THE KINDS OF GENETIC VARIABILITY IN RELATION TO SELECTION RESPONSES IN *DROSOPHILA* FECUNDITY¹

R. H. RICHARDSON² AND KEN-ICHI KOJIMA

Department of Genetics, North Carolina State University at Raleigh, Raleigh

Received April 19, 1965

A series of experiments have been carried out over the past six years at North Carolina State University by KOJIMA and his associates. The objective of these experiments was to evaluate the effectiveness of reciprocal recurrent selection between two populations relative to purebred selection. KOJIMA and KELLEHER (1963) reported a marked superiority of reciprocal recurrent selection (RRS) between the two populations over full-sib family selection (FFS) within each of the two populations in the improvement of fecundity in the cross. However, there still remained the question of the type of genetic variations that were involved in the selection progress.

The selection experiments reported in this paper were designed to elucidate some critical properties of the genetic systems involved in the previous selection study. These experiments, consequently, were not primarily intended to test a general theory of directional selection, but were to determine the genetic structure of two specific pairs of initial and final populations in the earlier selection study.

MATERIALS AND METHODS

KOJIMA and KELLEHER (1963) chose two populations of *Drosophila pseudoobscura*, Mather and Mono, on the basis of their being similar in their egg production and exhibiting very little or no heterosis. These populations were derived from flies collected by DR. TH. DOBZHANSKY and his co-workers at two locations, Mather and Mono, near Yosemite National Park. Several strains from each location were mixed *inter se* with respect to location, forming the two cage populations. At the initiation of the experiments, the Mather and Mono populations had been in cages for over two years (at least 20 generations). Throughout the series of experiments they were maintained under laboratory conditions at about 19°C with a high relative humidity, and were cultured on a cream-of-wheat medium.

During selection, the strains were cultured in half-pint milk bottles, with one bottle corresponding to a full-sib family. The cream-of-wheat medium was seeded with a drop of dense water suspension of dried yeast. Before pupation, one half of a Kleenex tissue soaked in a yeast suspension was added to each culture bottle. After eclosion was well under way, adult flies were discarded and three days later adult males and females (full-sibs) from a bottle were collected together into a vial containing medium. These flies remained together for about five days, until they were etherized, the males discarded, and the females placed in vials containing fresh food. The females were subsequently transferred to egg-laying-test bottles.

¹ Contribution from the Genetics Department, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the Approval of the Director of Research as Paper No. 1978 of the Journal Series. The work was financed by Public Health Grant GM 11546 and computer time was made available by Public Health Service Grant Fr-00011 to the Institute of Statistics.

² Present address: Department Zoology, University of Texas, Austin.

Three full sisters were transferred to a test bottle on the eighth day after original collection. Dried yeast suspension was sprayed on an agar-molasses-charcoal medium which was in a thin layer on the inside surface of a commercial paper milk-bottle cap. Fresh caps were placed on the test bottles daily. The first day's caps were discarded, and egg counts were made on caps for the following four days (9th through 12th day after collection). This test period corresponded to the first peak egg laying period after eclosion, and fertile eggs were layed throughout the test.

During all selection experiments, one of two designs was used. One design included two replicate test bottles (a total of six full sisters tested), and the half-sib family (a common sire) consisted of two full-sib families. This was the design used by KOJIMA and KELLEHER (1963). In the other design, there was one test bottle per full-sib family (a total of three full sisters tested), and the half-sib family (a common sire) consisted of three full-sib families.

In either design, selected individuals used as progenitors of the next generation were full-sibs to the females tested. For the intermix experiments to be described, males and virgin females were collected, fed 24 hours, and stored during the remaining test interval in a refrigerator at 4°C. The collection of these potential parents began immediately after collection for the egg-laying test. As a result of the storage, selection every generation was possible and progenitors of the next selection cycle (generation) were full-sibs to the tested full sisters. In the other selection experiments, there was an alternating scheme of a mass-culture generation with a selection generation, where the mass culture was within selected full-sib families and progenitors of the next selection cycle were offspring of the mass-cultured families. The two selection systems are essentially equivalent with respect to expected response and to inbreeding changes per selection cycle, but differ in chronological rate of change, in recombination effects within full-sib families, and in within-full-sib-family natural selection possibilities.

RESULTS

RRS and FFS upward selection experiments: According to KOJIMA and KELLEHER (1963), reciprocal recurrent selection (Figure 1) for improved crossbred performance between these two populations was extremely effective (for the first ten cycles, an increase in daily production of over 1.4 eggs per cycle) compared to selection within each population (a daily production increase of less than 0.4 eggs per cycle, which was statistically nonsignificant). For the first ten cycles

FIGURE 1.—A simplified diagram of RRS scheme. The mating design for making crossbred test A $\times$ B and maintaining purebred A' for one A male is shown. There were 60 males for A $\times$ B and A', and 60 males from B were used in the reciprocal side.

FIGURE 2.—The average RRS purebred egg productions expressed in terms of deviations from control values. For the comparison, the RRS crossbred results are given by regression lines (Y_1 for cycle 0–15; Y_2 for cycle 15–21). The detailed data of the purebred points are given in Table 1, and squares in this figure are averages of three sequential cycles plotted on the middle cycle.

the realized heritability computed on the basis of cumulative selection differentials was 0.16, and after this period there occurred a plateauing with a total egg production increase of over 25% (level of the regression line for last nine cycles in Figure 2).

For purebred selection (FFS, selection on the basis of average full-sib-family performance), after adjustment for inbreeding depression, there was a realized heritability of 0.03 for Mather and 0.04 for Mono. Even after this adjustment there was a negligible change in purebred performance.

The new information, which was not included in the paper by KOJIMA and KELLEHER (1963), is the behavior of purebred performance in the RRS study. As an indirect effect of selection for crossbred performance, the performance of the crossbred-selected purebred populations in the RRS experiment (A, A', . . . and B, B', . . . in Figure 1) increased after a delay of about 11 cycles, and eventually plateaued at a level about three to four eggs below the plateaued crossbred performance. (As indicated at the bottom of Table 1, the final test result was 1.5 eggs less for the purebreds than for the crossbreds.) This change took place in a parallel fashion between Mather and Mono (Table 1), so that the average performance of the two purebreds describes the pattern and extent of change (Figure 2). This particular pattern observed on purebred performance correlated with the change of crossbred performance was one of the most unexpected results of the RRS experiments, and was the major problem to be explained by the analysis of the genetic systems involved.

Two-way selection within Mather and Mono: Mather and Mono were selected

TABLE 1

Purebred performance of RRS experiment, expressed as average eggs per female per day

RRS selection cycle	Mather	Mono	Control
5*	43.8	38.7	41.5
10*	42.6	40.8	41.5
10†	32.4	36.2	31.3
12	34.3	35.5	30.7
13	39.4	41.6	34.3
14	38.4	38.8	34.6
16	34.0	27.5	22.8
17	38.4	38.6	36.4
18	40.7	45.0	33.7
21‡	45.8	46.4	39.0

* 3-day count corresponding to second, third, and fourth days of other cycles.

† 4-day count retest of previous 3-day test.

‡ Final RRS test: crossbred averages were Mather males $\times$ Mono females 45.7, and Mono males $\times$ Mather females 49.5. These data indicate that the average egg production of crossbreds was 1.5 eggs over the average of the purebreds.

upward for ten cycles, and then two-way selection was initiated, giving rise to a "high line" selected for increased egg production simultaneously with a contrasting "low line." The high line was the same as that previously reported for FFS (KOJIMA and KELLEHER 1963) and the low line originated as the lower portion of the population tested in the eleventh cycle of purebred selection. The testing and selecting techniques, using full-sib replicates, were modified by halving the original population size for each line to 20 male and 60 female parents with 360 daughters tested per selection cycle per line.

Two-way selection was continued for five cycles with an average divergence (strongly asymmetrical downward: see Figure 3) of 2.2 eggs per female per day in Mather and 1.6 eggs per female per day in Mono. A final, large-scale test including crosses between high and low lines within populations, and crosses between corresponding lines (high $\times$ high and low $\times$ low) of different populations contributed information about the genetic nature of the divergence.

The results of crosses between the selection lines (Figure 4) fell near one or between the parental mean performances, with the exception of the low $\times$ low cross between Mather and Mono. In this case, the cross was slightly above both parents, but not to a degree that was statistically significant. The only cross differing materially from both parents was the high $\times$ low cross within Mono, where the cross was midway between the parents. The corresponding cross in Mather was very near the performance of the high parent.

KOJIMA (1963) has interpreted these data as indicating Mather and Mono were approximately plateaued for high egg production, but still retained considerable genetic heterogeneity within the population. There was an indication of upward dominance in Mather, but none in Mono, and a hint of heterosis between the populations (supposedly an expression of partial to complete dominance at the locus level, with the two lines carrying the dominant alleles for different loci). Generally, there appears to be some directional dominance, but no strong indication of overdominance.

FIGURE 3.—Results of two-way selection in Mather and Mano FFS experiments. Prior to the initiation of two-way selection each population was selected for the upward direction for ten cycles. Plots are made on deviations of mean performances from control values against cumulative selection differentials from the beginning of upward selection.

FIGURE 4.—Results of final test made on the two-way FFS experiments in Figure 3. The top and bottom are the results of high $\times$ high, high $\times$ low and low $\times$ low crosses within each population. The middle represents high $\times$ high and low $\times$ low crosses between the Mono and Mather line.

Formation of the intermix populations: The intermix populations were of two types—Base Intermix and RRS Intermix. Base Intermix was derived from samples of the same Mather and Mono cages as were the selection experiments reported by KOJIMA and KELLEHER (1963). The Mather and Mono populations were maintained in population cages under fairly constant stockroom conditions during the earlier experiments, and they were resampled and crossed to produce Base Intermix.

Two hundred males and a like number of virgin females were collected from each cage as adults emerged from food cups taken from the cages. Ten culture bottles were set up, each containing ten random Mono virgin females and ten random Mather males (AB) together with another ten bottles with the reciprocal cross (BA). Reciprocal groups were intercrossed in the F_3 , so that the resulting 20 bottles included five bottles for each cross permutation of the four groups (males and females from AB and BA). Then during the following generations an effort was made to maintain adequate population sizes in individual culture bottles for a low rate of inbreeding and to retain representative genetic samples. The material for the present selection study was the F_6 generation. The intermix population supposedly had reached a state of approximate linkage equilibrium through the random mating generations.

The RRS Intermix population was initiated by intercrossing selected families of the 19th selection cycle of RRS. These consisted of the ten half-sib purebreds in each of Mather and Mono which gave the highest crossbred results. Ten males and ten virgin females were collected from each family, and the two populations were crossed pair-wise, family by family. Random mating generations followed as in the case of Base Intermix, except that the reciprocal progenies were combined at the F_2 generation. Population sizes were kept about the same as in Base Intermix, and selection was initiated at the F_4 generation.

During the random mating generations, mean egg counts of both intermixes were determined for each generation. The results are plotted in terms of deviations from control egg counts in Figure 5. The RRS Intermix behaved in an ex-

FIGURE 5.—Average population performances of RRS and Base Intermixes as deviations from control values (two solid lines). The dotted line is performances in Base Intermix retest (see text for the details).

pected manner on the basis of the result from the previous RRS experiment. That is, the F_1 was slightly superior to the parental average, and performance of subsequent generations stabilized at about the level of the parental average.

The result from the Base Intermix was quite unexpected; the mean egg counts increased to the F_3 generation and regressed back to a level of the parental average. The behavior of population means such as this cannot be explained with simple genetic models involving only additive and dominance effects of genes. However, at this stage of the experiment, it seemed the oddity of the result was likely due to the coincidence of peculiar circumstances, rather than due to an expression of some intrinsic genetic situation. Thus, another test of the mean performance of the critical generations was planned.

Such odd results might occur if a trend over the individual generations was not equally reflected in the intermix and the control (i.e., different genotype-by-environment interactions for the intermix and for the control). To eliminate such a possibility in the retest, a direct comparison of the advanced generation means was obtained by testing all generations simultaneously.

The Mather and Mono cages were resampled. From these flies, bottle cultures with a heavy culture density (simulating cage conditions) were established to be repeatedly sampled. Each generation following a new sample, the F_1 was remade, the F_2 made from the previous F_1 , etc., so that after five generations it was possible to test six groups—the two parents and the F_1 through the F_4 . About 50 bottles, each with three females, were tested for each group. A control was not necessary in this test, since the comparisons were to be made among the generations within the test.

Results of this test, together with the results of intermating generations prior to the initiation of selection for RRS and Base Intermixes, are plotted in Figure 5. Since there was no control material in the Base Intermix retest, the F_3 means for the two Base Intermix curves are arbitrarily placed together, and the other points for the retest are plotted relative to the F_3 mean.

The results of the retest generally agreed with the results of the first test series with respect to a marked F_3 peak. The performance of the F_1 in this test was lower than the parental average, rather than slightly higher as in the original test, but the discrepancy between the tests is somewhat discounted by the consistency of the other generations. Within the retest, the statistically significant differences ($P < 0.01$) of the F_3 mean versus the parental mean, the F_1 mean and the F_2 mean support the conclusion for an increasing, though not necessarily monotonic, pattern to the F_3 generation. The difference between the F_3 and the F_4 is not quite statistically significant ($P \approx 0.08$), but considering the confirmation of the first results through the F_4 , and the subsequent magnitude of decrease in the first test, there is little doubt that the trend of the decline is also a real one.

Two-way selection in the intermix populations: In the first generation of selection (the F_6 and F_4 in Base and RRS Intermixes, respectively), the test consisted of 60 half-sib families for Base Intermix and 62 for RRS Intermix, with two full-sib families per half-sib family. The test design used two replicate bottles per full-sib family, as previously described. Therefore, each half-sib family mean

was determined by 12 females. In this generation both ends of the half-sib-egg-count distribution was selected separately, giving rise to the high and low lines in each intermix, with 12 selected half-sib families for each end of the distribution. From each selected half-sib family at least three males and nine virgin females, full-sibs to the six females tested, were taken to make up parents of the next generation.

In subsequent generations, there was a constant type of breeding scheme (Figure 6) among the four selection lines, Base High, Base Low, RRS High, and RRS Low. One male mated with three females formed the half-sib family (the unit of selection). The complete test, without family losses due to sterility or poor cultures, was 30 half-sib families per selection line. Three female progenies for each full-sib family were tested, and enough males and females, full-sibs to the females tested, were collected to be mated with flies from other selected families.

The selection proportion was 1/6 (five selected half-sib families out of 30 tested) for the complete test. However, in the event of less than 30 half-sib families tested, five half-sib families still were selected. Where there were only two full-sibs in the half-sib selection, each full-sib family was replicated (two test bottles with three full sisters in each) in the test if possible, and then used correspondingly more frequently (usually three males and nine females instead of two males and six females) in crossing among other selected half-sibs. Thus each selected half-sib was equally represented in the crosses forming the next generation. Subsequent unequal representation of the half-sib families might come from sterile crosses or cultures too poor to produce enough flies to test and then mate if selected. However, selection differentials corrected for any unequal

FIGURE 6.—Selection scheme used for half-sib family selection in intermix populations. Two matings shown after selection are two examples out of a total of 30 such crosses made among selected families.

half-sib family representation were almost identical to uncorrected ones, indicating this possibility for "natural selection" (any selection other than as in the experimental design) was not realized.

Complete pedigrees were kept and used to compute the coefficients of inbreeding for all possible crosses among selected flies. Every generation matings among selected flies were made in such a way that the increase of inbreeding was somewhat less than that expected from random matings. Also the degree of inbreeding was held fairly constant among families to be tested in a given generation, allowing valid comparisons of families within generations without adjusting for differential inbreeding depression. These three restrictions on a mating pattern—reduced rate of inbreeding, uniform inbreeding, and equal representation of selected families—are partly antagonistic, so in practice, a compromise in the enforcement of the first two restrictions was reached.

The result of the eight cycles of selection in Base Intermix is seen in Figure 7.

FIGURE 7.—Results of RRS and Base Intermix selection studies. Deviations from control values are plotted against cumulative selection differentials.

The high line failed to respond to selection, and actually decreased slightly (about -0.02 eggs per cycle) according to the analysis of regression lines. (For details for the analysis of the two-way selection results see RICHARDSON 1965. The model fits a common intercept for the two selection lines of a given population, and also accounts for a correlation of the deviations of different selection lines from the control arising when the lines are tested together with a common control.) Conversely, the low line responded markedly to selection (about -2.06 eggs per cycle). The asymmetry is in part, no doubt, due to the inbreeding depression, since the inbreeding coefficient for each line at the end of the eight cycles was 0.35, and inbreeding depression is the rule for fecundity. However, for short term selection on traits of low heritability (which suggests only small changes in gene frequencies) with similar inbreeding in each line, the divergence between the high and low lines presumably is not materially affected by the inbreeding depression. The average rate of divergence for Base Intermix is 1.94 eggs per cycle, which is different from zero with a statistical significance of $P < 0.01$. In the last three generations of selection, crosses between the low and high lines were made, and the hybrids were tested for egg production. For the Base Intermix, the average performance of the cross was always between the mean egg counts of the selection lines, and near the average of the two lines for that generation, which further verifies the divergence of the selection lines.

The results of eight cycles of selection in RRS Intermix are also shown in Figure 7. The high line failed to respond to selection (-0.10 eggs per cycle), as in Base Intermix, and the inbreeding coefficient in this line at the end of the eight cycles of selection was 0.27. The low line of RRS Intermix responded to selection (-1.07 eggs per cycle), but to a lesser extent than the low line of Base Intermix. The inbreeding coefficient of this line was 0.29 at the end of the eight cycles of selection. Divergence of the two Lines for RRS Intermix was 0.90 eggs per cycle, which was different from zero with a statistical significance of $P < 0.01$, and also significantly less than the divergence of Base Intermix ($P < 0.05$). The results of the high $\times$ low line crosses in RRS Intermix were not as consistently in the middle of the selection lines as those in Base Intermix, but on the average, somewhere between the high and low lines. Consequently, the cross performance supported the conclusion of divergence but not quite as strongly as for Base Intermix.

DISCUSSION

Several sources of experimental results can be used to illuminate the genetic structure of Mather and Mono populations before and after the reciprocal-recurrent-selection process. Consequently, for review and summarization, a list of the relevant results is given.

1. *FFS purebred response*.—Both Mather and Mono failed to show a significant response to upward selection, but responded moderately to downward selection. The divergence, consequently, indicated some genotypic variance attributable to dominance and possibly nonallelic interactions within each population.
2. *RRS crossbred response*.—The crossbred response was marked at the beginning, and continued

for about 11 cycles of selection. Then the response decreased for 4 or 5 cycles and was essentially plateaued for the last 6 or 7 cycles of selection. This process indicated that there was genetic variation among the crosses between the two populations, which was extensively exploited in the early period of the selection experiment, so that there was little utilizable variation in the latter stage. There was a substantial increase in the percentage of sterility during the first several cycles of selection.

3. *RRS purebred performance*.—The purebred performances changed very little until some midway point of the crossbred-selection process, then rapidly increased until they plateaued and remained fairly stable from around the 18th cycle of the RRS selection. This plateau was 1.5 to 4 eggs per female per day below the plateaued crossbred performance. These results indicate that each of the original populations contained the genetic potential to improve their egg production to a level approaching that of their improved hybrid performance. The behavior of these two populations presented a striking contrast to the behavior of the FFS lines of the same original populations.

4. *Inbreeding depression*.—There was an inbreeding depression of about 1.5 eggs per 10% increase in the inbreeding coefficient in the Mather and Mono populations, and from the negative response to upward selection in both Base and RRS Intermixes, it can be deduced that there was inbreeding depression in these populations as well. The depression indicated the presence of genetic variation due to allelic and/or nonallelic interactions in each population.

5. *Performance during random mating phases of the intermixes*.—During the six generations of random mating in Base Intermix, there occurred a sharp rise in egg production to the F_3 generation, followed by a similarly sharp decline. This pattern of transitory nature suggests an association with a transitory genetic phenomenon such as linkage disequilibrium, but must be complicated further by nonallelic interactions. The pattern of the RRS Intermix was much more stable, with a slight peak in the F_1 , followed by a stable performance approaching the average of the parent populations. Mild heterosis and a subsequent decline in performance indicated a fairly simple mode of inheritance mechanisms, such as segregation of dominant and overdominant loci.

6. *Base Intermix divergence*.—The divergence of Base Intermix indicated about a 30% increase in additive genetic variation over the parent populations. (The ratio of the "realized additive genetic variances" of two-way selection in Base Intermix versus that in Mather, Mono, and pooled Mather and Mono was 1.17, 1.48, and 1.30, respectively, assuming previous upward selection, FFS, was essentially ineffective.) Consequently, it can be assumed that Mather and Mono were genetically different.

7. *RRS Intermix divergence*.—The divergence in RRS Intermix indicated the presence of genetic variation, but considerably less than for Base Intermix. The asymmetry of the divergence was also less than for Base Intermix, since there was less response to downward selection with neither population responding to upward selection.

One of the basic theorems of evolution, as formulated by FISHER (1930), is that the process of natural selection continues to maximize the mean fitness of populations. For models involving interaction of nonalleles, this theorem is known to lack universality (KOJIMA and KELLEHER 1961; MORAN 1964), though in certain restricted contexts it has led to several analytically useful corollaries. One of these corollaries is that a population genetically segregating for many genes affecting fitness tends to converge to a peak point of mean fitness when under a constant environment. Such a point corresponds to a peak on an adaptive surface (WRIGHT 1932, 1935). The number of possible peaks in a given genetic system in a given environmental condition depends upon the number of gene loci and the mode of gene interactions among these loci. In general, it is important to note that a multiple peak surface is possible only with nonallelic interactions (there are some known *exceptional* cases where multiple equilibrium systems may result

from causes other than nonallelic interaction, e.g., OWEN 1953). With tightly linked genes with epistasis the stationary points of mean fitness may not be exactly at peaks, but the number of stationary points probably will not decrease, and especially when the generalization includes more than two loci, the stationary points certainly become numerous.

Egg production, the trait studied, is undoubtedly one of the major components of fitness. Thus it is highly probable that the population mean of this trait under a population cage condition tends to lie ultimately near some stationary state. Consequently, it may be assumed that the genetic structure of Mather and Mono populations were approximately in such a state where the population means were near local peaks after having been kept in cages for more than two years. This assumption is supported by the lack of significant upward response but considerable downward response of FFS. The possibility that this equilibrium was maintained during FFS by counteracting natural selection is unlikely, since there was no obvious reduction in other fitness traits during FFS.

The base populations of Mather and Mono must have been segregating with respect to genes affecting egg production. This fact is demonstrated by the progress made by downward selection in each of them, the presence of inbreeding depression, and the changes in the level of purebred performance made as a correlated response to the crossbred progress in RRS. The genetic mechanism which maintained persisting segregation in Mather and Mono must be of the kind associated with "heterozygote superiority" through allelic and nonallelic interactions (KOJIMA 1959).

The Mather and Mono populations were of different genetic makeups, and must have been located at different points of equilibrium or quasi-equilibrium. This difference between the two populations is observed as an increase in genetic variation when the Mather and Mono populations are crossed to form Base Inter-mix (the greater "realized additive genetic variance" than in Mather and Mono separately).

After characterizing the genetic status of the initial Mather and Mono populations, the stage is set for comparing their variation patterns. Then among the alternative constitutions which could explain the RRS results, one can speculate on the variation sources present in Mather and Mono.

It is claimed, on a theoretical basis, that the method of RRS changes allelic frequencies in the following ways:

- (a) At gene loci with additive and dominance effects but without overdominant and nonallelic interaction effects, the frequencies of favorable alleles increase toward fixation in both purebreds.
- (b) At gene loci with overdominance effects, the frequencies of favorable alleles tend toward fixation in one purebred population and toward zero in the other population. Thus for overdominant loci, frequencies of heterozygotes tend to move toward one in the crossbred progenies (COMSTOCK, ROBINSON and HARVEY 1949).
- (c) At loci with nonallelic interaction effects, the frequencies of alleles in the entire interacting system in the two purebred populations are adjusted in such a way that the frequency of the best gene combination tends to one in the crossbreds. When the best genotype is heterozygous at some loci, the allelic frequencies at these loci tend to unity in one purebred population and

zero in the other. With respect to the loci which are homozygous in the best genotype, the fixation of these homozygotes will result in both purebred populations.

It is important to note that all the gene effects in the above statements are those expressed in the crossbred populations.

The fact that there was not a great deal of realized additive genetic variance in Mather and Mono suggests that there could not be too many important loci segregating where the gene effects were only additive and simple dominance in the purebred population. Most loci which fall in the category (a) under the crossbred gene action classification must have had neutral or overdominant effects in both purebred populations to have retained genotypic variation in a plateaued state but additive-with-dominance effects in the crossbred, to have allowed selection progress. Such a situation is possible with a system of multiple alleles, some of which are not common to both populations. Thus, it is possible that loci in this category existed in Mather and Mono, but there is no positive evidence for their existence in the present study.

On the other hand, there are more positive evidences for the existence of loci of the type discussed in categories (b) and (c). The selection progress of RRS crossbred performance and the correlated improvement of the purebred performance at a later stage can be explained only by the behavior of gene loci of category (c), when new mutations are excluded from consideration. Mather and Mono could have been maintained near local peaks, but the crossbred between them was not so located. When selection was applied on crossbred performance, the two populations gradually moved to another set of peaks that were evidently higher with respect to egg production than either the Mather or the Mono peak.

Another result compatible with the existence of a gene system with nonallelic interactions is given by the increase of egg production through F_3 and then a decline during the random mating cycles of Base Intermix. An interacting system with linkage can produce a pattern of performance such as seen in this phase (see RICHARDSON 1965, p. 58, for one possibility).

Base Intermix exhibited a release of genetic variation as a result of crossing two populations at different equilibrium points. If there were loci fixed for different alleles in the two base populations, segregation would result in the intermix. No new variation would be found where both populations were at the same equilibrium for given loci, but if one was segregating at equilibrium and the other fixed or the two were segregating at different equilibrium points, the intermix would show new additive variance.

The strong asymmetrical response observed in Base Intermix indicates a possibility of many loci with dominance in the direction of high egg production and with gene frequencies intermediate to high for favorable alleles in the intermix. There are other possibilities for asymmetrical response, but the present data are not definitive enough to distinguish among them.

When the plateaued Mather and Mono from RRS were crossed, the high and low response of the RRS Intermix was not as strongly asymmetrical as with Base Intermix, primarily because of less progress in the low line. Likewise, there was correspondingly less divergence. Thus, the RRS Intermix exhibited less genetic

variation than the Base Intermix. Less genetic variation in RRS Intermix, the approximately plateaued condition of the component populations of RRS Intermix, and the stable performance of the random mating phase of RRS Intermix indicate a lack of segregation among loci with nonallelic interactions which existed between the original Mather and Mono populations, probably as a consequence of extreme frequencies for the same alleles in both RRS purebred populations. Gene loci exhibiting dominance (with the favorable dominant allele originally absent or lost in one purebred population by drift) and overdominance (category b) remain as the major source of variation in the RRS Intermix, admitting the possibility of segregation of some multiple-allelic loci in which the component populations differed in allelic makeup from the beginning. Considering the degree of asymmetry of RRS Intermix, without any correction for inbreeding depression, a sufficient mechanism seems to be the segregation of overdominant or possibly of dominant loci.

For this interpretation, only one of several potentially possible consequences of previous selection has been considered. A complex system may take alternative paths while responding to selection pressures of similar kinds, but due to the lack of replication of selection lines in these experiments, only one of the alternatives could be expressed. Lack of replication, on the other hand, does not present the usual degree of impediment for reliable conclusions, since there are different sources of information from several logically related experiments which lead to generally consistent conclusions. This consistency serves to reinforce the reliability of the composite interpretation presented.

Addendum: A detailed analysis of the random mating generations of Base Intermix currently being conducted exposed the explanation for the discrepancy of the original and "retest" results reported in this paper (see Figure 5). In the parental generation, the flies to eclose first in a culture on the average lay more eggs per day than flies from a period of rapid eclosion (33.3 *vs.* 25.5, respectively).

The flies in the Base Intermix Retest were collected concurrently from cultures initially synchronized. Synchrony was lost by the time of eclosion, since the developmental rate of Mather by Mono hybrids was faster than that of the parental strains. Tested flies from the parental cultures were among the first to eclose, while those in hybrid generations were from a later period of eclosion. This difference did not exist in the original tests, where each generation was tested separately, and the flies were collected during a period of rapid eclosion.

In the current analysis, when flies collected at the period of rapid eclosion were compared, the trend of increasing egg production after the F_1 was again found, and the results are as follows: 25.5, 27.0, 31.3 eggs/female/day for parents, F_1 , and F_2 , respectively.

SUMMARY

Crossbred selection using reciprocal recurrent selection (RRS) and purebred selection using full-sib family selection were compared by KOJIMA and KELLERHER (1963) for their relative effectiveness in increasing fecundity in two populations of *Drosophila pseudoobscura*. Their results indicated a superiority of crossbred selection, where about a 25% increase in egg production was obtained contrasted to almost no change for purebred selection. The purebred populations of the crossbred selection experiment did not improve with respect to fecundity

until midway of the experiment, but later improved to a level slightly below the performance of the crossbred material. Both purebred and crossbred performances plateaued in this experiment.

For the purpose of elucidating the genetic characteristics of these populations, a pair of intermix populations were formed, one from the cross of the initial populations in the selection experiments of KOJIMA and KELLEHER, known as Base Intermix, and the other from the cross of the final populations from the crossbred selection scheme, known as RRS Intermix. Two-way selection was initiated on each of these intermix populations after a period of random mating without artificial selection.

An unexpected result appeared during the random mating phase in the Base Intermix population, where the performance of the F_1 to the F_3 increased markedly, and then declined by the F_6 to a performance level about like the F_1 . This peculiarity was retested and verified. It was concluded that this response pattern could best be explained by interaction of nonalleles and linkage.

These results, together with the divergence patterns of the intermix selection experiments (relatively greater divergence and asymmetry in Base Intermix than in RRS Intermix and two-way full-sib-family selection within purebred populations) were considered in speculating on a general description of the genetic systems of the initial and final populations in the crossbred selection experiment. It was suggested that the two initial populations were near their own points of adaptive maxima in the Wrightian sense with intermediate gene frequencies for mean performance in fecundity. Such situations can be expected with nonallelic interactions. Crossbred selections must have exploited the new genetic variation existing in their crosses in such a way that crossbred fecundity increased considerably. At the same time, gene frequency changes resulting from crossbred selection forced the individual purebred populations to move to a new peak, where purebred fecundity was higher than before, and the populations were more homozygous than previously. Each population went to essentially the same peak, with a majority of interacting loci near fixation for a synergistic combination of alleles. There was apparently some segregation of "overdominant" and/or dominant loci in the RRS Intermix populations, indicating that the final populations were being fixed in a complementary fashion with respect to these alleles.

LITERATURE CITED

- COMSTOCK, R. E., H. F. ROBINSON, and P. H. HARVEY, 1949 A breeding procedure designed to make maximum use of both general and specific combining ability. *Agronomy J.* **41**: 360-367.
- FISHER, R. A., 1930 *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- KOJIMA, K., 1959 Role of epistasis and overdominance in stability of equilibria with selection. *Proc. Natl. Acad. Sci. U.S.* **45**: 984-989. — 1963 Selection for interpopulation heterosis. (Abstr.) *Proc. 11th Intern. Congr. Genet.* **1**: 160.
- KOJIMA, K., and T. KELLEHER, 1961 Changes of mean fitness in random mating populations when epistasis and linkage are present. *Genetics* **46**: 527-540. — 1963 A comparison

- of purebred and crossbred selection schemes with two populations of *Drosophila pseudoobscura*. *Genetics* **48**: 57-72.
- MORAN, P. A. P., 1964 On the nonexistence of adaptive topographies. *Ann. Human Genet.* **27**: 383-393.
- OWEN, A. R. G., 1953 Genetical system admitting of two distinct stable equilibrium under natural selection. *Heredity* **7**: 97-102.
- RICHARDSON, R. H., 1965 A study of genetic variations of *Drosophila* fecundity by analysis of selection responses. Ph.D. thesis, North Carolina State University at Raleigh.
- WRIGHT, S., 1932 The roles of mutation, inbreeding, crossbreeding and selection in evolution. *Proc. 6th Intern. Congr. Genet.* 365-366. ——— 1935 Evolution in populations in approximate equilibrium. *J. Genet.* **30**: 257-266.