only a change of gene frequencies within the pool of sperm.

Our data do not permit us to estimate the genotypic fitnesses in the three Mexican populations, but the Δps are large enough that genotypic differences in male mating success must have been large. This form of selection is known to occur for *D. pseudoobscura* karyotypes in experimental populations (15–18), and it is sometimes quite powerful. Male mating success in the laboratory is often—even *usually*—dependent on genotypic frequency, with mating success increasing as frequency declines. Our data offer little insight into this matter of rare male mating advantage. The Δps for rare gene arrangements are expected to be small, even with frequency-dependent selection favoring them, and sampling errors would tend to obscure any selective changes.

We are not the first to obtain data like that in Table 1. Chromosome frequencies in adult males have long been used to supplement those in larvae produced by wild females, particularly when collections were poor. Unfortunately, male and larval frequencies were usually added together and presented as a single generation's sample. The only exception we have found in the older literature is the study of Epling *et al.* (3), which gives male and zygotic frequencies for repeated collections at two sites on Mt. San Jacinto in California. The dominant inversions were Standard, Arrowhead, and Chiricahua. In none of seven samples was there evidence of the frequency differences we found in Central Mexico. As our study neared completion, Crumpacker *et al.* (20) reported comparisons between males and zygotes from a locality in Colorado where Arrowhead and Pikes Peak were the common gene arrangements. In one of two collections they found a significant difference, comparable in magnitude to the differences we observed at Zirahuen and Tulancingo. They have clearly recognized the selective basis of such differences and ascribed them to the fertility component of selection, which includes mating ability. Their study shows that the phenomenon we have observed and related to selection by male mating success is a general one, not confined to Mexican populations by virtue of local peculiarities in population structure.

Gene arrangement frequencies undergo large fluctuations in the three populations we have studied. Within any locality the frequencies change by as much as 20–30% over a year. Some aspects of these temporal frequency changes appear to be regular, although the frequencies do not cycle smoothly like those at Mt. San Jacinto in California (2). The continuing flux of frequencies is clearly too great to be the result of sampling drift, because the number of flies that can be trapped in a small area is in the thousands for much of the year. The significant Δps in Table 2 usually indicate the direction in which the population's gene arrangement frequencies are moving. This concordance may be misleading, however. Our data are taken at 3-month intervals, and the flux of frequencies is great enough that changes in one generation may not be reliable indicators of frequencies several generations in the future.

The inversion polymorphism in the *D. pseudoobscura* populations we studied, and in many others as well, is "flexible" (31), responding to physical and biological components of the environment by changes in chromosomal frequency. Such fluctuations in frequency are often large but seldom lead to loss of gene arrangements; in fact, populations in the western United States show a remarkable persistence of rare or moderately rare gene arrangements (30, 32). Some form of selection must operate to maintain the diversity of gene arrangements in the face of this broad flux of frequencies. But the frequencies do not appear to be directed towards any single set of equilibrium frequencies. Rather, the gene arrangement polymorphism seems to resemble what Prout (33) has termed a "protected polymorphism," one in which selection acts so that alleles (or in this case, gene arrangements) are not lost, but in which the dynamics in the interior space of frequencies may take various forms, including even continual movement. We feel that male mating success is an important element among the selection components that act to retain this "fluctuating polymorphism" of gene arrangements in the Mexican populations we have recently studied and in populations elsewhere as well. Certainly our evidence that adult males and zygotes of the next generation sometimes differ in gene arrangement frequency, and sometimes do not, is consistent with this view. And it is likely that this selection is not constant, but changes continually, both in direction and intensity, in response to a changing environment and to the changing genetic constitution of each population.

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1. Guzman, J., Levine, L., Olvera, O., Powell, J. R., de la Rosa, M. E., Salceda, V. M., Dobzhansky, Th. & Felix, R. (1975) *Ann. Inst. Biol. Natl. Autónom. Univ. Mexico* **46**, 75–86.
2. Dobzhansky, Th. (1943) *Genetics* **28**, 162–186.
3. Epling, C., Mitchell, D. F. & Mattoni, R. H. T. (1953) *Evolution* **7**, 342–365.
4. Epling, C., Mitchell, D. F. & Mattoni, R. H. T. (1957) *Evolution* **11**, 225–247.
5. Dobzhansky, Th. (1947) *Heredity* **1**, 53–64.
6. Dobzhansky, Th. & Levene, H. (1948) *Genetics* **33**, 537–547.
7. Prout, T. (1965) *Evolution* **19**, 546–551.
8. Prout, T. (1969) *Genetics* **63**, 949–967.
9. Prout, T. (1971) *Genetics* **68**, 127–149.
10. Prout, T. (1971) *Genetics* **68**, 151–167.
11. Bundgaard, J. & Christiansen, F. B. (1972) *Genetics* **71**, 439–460.
12. Christiansen, F. B., Frydenberg, O. & Simonsen, V. (1973) *Hereditas* **73**, 291–304.
13. Polivanov, S. & Anderson, W. W. (1969) *Genetics* **63**, 919–932.
14. Clegg, M. T., Kahler, A. L. & Allard, R. W. (1978) *Genetics* **89**, 765–792.
15. Anderson, W. W. & Watanabe, T. K. (1974) *Genetics* **77**, 559–564.
16. Ehrman, L. & Parsons, P. A. (1976) *The Genetics of Behavior* (Sinauer, Sunderland, MA).

17. Spiess, E. B. (1970) in *Essays in Evolution and Genetics*, eds. Hecht, M. K. & Steere, W. C. (Appleton-Century Crofts, New York), pp. 315–379.
18. Anderson, W. W. & McGuire, P. R. (1978) *Evolution* **32**, 416–423.
19. Dobzhansky, Th. (1947) *Genetics* **32**, 142–160.
20. Crumpacker, D. W., Pyati, J. & Ehrman, L. (1977) in *Evolutionary Biology*, eds. Hecht, M. K., Steere, W. C. & Wallace, B. (Plenum, New York), Vol. 10, pp. 437–469.
21. Anderson, W. W. (1974) *Am. Nat.* **108**, 709–711.
22. Milkman, R. & Zeitler, R. R. (1974) *Genetics* **78**, 1191–1193.
23. Cobbs, G. (1977) *Am. Nat.* **111**, 641–656.
24. Stalker, H. D. (1976) *Genetics* **84**, 375–384.
25. Levine, L., Asmussen, M., Olvera, O., Powell, J. R., de la Rosa, M. E., Salceda, V. M., Gaso, M. I., Guzman, J. & Anderson, W. (1979) *Am. Nat.*, in press.
26. Lefevre, G. & Johnson, U. B. (1962) *Genetics* **47**, 1719–1736.
27. Dobzhansky, Th. (1944) in *Contributions to the Genetics, Taxonomy, and Ecology of Drosophila pseudoobscura and Its Relatives*, eds. Dobzhansky, Th. & Epling, C. (Carnegie Institution of Washington, Washington, DC), Publ. 554, pp. 47–144.
28. Kastritsis, C. D. & Crumpacker, D. W. (1966) *J. Hered.* **57**, 150–158.
29. Kastritsis, C. D. & Crumpacker, D. W. (1967) *J. Hered.* **58**, 112–129.
30. Olvera, O., Powell, J. R., de la Rosa, M. E., Salceda, V. M., Gaso, M. I., Guzman, J., Anderson, W. W. & Levine, L. (1979) *Evolution*, in press.
31. Dobzhansky, Th. (1962) *Am. Nat.* **96**, 321–328.
32. Anderson, W. W., Dobzhansky, Th., Pavlovsky, O., Powell, J. & Yardley, D. (1975) *Evolution* **29**, 24–36.
33. Prout, T. (1968) *Am. Nat.* **102**, 493–496.