Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9870–9874, December 1988
Population Biology

Rare male mating advantage in a natural population of *Drosophila pseudoobscura*

(components of selection/sexual selection/evolution/population genetics)

VICTOR M. SALCEDA*† AND WYATT W. ANDERSON*‡

*Department of Genetics, University of Georgia, Athens, GA 30602; and †Vicente Garcia Torres Number 130, Coyoacan 04030, Mexico D. F., Mexico

Contributed by Wyatt W. Anderson, September 16, 1988

ABSTRACT **The natural selection acting on chromosomal inversions was studied in a natural population of *Drosophila pseudoobscura*. Females from this population were allowed to produce offspring from their matings in nature. They were then remated to males from a laboratory strain and again allowed to produce offspring. Offspring were also produced from matings of males from nature to laboratory females. Diagnosis of salivary chromosomes in these several sets of larval offspring allowed us to deduce the karyotypes of adult females and males from nature as well as the karyotypes of the offspring of these females by their matings in nature. We reason that the males collected with the females are a reasonable sample of those that mated the females and deposited the sperm they carried on capture. Chromosome frequencies in the offspring of wild females by their matings in nature were decomposed into male and female parental contributions. Changes in chromosome frequency due to male mating success were calculated by comparing chromosomal frequencies in adult males with those in the chromosomes they contributed to their offspring. These changes were sizable and provide direct evidence that male sexual selection is an important component of selection on the inversions in this natural population. We proceeded further to classify karyotypes on the basis of their frequencies and to calculate the fraction of offspring fathered by rare or common males. Rare male karyotypes as a group had a selective value nearly twice that of the common male karyotypes.**

The analysis of selection has proceeded much further in the laboratory than in nature, and it has proven difficult to test in nature for some kinds of selection that can be demonstrated rather easily in the laboratory. The rare male mating advantage of *Drosophila* species is a case in point. Since the 1950s, repeated experiments in laboratory populations and in specially designed mating chambers have shown that male genotypes mate more frequently when they are rare than when they are common (1–4). These experimental studies have involved a sizable array of genotypes in a number of *Drosophila* species as well as several other insect species and possibly a few vertebrate species (5–9). Only for the milkweed beetle *Tetraopes tetraophthalmus* (10) and the ladybird beetle *Adalia bipunctata* (11) is there evidence for a rare male mating advantage in nature. The mechanism of this mating advantage is behavioral, including such aspects of the mating process as female preferences; recognition of male types by olfactory, auditory, and tactile cues; and vigor of male and female types (5–9).

Rare male mating advantage is a kind of sexual selection, which, in turn, is a component of fitness known to play an important role in selection on *Drosophila* genotypes (12–15). In *Drosophila pseudoobscura*, male sexual selection accounts for a large part of the overall selection that acts on the supergene inversions of the third chromosome (14, 15). Rare male advantage has been studied as intensively as any other specific component of selection in *Drosophila*, and a large fraction of this work has utilized *D. pseudoobscura*. All of these studies have been laboratory experiments, however, and the situation with regard to natural populations is the same now as it was in 1970 when Dobzhansky (ref. 16, page 174) wrote:

> Nothing is known about possible mating advantages of rare genotypes in natural environments. If they exist in the natural habitats of the flies, the resulting frequency-dependent selection may be a potent instrumentality for maintaining the polymorphic equilibria of gene alleles without heterosis. Even mildly deleterious alleles could be maintained in natural population by these means. Rare alleles will grow in frequencies until the mating advantages of their carriers decrease and disappear. More research in this field is evidently needed.

Dobzhansky's statement aptly summarizes the potential importance of rare male mating advantage. It is of particular interest to us as one aspect of the component of overall selection acting through differences among male genotypes in mating success.

The purpose of this paper is to provide evidence of male sexual selection in a Mexican population of *D. pseudoobscura* and, more specifically, to document the occurrence of rare male mating advantage in this natural *Drosophila* population.

MATERIALS AND METHODS

We used inversions of the *D. pseudoobscura* third chromosome as our genetic system. Recombination in the third chromosome is greatly reduced in heterozygotes for these inversions. Almost the entire chromosome, some 20% of the genome, is tied together as a supergene, with each inverted gene arrangement a superallele. The frequencies of the gene arrangements vary with environmental gradients, with seasons, and with altitude (17). At least some of these changes can be reproduced in experimental populations in which ecological factors such as temperature, population density, and nutrition are manipulated (18, 19). Powerful natural selection operates on these inversions, in nature and in the laboratory, making this genetic system particularly well suited for the analysis of selection.

We collected *D. pseudoobscura* late in January 1977 at Amecameca, Mexico. This site is a nursery for pine trees located on the southeastern edge of the Valley of Mexico. *Drosophila* were abundant, with *D. pseudoobscura* the dominant species, and we collected all of the flies for our

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ST, Standard; CU, Cuernavaca; TL, Treeline; and EP, Estes Park; all inverted gene arrangements of the third chromosome of *D. pseudoobscura*.

‡To whom reprint requests should be addressed.

study in a single evening. Pails of fermenting bananas were set out and a group of 10 workers swept flies from them every 10 min. Flies were taken to a microscope in the field as soon as they were collected, and females were separated from males. We believe this procedure eliminated matings among the flies after capture, so that the only sperm stored in females would have been deposited during matings in nature.

The collections were taken to the laboratory and each fly was placed in an individual vial at 15°C. To each vial containing a male from nature were added several virgin females homozygous for the Standard (ST) gene arrangement, which is not found in central Mexico. The wild females were allowed to lay eggs from their matings in nature for 2 weeks, during which time they were transferred to fresh vials every few days. We dissected salivary glands from at least five larvae from each male culture and from at least eight larvae from each female culture. We stained them in aceto-lactic-orein and made squash preparations of the salivary chromosomes. We continued to transfer cultures of wild females every few days, and after 2 weeks several males of the ST/ST karyotype were added to each culture to give the female an opportunity to remate with the laboratory stock. A few days later, we prepared additional slides of salivary chromosomes from the cultures of wild females, again dissecting at least eight larvae from each female culture. Each of us diagnosed the entire set of salivary chromosomes, using the established descriptions (20–23) for reference.

RESULTS AND DISCUSSION

Ninety-six percent of the females collected at Amecameca were inseminated and produced offspring; 99% of these inseminated females remated with the ST/ST males.

We were able to deduce the karyotypes of the males from nature by identifying the salivary chromosomes in at least five larvae from the offspring of each wild male by the ST/ST females. In a similar manner, we deduced the karyotype of each wild female from the chromosomes in at least five larval offspring she produced after remating with the ST/ST males. Since the ST gene arrangement is unknown in central Mexico, we could be certain that remating occurred when we saw ST in heterozygous combination with one of the gene arrangements known from Mexico.

Karyotypic frequencies in the males and females collected in nature, and in eight offspring produced by each female from her matings in nature, are presented in Table 1. We compared karyotypic frequencies in the males and females, since a significant difference between them would suggest that viabilities differed in the sexes. The sexes did not differ significantly in karyotypic frequencies. The χ^2 for homogeneity was 5.0 with 4 degrees of freedom, and the associated probability was >0.1.

Table 2 shows how we can calculate the frequencies of chromosomes contributed by males to their offspring and, following that, the change in chromosome frequency due to male mating success. The frequencies of chromosomes in the offspring of females who resulted from matings in nature are given in line 1. In rows 2 and 3 they are decomposed into male and female parental contribution of 50% each. The frequencies of chromosomes contributed by the female parents to the offspring are the same as the frequencies in the female parents, since the same number of larvae was studied from each female. The contribution of males is obtained by subtracting the maternal contribution from the offspring frequencies. These may in turn be compared with the frequencies in adult males. The turnover of sperm stored in females is rapid in *D. pseudoobscura*, because multiple matings are frequent (24) and fecundity is high. Thus, the males collected at any time in nature should be a representative sample of the males that deposited the sperm.

Table 1. Karyotypic frequencies (as %) in *D. pseudoobscura* adults from nature and in their offspring and *n*, the number of karyotypes observed

	CU/CU	CU/TL	TL/TL	CU/OT	TL/OT	OT/OT	*n*
Male	34.62	42.31	8.33	10.26	1.92	2.56	156
Female	44.26	33.11	7.54	11.15	3.28	0.66	305
Offspring	35.78	37.13	7.58	10.21	7.58	1.72	2440

Gene arrangements are Cuernavaca (CU), Treeline (TL), and Others (OT), including Estes Park (EP), Popocateptl, Ozumba, Santa Cruz, Olympic, Hidalgo, and Chiricahua. Offspring frequencies are based on eight larvae from each of the 305 females.

Line 6 in Table 2 shows the differences in frequency between chromosomes in adult males and chromosomes these males have contributed to the next generation. These differences are the changes in male chromosome frequency that we ascribe to male mating success. They should be caused mostly by differences in frequencies with which the male karyotypes mate, although any meiotic drive (25) or sperm precedence (26) would also be included. Although there is no evidence of meiotic drive acting on these inversions, differences in sperm predominance have been demonstrated in the laboratory (27) and may play a role in male sexual selection in nature.

The CU chromosome decreased in frequency, whereas the other gene arrangements increased in frequency. The variance of Δp may be calculated as a function of the variances of its component frequencies and may be used to test the hypothesis that the Δp for CU is statistically significantly different from zero. The test statistic is a standardized normal deviate, $Z = 2.35$, and the probability that Δp would be at least as large as it was by chance alone is only 0.02. CU and TL constitute about 90% of the chromosomes in the population, and EP and "Others" may fairly be combined as rare chromosomes. The increase in frequency of these rare chromosomes, between adult males and their contribution to offspring, is statistically significantly different from zero ($Z = 2.27$, $P = 0.02$) and provides the first clue that rare males may have a mating advantage. It is clear that differences in male mating success operate as a component of selection in the Amecameca population. Chromosome frequencies in this population undergo cycles, and selection by male mating success is strong enough to account for a major part of the changes in chromosome frequency. The selective changes in chromosome frequency we report here are based on direct comparisons of frequencies in adult males and the chromosomes they contributed to their offspring. They confirm our earlier evidence (15) for selection by male mating success, based on comparisons between adult males only and their offspring, in which the contributions from female parents could not be assessed.

As seen in row 7 of Table 2, the greatest proportional changes in gene arrangement frequency are for the Others, a group of six inversions whose individual frequencies were each <1%, and for EP, whose frequency was ≈8%. The genotypes that carry these "rare" chromosomes will also be rare by comparison with CU/CU and CU/TL and, usually, by comparison with the homokaryotype TL/TL as well. Since we have reasoned that the changes in chromosome frequency in row 6 of Table 2 are the result of differences in male mating success, we are led to examine the mating advantage of the rare male genotypes.

Suppose we could calculate the contribution of each male karyotype to the offspring that form the next generation. Since we already know the frequencies of the karyotypes among adult males, it seems that we should be able to estimate the component of fitness due to male mating success

Table 2. Gene arrangement frequencies during the breeding cycle of *D. pseudoobscura* from a natural population in Mexico: estimation of frequencies (as %) in sperm stored in females and Δp_m, the change in frequency among males due to differential male mating success

	CU	TL	EP	Others	Total %	*n*
1. Frequencies in eight offspring from each of 305 females	59.45	29.94	8.30	2.32	100	4880
2. Contribution of female parents to offspring	33.20	12.87	3.20	0.74	50	610
3. Contribution of male parents to offspring = (row 1 − row 2)	26.25	17.07	5.10	1.58	50	
4. Male contribution scaled to 100% = frequencies in sperm stored in females (2 × row 3)	52.50	34.14	10.20	3.16	100	
5. Frequencies in 156 adult males from nature	60.90	30.45	7.05	1.60	100	312
6. Δp_m due to differences in male mating success = (row 4 − row 5)	−8.40	3.69	3.15	1.56	0	
7. % change in frequency = 100 × (row 6/row 4)	−16.0	10.8	30.9	49.4		

All female parents contributed equally (eight larvae) to the offspring that were studied, so gene arrangement frequencies in row 2 are the same as those in the adult females of Table 1. Abbreviations for gene arrangements as in Table 1. *n*, Number of chromosomes on which frequencies are based.

for each karyotype. Unfortunately we cannot make these estimates because multiple insemination is frequent in this population. The sperm that fertilize eggs in a single female often will come from two different males, and we usually cannot diagnose the male genotypes. We estimate the frequency of such mixed broods in the Amecameca population to be nearly 70%. It is possible, however, to divide the karyotypes into rare and common classes and to use the male contributions of gene arrangements (line 4 of Table 2) to estimate the mating success of males in the rare and common groups.

In the adult males that we studied, CU and TL constitute about 91% of all chromosomes. Next in frequency is EP, at only 7%, followed by a group of six other gene arrangements, each at frequencies much smaller than 1.0%. The karyotypes divide naturally into those involving only CU and TL (the common group), and others (the rare group). The common group includes CU/CU (34.6%), CU/TL (42.3%), and TL/TL (8.3%), for a total frequency of 85.2%. All other karyotypes are grouped as rare, including CU/EP (8.3%), TL/EP (1.3%), and six others at individual frequencies of <1.0%. The total frequency of these rare karyotypes is 14.8%.

EP is the only rare gene arrangement occurring frequently enough to cause concern about contributions from homokaryotypic males. EP/EP is the only homokaryotype for a rare chromosome found among the 156 males and 305 females collected in nature. Since karyotypic frequencies in the two sexes did not differ significantly, we can use the pooled adult data to estimate the fractions of EP chromosomes occurring in homokaryotypes and in heterokaryotypes. A total of 61 EP chromosomes were observed in the 461 adults from nature; 8 occurred in homokaryotypes and 53 occurred in heterokaryotypes. Thus we apportion the male contributions of EP chromosomes as follows: 8/61 = 0.1311 from homokaryotypes for EP; and 53/61 = 0.8689 from heterokaryotypes for EP.

In Table 2, line 4, we have already calculated that the frequency of EP in the sperm stored within females at capture (the male contribution to offspring) was 0.1020. Hence the male parental contribution of EP gene arrangements from EP/EP males is estimated to be 0.1020 × 0.1311 = 0.0134, and the contribution from heterozygotes for EP is estimated to be 0.1020 × 0.8689 = 0.0886. All rare chromosomes other than EP occurred only as heterokaryotypes with another chromosome, and the frequency of these other rare chromosomes in the paternal contribution was 0.0316. We add to this 0.0316 the 0.0886 contribution from heterozygotes for EP, to get a total estimate of 0.1202 for the frequency of rare chromosomes contributed by rare male heterozygotes.

Mendel's law of segregation tells us that males heterozygous for a rare chromosome will, on the average, contribute a common chromosome for each rare one they contribute. Thus, we double the frequency of rare chromosomes contributed by rare heterozygotes to account for the common chromosomes (CU and TL) they also contributed: 0.1202 × 2 = 0.2404, which is the estimated total frequency of chromosomes contributed by rare male heterozygotes. The total contribution of chromosomes by rare males is the sum of contributions by rare heterozygotes and by the EP/EP homokaryotype: 0.2404 + 0.0134 = 0.2538, the estimated total frequency of chromosomes contributed by rare male karyotypes. Since each chromosome contributed by a male equals one offspring fathered, the fraction of offspring fathered by rare male karyotypes is estimated to be 0.2538. Because we studied an equal number of offspring from each female, there was no opportunity for female fecundity to influence this calculation.

We want to formalize this reasoning to test statistically for rare male mating advantage. Let a denote the frequency of rare karyotypes in adult males and A denote the fraction of offspring that they fathered. The value of a, calculated from Table 1, is 0.1474. A comparison of a and A provides a direct test for rare male mating advantage. A is a function of the frequencies of rare gene arrangements in the male contributions of line 4, Table 2. These male contributions, in turn, are functions of the rare gene arrangement frequencies in the female parents and their offspring given in Table 2. To write a formula for calculating A, we define the following terms: $\hat{v}$ = estimated frequency of EP chromosomes contributed by males; $\hat{u}$ = estimated frequency of other rare gene arrangements contributed by males; $\hat{w}$ = estimated proportion of EP chromosomes contributed by EP/EP males; and $(1 - \hat{w})$ = estimated proportion of EP chromosomes contributed by males heterozygotes for EP. By our reasoning above, A may be estimated as $\hat{A} = 2\hat{u} + 2\hat{v}\hat{w} + \hat{v}(1 - \hat{w})$. The variance of $\hat{A}$ can be found by the delta method, or method of statistical differentials (28). For our data $\hat{A} = 0.2538$ and its variance is ≈ 0.0012. We test for rare male mating advantage by forming the statistic $Z = (\hat{A} - \hat{a})/(\text{var}\,\hat{a} + \text{var}\,\hat{A})^{1/2}$, which should be normally distributed under the null hypothesis that $\hat{A} = \hat{a}$.

Our data give $Z = 2.36$, with an associated probability of 0.025. The fraction of offspring fathered by rare male karyotypes is statistically significantly higher than the frequency of rare karyotypes among the adult males collected in nature. A model for estimating the mating success of the rare male karyotypes relative to that of the common karyotypes is outlined in Table 3. Applying the model to our data, we estimate that the relative mating success of the rare male karyotypes to be 1.97, nearly twice that of the common karyotypes.

In this population, then, rare male karyotypes showed a large advantage in mating success over the common karyotypes. The effect of this one component of selection will be to increase the frequencies of rare gene arrangements. Other selection components will come into play as well, of course, and there could be counteracting selection by viability or fecundity or any other component. Rare gene arrangements would not be rare if their frequencies continued to increase under selection, and our data from the Amecameca population show continually low frequencies of the rare chromosomes in samples taken every few months over 3 years. Thus, it seems unlikely that the rare gene arrangements at Amecameca are favored by an overall selective advantage of the flies carrying them. It seems more likely that the increased mating success of the rare karyotypes favors the retention of rare gene arrangements in the face of opposing forces such as genetic drift. If so, and if this rare male mating advantage occurs in other populations, then it may indeed be the "potent instrumentality" for maintaining genetic variability in the quote by Dobzhansky (16). Again and again, very rare chromosomal variants, found in only one or two flies, have been recovered in samples of *D. pseudoobscura* from the same population 25–100 generations later (23, 29). It would not be surprising if the rare male mating advantage were a major factor in retention of these rare chromosomal gene arrangements.

The rare male mating advantage that has been studied in the laboratory is frequency-dependent; its intensity increases as male genotypic frequency decreases. Our measurements in the Amecameca population involve only one set of frequencies, and they do not allow us to determine whether the selection we have found is frequency-dependent. We suspect that it is, by analogy with the laboratory studies. Proof would require measurements of mating success in the same population at different karyotypic frequencies, and data of this kind will take some time to obtain (see ref. 10).

Table 3. Calculation of rare male mating advantage in nature

	Rare karyotype	Common karyotype
The model		
Initial male frequencies	a	b
Relative mating success	M	1
Expected frequency of offspring fathered	$aM/(aM + b)$	$b/(aM + b)$
Observed frequency of offspring fathered	A	B
Our data for *D. pseudoobscura*		
% frequencies in adult males	14.74	85.26
% of offspring fathered	25.38	74.62
Relative mating success	1.97	1

CU/CU, CU/TL, and TL/TL were grouped as common karyotypes. The remaining 20 karyotypes were grouped as rare karyotypes, having individual frequencies among adult males of <2% except for CU/EP, which had a frequency of 8.3%. Frequencies of rare and common karyotypes were estimated in a sample of 156 males from nature, whereas the fractions of offspring contributed by rare and common males were assessed in eight larvae from each of 305 females inseminated in nature. The frequency of rare karyotypes in adult males was statistically significantly different from the frequency of offspring that they fathered. The relative mating success of rare males is estimated according to the model as $\hat{M} = \hat{b}\hat{A}/(\hat{a}\hat{B})$.

What our results do show, however, is that changes in gene arrangement frequency due to male mating success are large enough at Amecameca that this component of selection must play a major role in the overall selection on the inversions in this natural population. Moreover, rare male karyotypes as a group enjoy a substantial advantage in mating success over the three most common karyotypes. These results are a step toward our goal of providing a full analysis of the selection acting on the gene arrangements of *D. pseudoobscura*, in the laboratory and in nature.

We are grateful to Dr. Margaret Anderson for guidance and assistance with the statistical analysis and to Drs. Jonathan Arnold, Francisco Ayala, Stuart Barker, Lee Ehrman, Claudine Petit, Timothy Prout, Eliot Spiess, and Michael Turelli for critically reviewing the manuscript. We owe a special debt of gratitude to Francisco Ayala for the hospitality of his laboratory during the time much of this paper was written. We are grateful to our colleagues in this project for assisting in the collection. This work was supported by the National Science Foundation under Grant DEB-7918493. The field collection was made under the auspices of a binational cooperative research program supported by U.S. National Science Foundation Grant 0IP75-06738 and by El Consejo Nacional de Ciencia y Tecnología de Mexico, Contract 651.

1. Petit, C. (1958) *Bull. Biol. France Belg.* **92,** 248–329.
2. Ehrman, L. (1966) *Anim. Behav.* **14,** 332–339.
3. Spiess, E. B. (1968) *Am. Nat.* **102,** 363–379.
4. Anderson, W. W. & Brown, C. J. (1984) *Genetics* **107,** 577–589.
5. Petit, C. & Ehrman, L. (1969) in *Evolutionary Biology*, eds. Dobzhansky, Th., Hecht, M. K. & Steere, W. C. (Appleton–Century–Crofts, New York), Vol. 3, pp. 177–223.
6. Ehrman, L. & Parsons, P. A. (1981) *Behavior Genetics and Evolution* (McGraw-Hill, New York).
7. Spiess, E. B. (1987) in *Kin Recognition in Animals*, eds. Fletcher, D. J. C. & Michener, C. D. (Wiley, New York), pp. 75–119.
8. Knoppien, P. (1985) *Biol. Rev.* **60,** 81–117.
9. Partridge, L. & Hill, W. G. (1984) *Biol. J. Linnean Soc.* **23,** 113–132.
10. Eanes, W. F., Gaffney, P. M., Koehn, R. K. & Simon, C. M. (1977) in *Measuring Selection in Natural Populations*, eds. Christiansen, F. B. & Fenchel, T. M. (Springer, Berlin), pp. 49–64.
11. Muggleton, J. (1979) *Heredity* **42,** 57–65.
12. Prout, T. (1971) *Genetics* **68,** 127–149.
13. Prout, T. (1971) *Genetics* **68,** 151–167.
14. Anderson, W. W. & Watanabe, T. K. (1974) *Genetics* **77,** 559–564.
15. Anderson, W. W., Levine, L., Olvera, O., Powell, J. R., de la Rosa, M. E., Salceda, V. M., Gaso, M. I. & Guzman, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1519–1523.
16. Dobzhansky, Th. (1970) *Genetics of the Evolutionary Process* (Columbia Univ. Press, New York).
17. Dobzhansky, Th. (1947) *Evolution* **1,** 1–16.
18. Wright, S. & Dobzhansky, Th. (1946) *Genetics* **31,** 125–156.
19. Dobzhansky, Th. (1971) in *Ecological Genetics and Evolution*, ed. Creed, R. (Blackwell, Oxford), pp. 109–133.
20. 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.
21. Kastritsis, C. D. & Crumpacker, D. W. (1966) *J. Hered.* **57,** 150–158.
22. Kastritsis, C. D. & Crumpacker, D. W. (1967) *J. Hered.* **58,** 112–129.
23. 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* **33,** 381–395.
24. Levine, L., Asmussen, M., Olvera, O., Powell, J. R., de la Rosa, M. E., Salceda, V. M., Gaso, M. I., Guzman, J. & Anderson, W. W. (1980) *Am. Nat.* **116,** 493–503.

25. Sandler, L. & Novitski, E. (1957) *Am. Nat.* **91,** 105–110.
26. Prout, T. & Bundgaard, J. (1977) *Genetics* **85,** 95–124.
27. Turner, M. E. & Anderson, W. W. (1984) *Evolution* **38,** 983–995.
28. Elandt-Johnson, R. C. (1971) *Probability Models and Statistical Methods in Genetics* (Wiley, New York).
29. Anderson, W. W., Dobzhansky, Th., Pavlovsky, O., Powell, J. & Yardley, D. (1975) *Evolution* **29,** 24–36.