EXPERIMENTAL CONTROL OVER THE EVOLUTION OF FITNESS IN LABORATORY POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

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AN experimental method sensitive enough to detect fitness changes caused by natural selection over experimentally practical short periods of time has been previously described (STRICKBERGER 1963). In brief, experimental populations of *Drosophila pseudoobscura* containing strains of the third chromosome arrangements, Arrowhead (AR) and Chiricahua (CH) from Pinon Flats, California, will under prescribed circumstances, reach uniform equilibrium frequencies of AR and CH. If, however, the AR and CH strains have been subjected to different selective histories for a period of 1½ years or longer, the frequencies achieved and the rates at which they are achieved may now differ considerably from those normally observed.

The purpose of the present experiments has been to use this method in evaluating some of the factors that may affect the evolution of fitness. Specifically, a group of AR strains were subjected to three different levels of recombination and to two levels of mutation as they evolved in population cages. The effect of each of these conditions was then evaluated by comparing the relative performance of each AR population against a set of standard CH strains. Since the evaluating tests also took place in population cages, this method offered the advantage of scoring the actual performance of genetic material competing for survival under the same selective conditions that had been used for its evolution. In addition to these conditions, evolution under half-pint bottle conditions at two levels of recombination was also tested.

MATERIALS AND METHODS

The strains of *Drosophila pseudoobscura* used in the present experiments had their origin in Pinon Flats, California, during the 1940's. They have since been kept as small mass cultures, each homozygous for AR and CH. In the latter part of 1960, males from a number of different AR strains of this collection were crossed to a balanced marker stock containing the genes Blade and Scute on the Standard (ST) third chromosome arrangement, and Lobe on the Santa Cruz (SC) arrangement. Since crossing over is virtually absent between SC and AR, the crosses shown in Figure 1 produced strains of AR that were highly, if not completely, isogenic for their third chromosome arrangements. The four most viable of these strains, for convenience called AR1, AR2, AR3, and AR4, were then treated in the following manner:

Condition A: Each strain was kept separately as small mass cultures in individual half-pint bottles at 16°C and transferred approximately every six weeks. In February, 1962, these cultures were transported from Columbia University, New York, to the University of California, Berkeley, and the cultures were then maintained at 18°C.

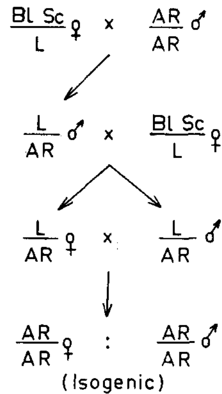

FIGURE 1.—Method used in isolating strains isogenic for the third chromosome Arrowhead arrangement.

Condition B: Crosses were made between all four isogenic strains and the progeny maintained as a small mass culture in a half-pint bottle treated exactly as the cultures in condition A.

Condition C: Individual samples of each isogenic strain were placed in separate plastic population cages of a standard size 12.5 × 10.5 × 4 inches, having room for 15 one-ounce food cups. The food cups were prepared with the same Cream of Wheat-molasses medium used for the half-pint cultures in conditions A and B, moistened with a few drops of an aqueous suspension of Fleischmann's Dry Yeast. The cages were maintained at 25°C and new food cups were inserted three times a week remaining in the cage for a 35-day interval before replacement. Under these conditions, egg to adult development is estimated to average 25 days. In February, 1962, the cages were transported to Berkeley, California, but were maintained on the same medium and at the same temperature.

Condition D: Reciprocal crosses were made between two isogenic strains (AR3 × AR4) and 200 F_1 progeny placed in an individual population cage. This cage was then maintained in the same fashion as those in condition C.

Condition E: Interstrain crosses between AR1, AR2, AR3, and AR4, which gave rise to the flies in condition B, were also used to furnish flies placed in an individual population cage maintained similarly to those in conditions C and D.

Condtion F: Approximately 500 each of virgin females and males were collected from the same interstrain crosses used in conditions B and E. The males were then irradiated with X rays at 8,000r given in two doses of 4,000r separated by three days. (The machine was set at 180 kv, 30 ma, without a filter. The dose rate was 1560r per minute, with a half-value layer of .26 mm copper and a target-to-fly distance of 18.5 cm. I am indebted to DR. A. E. MOURAD for handling this procedure.) After irradiation, the males and virgin nonirradiated females were placed together in an individual population cage maintained henceforth in the same fashion as those of conditions C, D, and E. All population cages in conditions C to F were begun within two weeks of each other in March, 1961.

Approximately 18 months later, in October 1962, egg samples were taken from each of the above populations in order to begin crosses for test populations in which AR from each condition was to compete against CH. This was done in a manner enabling the foundation stock of each test population to have the opportunity to carry within it descendants of the genes of all four isogenic AR strains. In the case of conditions A and C, reciprocal crosses were made between all four strains of each condition. For condition D, reciprocal crosses were made between flies from D and flies from cages AR1 and AR2 of condition C. The progeny of crosses from conditions A, C, and D, and the adults from egg samples of B, E, and F, were then crossed separately to the same group of 13 CH strains in order to provide six foundation stocks for six individual test populations. In each case, the foundation stock consisted of 600 CH/CH homokaryotypes and 400 AR/CH heterokaryotypes or a starting frequency of .80 CH and .20 AR.

In December, 1962, these six foundation stocks were placed into individual population cages, labeled as A to F according to the origin of their AR parents. Egg samples were then taken from these test populations at periodic intervals and the larvae raised at optimum conditions (for procedural details, see Strickberger 1963). Each sample provided 150 larvae enabling 300 chromosomes to be scored. Test populations A and B were terminated approximately 6 months later and Cages C to F were terminated at the time of the author's departure from Berkeley, approximately 8 months later.

RESULTS AND DISCUSSION

Six test population cages of *Drosophila pseudoobscura* containing AR and CH third chromosome arrangements have been scored in which the AR has been derived from four isogenic strains with different evolutionary experiences over an 18-month period. During this evolutionary period, some samples of these AR strains were exposed to bottle conditions (test cages A and B) and others to cage conditions (test cages C to F). Further evolutionary distinctions include the absence of recombination between all four strains (test cages A and C); recombination between two of the AR strains but not between the two others (test cage D); recombination between all four AR strains (test cages B, E, and F); and 8,000r irradiation added to one group of AR strains at the beginning of this evolutionary period (test cage F).

Chromosome frequencies in test populations A and B are graphed in Figure 2, and those in test populations C to F are graphed in Figure 3. For both sets of data, AR chromosomes derived from the recombinant conditions (B, E, F) maintain frequencies that are clearly higher than those derived from partially recombinant or nonrecombinant sources (A, D, C). The difference in performance between AR derived from recombinant and nonrecombinant sources, however, seems greater under cage conditions (Figure 3) than bottle conditions (Figure 2). Although the exact causes responsible for this are unknown, they seem to be asso-

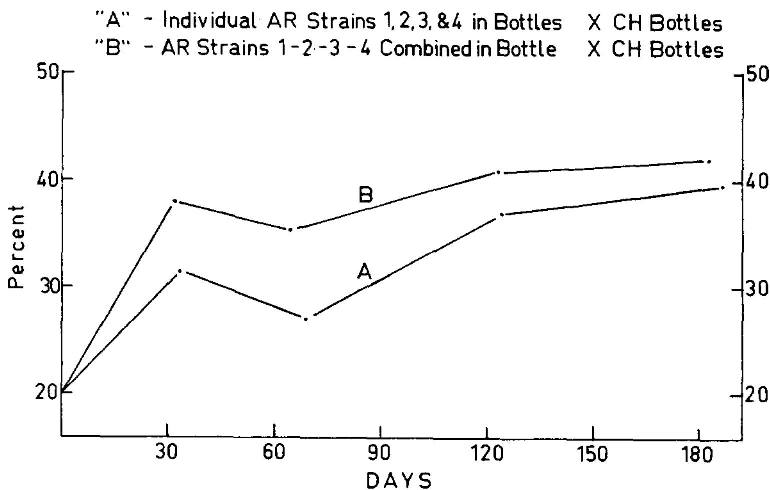

FIGURE 2.—Frequency changes of Arrowhead third chromosomes in test populations A and B based on 300 chromosomes per sample.

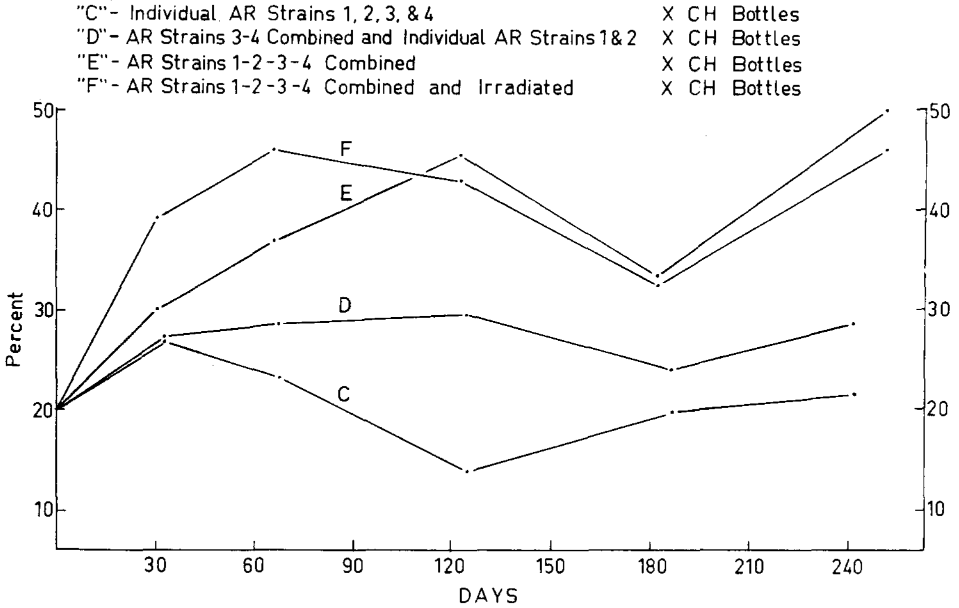

FIGURE 3.—Frequency changes of Arrowhead third chromosomes in test populations C to F based on 300 chromosomes per sample.

ciated with the relatively high performance of AR from nonrecombinant bottle conditions (cage A).

If we confine our attention to AR from cage origin (Figure 3), there is a clear stepwise gradation in fitness between nonrecombinant (C), partially recombinant (D), and full recombinant (E and F) AR chromosomes. The nonrecombinant Arrowheads in cage C still hover around the initial starting frequency of 20% at the end of 8 months, while the full recombinant Arrowheads in cages E and F have achieved frequencies of 45% or higher. The performance of the partially recombinant Arrowheads in cage D remains almost exactly midway between these two extremes throughout the entire experiment.

Thus, the degree of fitness evolved by AR chromosomes in these experiments appears to depend directly on the extent of genetic recombination they have experienced. In this sense, recombination offers the opportunity for genes on separate AR third chromosomes to form new combinations on the same third chromosome. The more new combinations are formed, the greater the opportunity for selection to choose those that are most advantageous. To a measureable degree, the evolution of fitness in these experiments is therefore a function of interaction between genes on the same chromosome. These experiments, therefore, support the previously proposed view (STRICKBERGER 1963) that the difference in fitness between chromosome arrangements from polymorphic and monomorphic cages may arise from the extent of recombination that a chromosome arrangement experiences in each source. Similarly, the relatively poor performance of chromosome arrangements derived from bottle sources in previous experiments is most likely caused by the absence of recombination between the individ-

ual strains in separate bottles. The relatively high performance of nonrecombined bottle strains in the present experiments is not understood, but it should be kept in mind that it is nevertheless lower than that of recombined bottle strains.

Another point of interest in the present experiments is the relatively low frequencies of AR even in the best cages (E and F). By contrast, AR in most previous competitions with CH reaches a frequency of 60% by the end of six months (DOBZHANSKY and PAVLOVSKY 1960; STRICKBERGER 1963). This difference between present and past results may lie in the number of AR strains that are used. At present, only four isogenic AR strains compete with 13 strains of CH, while in previous experiments at least ten strains of AR were involved in the same competition. In other words, the amount of initial genetic variability may determine the upper level to which fitness can evolve in these experiments. Thus, although recombination may offer a variety of advantageous AR chromosomes upon which selection may act, the number of different gene combinations available is limited by the number of different genes.

It might therefore appear as though mutation added by irradiation should have an enhancing effect on the evolution of fitness since a greater number of genes are thereby provided. However, the addition of irradiation to the Arrowheads used in test cage F does not appear to have increased evolutionary opportunities to any notable degree beyond that of cage E. Only for the first two points, 30 and 60 days, does AR in cage F show higher frequencies than AR in cage E. This may mean either that the added variability is not of the kind usable for the evolution of fitness, or that one dose of 8,000r is relatively small compared to the variability produced by recombination between four isogenic AR strains. At present, experiments are under way testing the evolution of fitness caused by different degrees of initial genetic variability when produced through differences in the number of initial strains as well as through differences in the degree of irradiation to single strains.

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SUMMARY

Four strains isogenic for the AR third chromosome arrangement were permitted to evolve for an 18-month period under different degrees of recombination in bottles and in population cages, and under two different degrees of mutation in the cages. The competitive ability of AR chromosomes from each of these sources was then tested under population cage conditions against strains carrying the CH arrangement. For both AR from bottle and cage origins, the degree of fitness evolved was enhanced by the extent of recombination that had been experienced. At the same time, the highest competitive frequencies achieved by

AR chromosomes appeared to be limited by the amount of their initial genetic variability. The mutation effect produced by 8,000r of X rays neither enhanced nor detracted from the evolution of fitness of the AR chromosomes to which it had been added.

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