THE ESTIMATION OF RELATIVE FITNESS OF DROSOPHILA POPULATIONS. IV. EXPERIMENTS ON *D. PSEUDOOBSCURA*

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Received January 5, 1965

VARIOUS experiments by DOBZHANSKY and his collaborators (e.g. DOBZHANSKY 1957; LEVINE, PAVLOVSKY, and DOBZHANSKY 1958; LEVINE 1955) have shown that experimental populations of *D. pseudoobscura* polymorphic for the Arrowhead (AR) and Chiricahua (CH) chromosome III gene arrangements are subject to strong selection. At 25°C, the heterozygote shows higher fitness than either homozygote, producing an equilibrium at about 70 to 75% AR. More recently, questions have centered on the relative fitness of such polymorphic populations, as compared with monomorphic populations (BEARDMORE, DOBZHANSKY, and PAVLOVSKY 1960; DOBZHANSKY and PAVLOVSKY 1961; STRICKBERGER 1963a), and on the evolution of fitness in these populations (BEARDMORE and LEVINE 1963; STRICKBERGER 1963b). The fitness estimates have generally been based on biomass productivity. It would be useful to have comparative estimates obtained by different techniques, particularly if these allowed further study of the evolution of fitness in monomorphic and polymorphic populations.

A model for estimation of relative fitness of *Drosophila* populations was described by CLARINGBOLD and BARKER (1961). This is based on interspecific competition, and has been evaluated by BARKER (1963a, b) for *D. melanogaster*, using the sibling species *D. simulans* as the competitor. To study this model for *D. pseudoobscura*, its sibling species (*viz.* *D. persimilis* and *D. miranda*) must be rejected because of their known incomplete reproductive isolation from *D. pseudoobscura* (DOBZHANSKY 1951; DOBZHANSKY and KOLLER 1938; MAYR 1946). This paper reports on a preliminary experiment designed to select a suitable species, and on a second experiment for the further evaluation of the selected species. In the second experiment, the survival rates of males and females of the initial cage populations were quite different, so sex ratio also was estimated at each cage census.

EXPERIMENT 1

Materials and methods: Three species were chosen for testing, namely, *D. nebulosa* (Belem strain), *D. serrata* (Moresby 9 strain—the descendants of many females captured by ТН. DOBZHANSKY in 1960), and a multiple mutant strain of *D. melanogaster* homozygous for scute (*sc*, 1–0.0), crossveinless (*cv*, 1–13.7), vermilion (*v*, 1–33.0), and forked (*f*, 1–56.7). Flies from stock cultures were set up in 5-ounce cream jars, and their virgin progeny collected for the initial adult populations of the cages. Only one strain of *D. pseudoobscura* was used. Egg samples were taken from DOBZHANSKY's Cage 211, which was polymorphic for AR and CH, and virgin flies emerging from the egg samples were used also for the initial cage populations.

Twelve experimental populations were established, four for each of the three species. Two cages for each species were initiated on the one day (27 October, 1961), the remainder one week later (3 November, 1961). Within each week, the age distribution of *D. pseudoobscura* was the same for all six populations. The age distributions varied slightly between the other three species, but were the same for the two populations of each. For all species, these flies were three to nine days old, and all cages were initiated with 200 flies in equal sex ratio and with 50% of each species.

The cages were a modified version of those described by VAN VALEN (1958), with 12 one-ounce food cups. A fresh food cup was inserted every three days. These contained Cream of Wheat-molasses medium (SPASSKY 1943), with sterilized paper towelling, and 1 ml of a suspension of 1 g Fleischmann's Dry Yeast in 20 ml of water was added before use. All cages were maintained at 25°C.

Weekly after initiation, a food cup was attached to each cage for 24 hours to obtain an egg sample. The top few millimetres of the medium were sliced off and, together with the eggs, transferred to culture bottles. For each sample, approximately 150 to 200 eggs were placed in each of two culture bottles. Here, development took place under uncrowded conditions, and all emerging adults were counted by species. Every four weeks after initiation, the total adult population was removed from each cage under low vacuum pressure, counted by species, and returned to the cage.

Results: Figure 1 shows, for each cage, the percentage of *D. pseudoobscura* in the weekly egg samples, and in the four-weekly adult population censuses. *D. pseudoobscura* was rapidly eliminated by *D. melanogaster*, and almost as rapidly it eliminated *D. serrata*. These eight cages were discarded after the adult census in Week 12. In competition with *D. nebulosa*, the percentage of *D. pseudoobscura* among adults increased more slowly to about 80%, followed by stabilization or a slight tendency to decline. The later censuses were only obtained through the kind cooperation of Dr. A. M. MOURAD, but unfortunately all four cages had to be discarded after the adult census of the first two at Week 20.

The most striking feature in Figure 1 is the lack of correlation between the results from egg sampling and adult population census. In competition with *D. serrata*, the percentage *D. pseudoobscura* in egg samples was generally higher than the percentage among adults. In the first three weeks, this could mean a lower mortality among the *D. pseudoobscura* founders than among the *D. serrata*. Overall, it indicates either higher fecundity or higher percentage emergence in *D. pseudoobscura*. All these factors would contribute to the superior fitness of *D. pseudoobscura*. For *D. melanogaster*, there was the inverse situation, with percentage *D. pseudoobscura* in egg samples generally less than the percentage among adults. When in competition with *D. nebulosa*, the percentage *D. pseudoobscura* was generally higher in the egg samples. In these, percentage *D. pseudoobscura* was generally very high in Weeks 1 and 2, suggesting that it has either lower mortality or higher fecundity. In Week 3, the percentage declined, presumably due to emergence of the first generation *D. nebulosa* before the *D. pseudoobscura*. The percentage increased for the next three weeks (4 to 6), declined in Weeks 7 and 8, then increased again. Thus *D. pseudoobscura* has apparently higher fecundity or lower mortality than *D. nebulosa* under this cage environment, but its superiority in these fitness components is countered by the shorter generation interval of *D. nebulosa*.

FIGURE 1.—Percentage *D. pseudoobscura* in weekly egg samples, and in four-weekly adult population censuses (with curves drawn on the latter), when in competition with *D. nebulosa*, *D. serrata*, or *D. melanogaster*. · and × = cages initiated 27 Oct. '61; ○ and △ = cages initiated 3 Nov. '61.

On the basis of these results, *D. nebulosa* warranted further investigation as a possible suitable species for studies of interspecific competition with *D. pseudoobscura*.

EXPERIMENT 2

Materials and methods: The strain of *D. nebulosa* was the same as used in Experiment 1. Four stains of *D. pseudoobscura* were selected, two each of strains homozygous for AR and CH. Each strain had descended from a single female captured in the wild, and all four were among those used in the initiation of DOBZHANSKY's Cage 211. They were selected on subjective estimates of the numbers of flies in culture bottles at each transfer, as one of relatively high fitness and one of low fitness for each gene arrangement.

The cages used were those described by BARKER (1960), and two media and media jar changing routines were incorporated as an experimental treatment. For ordinary medium (5 g agar, 720 ml water, 100 g cornmeal, 100 ml treacle (molasses), 0.8 g Nipagen-M), 15 jars were used in each cage, with a fresh jar added three times per week. A jar thus remained in the cage for 5 weeks. For fortified medium (Medium F of CLARINGBOLD and BARKER 1961), 12 jars were used,

changed three times per week, so that a jar remained in the cage for 4 weeks. Each jar contained 20 ml of medium, seeded with 6 drops of a 1:19 live yeast suspension immediately before use.

This design of 4 strains $\times$ 2 media was to be done in two replicates, totalling 16 populations. However, insufficient flies of the low fitness CH strain (CH 309) were obtained, and only two cages of it were initiated, one on each medium. All cages were initiated with 500 three to seven day old adult virgin flies, with 50% of each species, and equal sex ratio. The age distributions were the same for all populations of each *D. pseudoobscura* strain, but differed slightly between strains. The distribution was the same over all cages for *D. nebulosa*. All populations were initiated on the one day, and then weekly for three weeks, the total adult populations were removed from each cage under low vacuum pressure, counted by species and sex, and returned to the cage. After this, it was not possible to score all cages on the one day, and the weekly census was continued for only eight cages. The cages with CH 309 were eliminated because of its low fitness (see below), while all four with AR 126 were continued, as it apparently was of intermediate fitness. For AR 149 and CH 333, one cage each on ordinary and fortified media were continued. The cages were maintained at $25 \pm 0.5^\circ\text{C}$, with 12 hr light per day (6 A.M. to 6 P.M.).

Where the frequencies of two or more types in a cage population are being estimated from counts of adult flies, it would be convenient if estimates could be obtained from a sample of the total population. The following procedure was used to determine whether a random sample of adults could be obtained. At each sampling, any adults in the medium jars were emptied into the body of the cage, the flies were disturbed by gently bumping the cage, then approximately half the flies were removed and counted. The remainder were then collected and counted separately.

Results: (1) *Adult sampling technique.* For all cages, the percentage of males in the first collection was generally higher than in the second. For *D. nebulosa*, this occurred at practically every sampling, the average percentages of males in the two collections, over all cages and all samplings, being 53.3 and 48.2. This effect was not so marked for *D. pseudoobscura*. However, almost invariably, the percentage of this species was higher in the first collection than in the second. Although the population was disturbed both before and during collection, it was not possible to obtain a random sample of adults, either in regard to sex or species. It seems that the only satisfactory way to sample an adult population would be to collect all adults, anaesthetize, thoroughly mix, and then sample.

(2) *Interspecific competition.* The percentage *D. pseudoobscura* in each cage at each sampling is shown in Figure 2 (A and B). Of the three strains followed in detail, differences in competitive ability with *D. nebulosa* are obvious, although their fitnesses were higher on fortified medium. CH 309 had the lowest fitness, as on ordinary medium, it was absent from the cage in Weeks 2 and 3, and again in Week 13 when it was scored before being terminated. On fortified medium, the percentage CH 309 in Weeks 2 and 3 was much less than for other strains, and in Week 13 was only 2.4%. AR 149 had the next lowest fitness, being rapidly eliminated on both media. The fitness of AR 126 was intermediate, as it survived for 18 and 34 weeks on ordinary medium, and 33 and 49 weeks on fortified. On ordinary medium, the frequency of CH 333 declined to 4.1% in Week 17, then fluctuated between 4% and 18%, and was still present when the cage was terminated because of infestation with *Spilomicrus* spp. (Hymenoptera; Proctoteroidea), which proved parasitic to pupae of both *D. nebulosa* and *D. pseudoobscura*. On fortified medium, CH 333 increased in frequency for the first 2 to 3 generations, decreased over the next 2 to 3 generations, and then fluctuated from

FIGURE 2.—Percentage *D. pseudoobscura*, total population numbers, and numbers of *D. nebulosa* in weekly adult population censuses. Data for CH 309 not included. A, C, E = Cages on ordinary medium; B, D, F = Cages on fortified medium.

about 15% to 50% until the cage was terminated because of contamination. It is unfortunate that both CH 333 cages were inadvertently lost, preventing further study of this apparent equilibrium in an interspecific competition.

In all cages, the frequency of *D. pseudoobscura* was high in Week 1 (60 to 90%), when only survivors of the founding populations were counted. The initial survival rate of this species was thus invariably higher than that of *D. nebulosa*. In Week 2, particularly on fortified medium, the first generation *D. nebulosa* had started to emerge, and the percentage *D. pseudoobscura* had declined. By Week 3 on fortified, and Week 4 on ordinary, *D. pseudoobscura* had commenced emergence. After these first few weeks, as generations tend to overlap, the percentage *D. pseudoobscura* would not be expected to fluctuate greatly from week to week. However, on fortified medium, such fluctuations did occur.

(3) *Population numbers.* In those cages where *D. pseudoobscura* was eliminated, the weekly censuses were continued to Week 37 or 38. Results for total population numbers are shown in Figure 2 (C and D). The four cages on ordinary medium were remarkably consistent, with the number increasing from Week 2 to a maximum in Week 10, and then fluctuating fairly regularly with a peak every 3 to 4 weeks. In the cages on fortified medium, all showed the same trend in numbers in the initial weeks, rising to a maximum in Weeks 4 to 5, but after this, there was greater variation between cages than with ordinary medium, and the fluctuations were more marked. This probably accounts for the greater fluctuations in percentage *D. pseudoobscura* from week to week on fortified medium.

On both media, the variation in total population numbers (that is, between cages within weeks) was less than the variation in *D. nebulosa* numbers (Figure 2 E, F). This is more obvious for the cages on fortified medium, which maintained a higher percentage of *D. pseudoobscura* than did those on ordinary. Further, there were no obvious differences in the week to week fluctuations in population numbers between the cages in which *D. pseudoobscura* was still present and those in which it was eliminated. Thus for these two species, a cage apparently supports a given number of flies, and there are no marked antagonistic or facilitation effects when both species are present in a cage.

(4) *Sex ratio.* In many of the adult censuses, the sex ratio (expressed as number of males/total number) was significantly different from 0.5. Directional trends in sex ratio with time, population numbers, or percentage of the species were apparent in some cases, and weighted regressions of sex ratio on these variates were calculated (weighted according to the numbers of flies on which each sex ratio was based), separately for each cage. In no case did the estimates for a particular regression differ significantly among the four cages on either medium, so that estimates presented below are all pooled estimates over the four cages.

For *D. nebulosa*, the sex ratios that were significantly different from 0.5 are shown in Figure 3. Of the initial populations, the survival rate of males was much less than that of females, but higher on fortified than on ordinary. This medium

FIGURE 3.—Significant sex ratios for *D. nebulosa* in weekly adult population censuses, and weighted regression of *D. nebulosa* sex ratio on Weeks 3 to 24 (ordinary—A), and on Weeks 2 to 27 (fortified—B).

difference remains obvious throughout. When all sex ratios are considered for the cages on ordinary medium, this parameter increased to about Week 12, with possibly further increase to about Week 24, and then fluctuated around a mean value near 0.5. The regression coefficient of *D. nebulosa* sex ratio on weeks (for Weeks 3 to 24) was 0.00316 ($P < 0.001$). The regression for weeks 25 on was not significant, and mean sex ratio over all cages was 0.490. The trend of change in sex ratio was similar in the cages on fortified medium. For Weeks 2 to 27, the regression coefficient of sex ratio on weeks was 0.00293 ($P < 0.001$). Again, in later weeks, the regression was not significant, with mean sex ratio over all cages of 0.538. Regressions of *D. nebulosa* sex ratio on other variates were not significant for the cages on fortified, but for those on ordinary, the regression of *D. nebulosa* sex ratio on the numbers of this species was significant. However, both for Weeks 3 to 24 and for the total period, the partial regressions of *D. nebulosa* sex ratio on *D. nebulosa* numbers (correcting for variation due to weeks) were not significant. Thus the changes in sex ratio of this species on both media were associated only with time, showing an increase in the early history of the populations.

For *D. pseudoobscura*, the survival rate of males in the initial population was higher than that of females, particularly on ordinary medium (Figure 4), but unlike *D. nebulosa* this medium effect was not obvious in later weeks. However, the two strains of *D. pseudoobscura* that persisted (AR126 and CH333), differed

FIGURE 4.—Sex ratios for *D. pseudoobscura* in weekly adult population censuses of the cages on ordinary medium, and regression of sex ratio on Weeks 3 and later.

in sex ratio. Relatively few of the individual sex ratios for AR126 were significant, and these were generally in the early weeks with excess males. The mean values for Cages P-4 and P-5 (ordinary), and for Cages P-6 and P-7 (fortified) were 0.534 and 0.525. Over 80% of the significant sex ratios for CH333 showed excess females, and the mean values for Cages P-12 (ordinary) and P-14 (fortified) were 0.451 and 0.453. Thus for CH333, the relative viability of males is less than that of females, while the reverse may be true for AR126. Selective differences between the sexes have been found for example, by DOBZHANSKY (1950), LEVITAN (1954), MERRELL and UNDERHILL (1956), and WOOLF and CHURCH (1963), while OWEN (1953) and LI (1963) have investigated the gene frequency equilibria with such selection. If the particular differential viability observed here, where selection is of opposite direction in the two sexes, operates in populations in which these two gene arrangements are segregating, then it would contribute to the development of balanced polymorphism. On ordinary medium, there were significant regressions of *D. pseudoobscura* sex ratio on Weeks 3 to 52 (0.00138 , $P < 0.05$; Figure 4), numbers of *D. pseudoobscura* (-0.000185 , $P < 0.001$; Figure 5 A), and total population numbers (-0.000086 , $P < 0.01$; Figure 5 B). However, the partial regression on weeks (correcting for variation in *D. pseudoobscura* numbers) was not significant, while that on *D. pseudoobscura* numbers (correcting for variation due to weeks) remained highly significant. This regression is largely due to cage P-12 (CH333). The only significant regression of *D. pseudoobscura* sex ratio on fortified medium was on the percentage of this species (-0.00045 , $P < 0.05$).

DISCUSSION

One aspect of Experiment 1 warrants comment. STRICKBERGER (1963b) also studied competition between an AR/CH polymorphic population of *D. pseudoobscura* and the same mutant strain of *D. melanogaster* as used here. In his two cages, the percentage *D. pseudoobscura* was reduced in the initial generations to

FIGURE 5.—Regressions of *D. pseudoobscura* sex ratio on numbers of this species (A), and on total population numbers (B), for the cages on ordinary medium.

about 15 to 30%. But after 100 days, the frequency then increased to 65 to 75% at 170 days, when the experiment was terminated. The apparent higher fitness of STRICKBERGER's polymorphic population, as compared with that used here, may lie in their previous histories. The source flies for Cage 173 (used by STRICKBERGER) and Cage 211 (used here) were the same, namely 10 strains homozygous for AR and 13 strains homozygous for CH, all isolated from flies collected at Pinon Flats, San Jacinto Mountains, California. However, when flies were taken from Cage 173 by STRICKBERGER, it had been in existence for over four years, and had reached final equilibrium of approximately 75% AR: 25% CH. Cage 211 had only been in existence for 7 months when used to provide the polymorphic strain here, and had not reached final equilibrium. It is unlikely that the different fitnesses of the two polymorphic populations are due to sampling variation, but they may well reflect the expected increase in fitness of a polymorphic population as it approaches to equilibrium. The pattern of change in

D. pseudoobscura frequency observed by STRICKBERGER (i.e., initial decrease and later increase), has been commented on by BARKER (1963b). It may be due to the shorter generation interval of *D. melanogaster* giving it an initial advantage, which is later cancelled by the superiority of *D. pseudoobscura* in other fitness components. This possibility is now under investigation.

For Experiment 2, results have not been presented for those cages scored for the first 3 weeks only, as they were practically the same as for their respective replicate cages. They merely served to strengthen conclusions already noted for these early weeks. Detailed comparisons between replicate cages are possible for the AR126 cages. For P-4 and P-5 on ordinary medium, the week to week fluctuations were very similar, although generally the numbers of each species, total population numbers, and percentage *D. pseudoobscura* were higher for P-4. On fortified medium (cages P-6 and P-7), the fluctuations in numbers of *D. nebulosa* and total population numbers were similar, although P-6 numbers were higher. But the numbers of *D. pseudoobscura*, and the percentage of this species were markedly higher for P-7 to Week 16 (see Figure 2 B). Further, the variation between cages in population numbers was greater and there were wider fluctuations in percentage *D. pseudoobscura* on fortified medium. This medium is therefore less satisfactory for study of this interspecific competition.

The observed changes in sex ratio in these populations are of considerable interest. For *D. nebulosa*, the survival rate of males in the initial populations was less than that of females, but over the first 6 months or so, the sex ratio increased at approximately parallel rates on both media. This may appear to be the result of selective changes in the sex ratio, which could be expected as FISHER (1930) and SHAW and MOHLER (1953) have argued that natural selection should act to produce equality of the sexes at maturity. The apparent rapidity of the increase might argue against such selective changes, particularly as FALCONER (1954) found selection for sex ratio ineffective, except when the initial population happens to contain a major gene that affects sex ratio. That is, there is apparently little additive genetic variance for sex ratio in random bred populations. However, sex ratio may well be a homeostatic population character, so that in an unusual environment that changes the sex ratio, selection back to equal sex ratio may be quite rapid. But in the initial generations in the population cages, the cage environment will also be changing, perhaps in such a way as to increase the probability of male survival. This seems more likely as the sex ratio in Weeks 1 and 2 was higher on fortified, and remained higher throughout, and as most of the increase in sex ratio apparently occurred in about the first 12 weeks (Figure 3 A, B)..

The sex ratio of *D. pseudoobscura* on ordinary medium showed an effect of adult density, there being significant negative regressions on both numbers of *D. pseudoobscura* and total population numbers. This extends to population conditions the findings of SOKOLOFF (1955), who raised *D. pseudoobscura* and *D. persimilis* at densities from 1 to 80 larvae per vial, and found that male sex ratio at emergence tended to decrease at higher densities. ANDERSEN (1961), in a review of the effect of density on sex-ratio at emergence, states that this is the only

case of a significant effect in animals with chromosomal and random sex determination, and heterogametic males. Further, in population studies with *Drosophila* (e.g. THOMSON 1961), no case of a relation between sex ratio and population size has previously been recorded. No explanation is readily available to account for this density effect in the cage populations. The media jars are always overcrowded with larvae once a cage reaches peak population numbers, so that differential larval viability seems unlikely. If adult male viability decreases as numbers increase, why is this more related to numbers of *D. pseudoobscura* than to total population numbers? It is clearly not just adult crowding *per se*.

Even though the interspecific competition itself warrants further study, it is concluded that *D. nebulosa* is a suitable competing species to use in the estimation of relative fitness of *D. pseudoobscura* populations, and it will now be used to study the evolution of fitness in monomorphic and polymorphic populations.

I have indicated my debt to DR. A. M. MOURAD for the later censuses of the populations in Experiment 1. This experiment was done during the tenure of a U.S. Government (Fulbright) grant at Columbia University, and under Contract No. AT-(30-1)-1151, U.S. Atomic Energy Commission. I am grateful to PROFESSOR TH. DOBZHANSKY for his kind hospitality, interest, and advice. Experiment 2 was supported by a University of Sydney Research Grant, and ROBIN JOHNSTON, NEIL PODGER, and SHANE BULL provided valuable technical assistance. I am indebted to MR. L. N. BALAAM, Department of Agriculture, University of Sydney, for statistical advice, and to G. E. J. NIXON, Commonwealth Institute of Entomology, London, for the identification of the parasitic Hymenoptera as *Spilomicrus* spp.

SUMMARY

Results are presented of an experiment designed to select a suitable species for interspecific competition studies with *D. pseudoobscura* in population cages, and of a further experiment using the chosen species. In the first experiment, *D. pseudoobscura* was rapidly eliminated by a *D. melanogaster* strain homozygous for four sex-linked mutants (*sc*, *cv*, *v*, *f*), and almost as rapidly it eliminated *D. serrata*. With *D. nebulosa*, percentage *D. pseudoobscura* increased more slowly over a 20-week period, and this species was chosen for further study. This experiment emphasized the need for care in analyzing such experiments, as estimates of the proportions of the two species obtained from egg samples and total population censuses were often quite different.

In the second experiment, competition between *D. nebulosa* and each of four strains of *D. pseudoobscura* was studied on each of two media, the outcome of competition being analyzed through weekly adult population censuses. It was shown that the only satisfactory way to sample an adult population would be to collect all adults, anaesthetize, thoroughly mix, and then sample. Fitness differences between the *D. pseudoobscura* strains were marked, but all exhibited higher fitness on dead yeast fortified medium. Population numbers increased rapidly to a peak and then fluctuated with a general tendency to decrease. The variation between cages in population numbers was greater, and there were wider fluctuations in percentage *D. pseudoobscura* on fortified medium, so that this medium is less satisfactory for study of this interspecific competition. Many sex ratios (number of males/total number) were significant, and there were significant

trends in sex ratio with time, population numbers, or percentages of the species. *D. nebulosa* sex ratio on both media significantly increased over about the first 6 months of competition, a result that could be due to selective changes, or due to changes in the environment in the cages. *D. pseudoobscura* sex ratio on ordinary medium significantly decreased as numbers of this species, and total population numbers increased. No such relationship has previously been recorded, and it appears to be more than an effect of just adult crowding *per se*. However, *D. nebulosa* should be a suitable competing species for the estimation of relative fitness of *D. pseudoobscura* populations.

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