SELECTION FOR SEXUAL ISOLATION WITHIN A SPECIES

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Two mechanisms have been advanced for the origin of reproductive isolation between species. Muller (1939), dealing in the main with barriers to crossing in the later stages of species divergence, such as hybrid inviability and infertility, suggests that these arise almost by chance as a product of change in the genetic background either by genetic drift or as adaptation to different biological situations. This would lead to accelerating divergences as the process continues, or, as Muller puts it, "ever more pronounced immiscibility as an inevitable consequence of non-mixing." Dobzhansky's suggestion (1937), which is perhaps complementary rather than antagonistic to Muller's, is that when sufficient divergence between two species has arisen so that the hybrids are less well adapted for any available habitat than either parental type, there will be selection for sexual isolation. That is to say—if mating can take place and if the resulting hybrids are inviable or infertile, then natural selection will operate to reduce the chance that mating will occur, either by reducing the chance of encounter or the chance of mating with members of the other species when they are encountered.

Some writers, in discussing the mechanism proposed by Dobzhansky have suggested that "natural selection will favour any mechanism which prevents the wastage of gametes involved in unsuccessful hybridisation." This seems to be unduly teleological. Natural selection will only tend to suppress crossbreeding if those individuals which hybridise will in consequence pass on fewer gametes in the form of pure-bred offspring. It would seem probable that this would be more often the case in females than in males. In *Drosophila melanogaster,* for instance, females seem reluctant to mate again for a period of two or three days after an effective mating. If the first mating has been heterogamic, this will reduce the number of purebred offspring that she will produce in her lifetime. Gestation in mammals will have a similar effect. But the male, who must on the average have the same number of effective matings in his life as the female, is usually capable of many more if willing females are available. It follows then that willingness to cross-breed, which may merely be a sign of greater general sexual activity, will not necessarily reduce the number of purebred progeny that a male will leave. If Dobzhansky's mechanism for the establishment of sexual isolation is correct, it follows that it should be in the main a matter of female preference. Merrell (1954) has recently presented evidence that it is the female which exercises discrimination in matings between *D. pseudobscura* and *D. persimilis.*

Koopman (1950) has shown that selection leads to an intensification of the sexual isolation between these two species. Using marked stocks of the two species, he selected continually for purebred flies—the progeny of parents that had mated homogamically. He showed that the proportion of hybrids emerging declined dramatically after a few generations of selection. More recently, Wallace (1950) and King (private communication) attempted to demonstrate the production of sexual isolation by selection within a species. They used two stocks of *D. melanogaster,* from widely separated localities, which had each been marked by a different recessive gene. After 12 generations, when the experiment was first reported, little change in the proportion of wild-type flies emerg-

ing had been observed, but in subsequent generations the proportion declined significantly, showing that sexual isolation had been to some extent established. This was confirmed by observation of individual matings.

Our own experiment on very similar lines was started before we were aware of Wallace's work, and as our work was slightly different in conception, we decided to proceed with it. In Wallace's experiment, the mutants were used solely as markers, the stocks because of their origin presumably differing in many genes. As it happens, we had used in our work stocks marked with the autosomal recessive genes, ebony and vestigial, which has been extracted from a population in which the two had been segregating for many generations. The original stocks making up this population were actually those used by Rendel (1951) in his work on the effect of light on the mating of these mutants. Our two foundation stocks, both of which contained a considerable amount of genetic variability, were thus probably genetically very similar except for the marker genes. These genes were chosen because of the ease of scoring but they do react differently to light and, as Rendel has shown, ebony males mate more frequently in the dark than in the light.

In the first experiment of this type that we carried out, there appeared in the seventh generation some flies that were both ebony and vestigial, indicating that in previous generations either a non-virgin female or else a wild-type heterozygote had been used as a parent with the result that each mutant stock was contaminated with the other gene. Theoretical consideration of the effect of this showed that the proportion of double recessive flies should increase by a factor of four each generation until they reached a level of 11% of all flies emerging. At that point, the proportion of flies in each mutant stock that were heterozygous for the other gene would be ⅔. There would then be a continual interchange of genes between the two stocks. In addition, one-third of the apparently pure mutants used as parents would be derived from heterogamic matings, thereby reducing the selection for sexual isolation. We therefore discarded the line and started afresh with stringent precautions against non-virginity, parents being collected over a 7 hour period. In the two experiments presented here in detail, no double recessive flies were ever observed.

Description of Experiments

Box Experiment

Two mutant strains of *D. melanogaster* homozygous for the genes ebony and vestigial respectively were used. They had been extracted from the same population, after segregation for many generations. At the start, 54 males and 54 virgin females from each of the stocks were put together into a breeding box (size 18″ × 18″ × 7″) which contained 10 unstoppered ¼ pint bottles of maize meal–molasses–yeast–agar medium. Flies were etherised for counting, but were not put into the box until three hours after complete recovery from anaesthesia. The box was then placed in a constant temperature room at 25° C. All phases of the experiment were done at this temperature. The box was always put in the same part of the room, where, due to the direction of the light, two sides of the box near the edge were in slight shadow. The ebony flies, immediately the box was positioned, migrated towards the light source, that is, towards the shaded edge. After some time, the majority of them moved more freely about the cage.

After six days of mating, the ten food bottles were removed, cleared of any flies which remained inside, and stoppered. The parents were discarded. The count of the next generation was started five days afterwards, i.e., on the eleventh day after the parents were put into the box. Three types of flies emerged; hybrids from heterogamic matings, and the two mutants ebony and vestigial from

homogamic fertilizations. For 3½ days every fly which emerged was counted. The culture bottles were completely cleared at 10 A.M. Flies which emerged by 5 P.M. on the same day were segregated and mutants were kept in separate vials to be used as parents for the next generation when 1 to 4 days old. When insufficient virgins were obtained, those collected were bred with their own kind, and the experiment carried on from their progeny. In the box experiment, this was done three times in 38 generations.

In order to ensure that any changes in external conditions had not affected the course of the experiment, controls were done on the box experiment in the later generations. Parent virgin flies were obtained from the original stocks and put into a box of identical proportions to the experimental one. The control box and the experimental one received exactly the same treatment throughout. This was done seven times between the 25th and 35th generations.

Jar Experiment

An experiment on similar lines was run in conjunction with the cage one. A 2 lb. glass jar containing approximately 1″ of food was used as the breeding chamber. The number of parent flies employed in this case was between 20 and 30 of each sex of the mutants. Again, it was sometimes necessary to mate the virgins with their own kind to produce sufficient numbers for the next preferential mating. This was done three times in 33 generations. From generations 1 to 12 the parent flies were still under ether when put into the jar, as it was thought that they might otherwise escape. This was found, however, to be unsatisfactory. So from the 13th generation onwards the parents were introduced into the jar three hours after recovery from the ether. The jar was put into the same constant temperature room and at the same time as the box. Thereafter, all operations, such as clearing parents from the jar, counting and segregating flies of each generation, etc., were carried out at the same time and in an exactly similar manner to the box experiment.

Because of the small capacity of the jar compared with that of the box and the fact that there was little or no variation in the light within the jar, it was assumed that any tendency towards an eco-

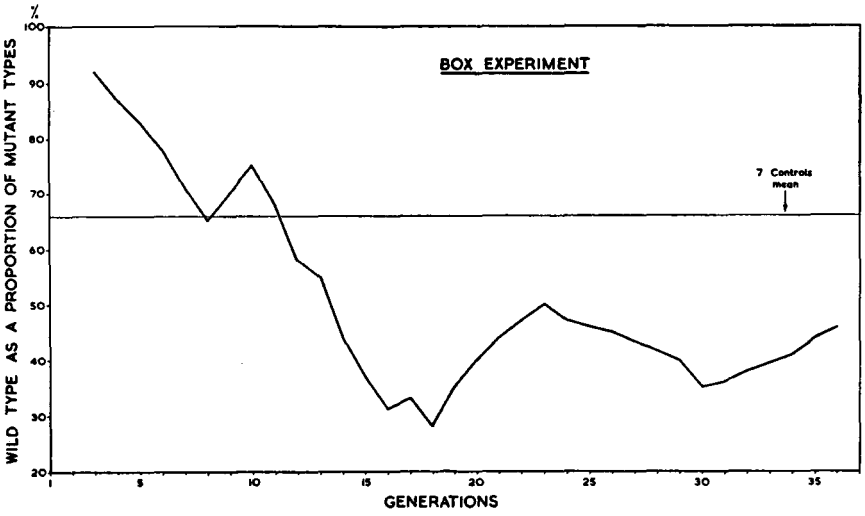

FIG. 1. Results of box experiment. Number of hybrids expressed as a percentage of sum of ebony and vestigial emergences.

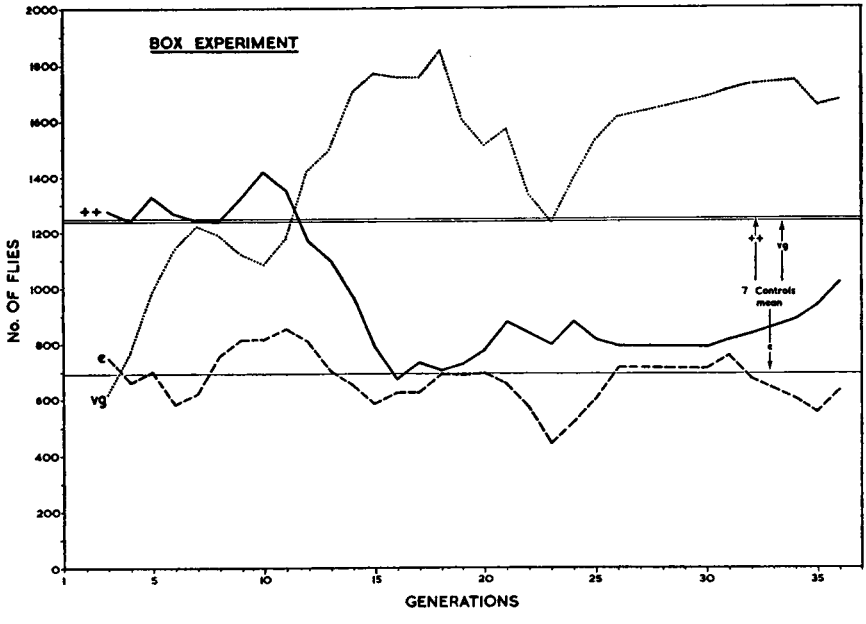

FIG. 2. Results of box experiment. Numbers of 3 types of flies emerging shown separately.

logical isolation between the ebony and vestigial flies would be eliminated.

RESULTS

One of the first impressions at the start of the experiments was of the great fluctuation in results from generation to generation. The jar experiment was in fact started to try to remove this by having all flies developing in one food mass. Our criterion of isolation has been the ratio of wild-type flies, produced by heterogamic matings, to the total number of mutants produced by homogamic matings. The standard deviation of this ratio due to chance fluctuation, determined from the mean square difference between successive generations, was 0.16 for the box experiment and 0.24 for the jar. This fluctuation is equal to that produced by random sampling of 160 units and 70 units respectively from a population made up of two types of objects with equal frequency. The total count was actually of the order of 2,000 flies in both cases. But the number of female parents was 108 and 50 in the box and jar respectively. The observed fluctuations suggest that the effective units are the initial inseminations of the individual females. In this respect, it is of interest that of the individual platings of females taken from the box after six days of mating, 660 gave offspring all of the same type and only 75 had mixed offspring. However, whatever the reason, it is still true that too many flies were counted each generation and a sample of a quarter of the size that we took would have been quite adequate.

Box Experiment

The results of the box experiment are set out graphically in figures 1 and 2. The graphs are moving averages over 5 generations to smooth out fluctuations. In figure 1, the number of hybrids is expressed as a percentage of the sum of the ebony and vestigial emergences. Figure 2 shows separately the numbers of the three types of flies emerging. From the first to the eighteenth generation a more or less steady decline in the percentage of hybrids is noted. The lowest percentage of hybrids in any individual gen-

eration was 10.3% at the eighteenth, with emergences of ++ 246, e 736, vg 1640. Only once afterwards, at the 23rd generation, does the vestigial line graph fall as low as the control mean for this mutant. Thereafter the values remain high for vestigial emergences. The hybrid figure drops, and is lowest between the 16th and 18th generations, only rising a little and slowly towards the end of the experiment. During the whole 38 generations the emergence values for ebony alternate slightly above and below the figure for the control mean. This suggests that the sexual isolation, after the 18th generation, is due mainly to the increase in the number of homogamic matings of the vestigial flies.

The average values for the seven control generations are also shown in figures 1 and 2. The proportion of wild-type flies to mutants averages 0.66, compared to the proportion in the selected population at the same period of 0.38. The figures for the individual mutants show that the change is due to a decrease of wild-type flies and an increase of vestigial.

It has been shown by Rendel (1951) that ebony reacts to light intensity in its mating behaviour. It seemed possible that the sexual isolation was due to an accentuation of this response. Towards the end of the experiment, therefore, duplicates of the selection box were made up from parents from the selected stock but were kept instead in complete darkness. The ratio of wildtype to mutant offspring was 0.48 compared to 0.46 for the three contemporary generations in the light. It seems therefore that the demonstrated sexual isolation is not concerned with phototropic response. However, there were many more ebony flies in the dark boxes—in fact the average of the three tests (1102 flies) had only once been exceeded by a single generation in the light, and the average in the last few generations of the latter was about 650. There was correspondingly a shortage of vg flies, but the proportional effect was not so great. This agrees with Rendel's observation that ebony males show greater sexual activity in the dark.

TABLE 1

Controls ♀	Inseminated by e	Inseminated by vg
e	71	69
vg	41	63
Selected stocks		
e	151	108
vg	77	142

Between the 20th and 30th generations, the females were placed in individual vials after they had been removed from the box, and their progeny were examined on emergence. This was done with 6 generations of the selected stocks and with three of the controls. The results in terms of effective matings are given in table 1.

There is a slight tendency to homo-

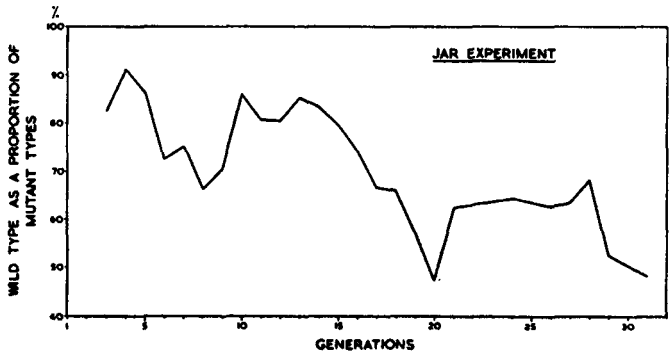

FIG. 3. Results of jar experiment. Compare figure 1.

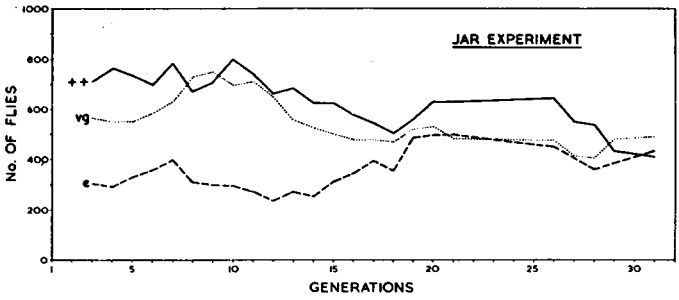

FIG. 4. Results of jar experiment. Compare figure 2.

gamic mating in the controls but the heterogeneity χ^2 is only 2.15. In the selected stocks the tendency is much more marked and the χ^2 value is 27.14. This is confirmatory evidence that some degree of sexual isolation has been obtained in the selected population.

It might have happened that this type of selection, picking out always the mutant flies, would have affected the segregation ratio by selecting those genes favouring the survival from egg to adult of the mutant types. However, a check based on several thousand flies at the end of the experiment showed no differences between control and selected stock in the segregation ratio for either mutant.

Jar Experiment

The results for the jar experiment are given in figures 3 and 4. In the jar, the light intensity was much more uniform than in the box and in addition the flies were more confined—the volume of the jar being of the order of $\frac{1}{50}$ of that of the box. Here again there is a decline in the proportion of wild-type flies as the experiment proceeds, although the proportion at the end is a trifle higher than in the box experiment. However, the ratio of wild-type to mutant flies has declined from 80% to between 50% and 60%. As was noted above, there was a change in method in the middle of this experiment. Up to generation 12, flies were put into the jar etherized but afterwards they were put in an active state. This change does not appear to have affected the sexual isolation. In the five generations before the change, the average ratio of wild-type to mutants was 0.86 and in the five after the change it averaged 0.80. It may however have affected the separate types. The numbers of vg and wild-type flies decline by about one-third as a result of the change, whereas the ebony count is unchanged. The subsequent change in the wild-type/mutant ratio appears in this case to be due to an increase in the number of ebony flies. Control experiments were not carried out on the jar population, as the latter was subsidiary to the main experiment in the box.

Sexual Preferences in Inbred Lines and Closed Populations

It is convenient to present here a small amount of data on sexual preferences between lines and populations chosen at random, with a bearing on the "chance" occurrence of sexual isolation. These experiments were carried out by the usual "male choice" method in which males are given equal numbers of two types of female, one of which is recognisable—in our case by a spot of silver paint. The females are then examined for the presence of sperm in the seminal tract. In the first case, two wild-type inbred lines of completely different origin were used, given in our stock list the symbols W20 and K7. The results are given in table 2, which shows the proportion of females inseminated. In all cases, the ♂♂ were equal in number to the ♀♀ of each of the separate lines.

A similar experiment was then done

using lines which, although of common origin, had been selected in different directions without inbreeding for 20 generations for number of chaetae on the 4th and 5th abdominal sternites. There was no overlap in chaetae count between the high and low lines used, so that this character could be used for identifications. The results are given in table 3, for matings between one high line, H1 and two low lines, L4 and L5.

In these rather meagre results, there is little suggestion of sexual isolation having developed by chance in either the inbred experiment or in that with the selected lines. In the latter, it is of interest that the selection for the quantitative character has caused a differentiation in mating ability. The Hl ♂♂ are poorer than those from the two low lines but on the other hand the high ♀♀ seem to be better. But this seems to be a general change in sexual drive, not specifically adapted to the other sex of the same line. Wallace (1955 in press, and personal communication) has also tested whether the mere isolation of two populations is sufficient to cause sexual isolation to arise between them. His populations had been separated for 80–100 generations, and differed in certain morphological characters (primarily abdominal pigmentation). In an extensive series of tests, no tendency towards preferentially mating could be detected nor does there seem to have been any evidence of differences in the intensity of general sexual drive.

TABLE 2

♀s marked	♂	W20 ♀	K7 ♀	Duration of mating
W20	W20	22/23	11/25	40 mins.
	K7	17/23	9/25	40 mins.
W20	W20	17/19	16/20	70 mins.
	K7	4/20	1/19	70 mins.
K7	W20	9/10	5/10	30 mins.
	K7	8/14	9/14	60 mins.
K7	W20	10/20	2/19	30 mins.
	K7	10/20	5/20	45 mins.

TABLE 3

♂	H1 ♀	L4 ♀	Duration of mating
H1	3/10	1/9	30 mins.
L4	9/14	3/14	30 mins.
	H1 ♀	L5 ♀	
H1	11/20	5/19	60 mins.
L5	20/24	17/25	60 mins.

DISCUSSION

Our results may be summarised in the statement that some sexual isolation developed when we selected for a tendency towards homogamic matings, but that none was found to have arisen by chance in a few lines which had been selected for abdominal chaeta number or inbred. Laboratory experiments on evolutionary mechanisms can, of course, only be indicative and not demonstrative—they can show what might happen in wild populations, rather than what has happened. As far as they go, our experiments lend support to the mechanism suggested by Dobzhansky rather than that discussed by Muller. But when attempting to apply these results to occurrences in nature, one must bear in mind the ways in which artificial populations may fail to imitate conditions in the wild.

It is perhaps misleading to put Muller's hypothesis of the chance origin of sexual isolation in antithesis to that of Dobzhansky, which attributes it to the action of selection. In all probability, both mechanisms have operated in the wild in different cases. Some evidence supporting Dobzhansky's hypothesis comes from Dobzhansky and Koller (1938) who found, in an analysis of crosses between *Drosophila pseudoobscura* and *D. miranda*, that the isolation was greatest between races close to each other in their range. King (1947) has similar evidence from the *guarani* group. However, even between races of the two species widely

separated in origin, the isolation was considerable. One has the suspicion that sexual isolation is common between species which have never had the opportunity to crossbreed, though the evidence is rarely conclusive since seldom if ever do we know the full evolutionary history of the populations.

It is perhaps not surprising that differences in sexual behaviour arose in the experiments involving selection, but were not found in the comparison of populations which has originated independently. If they had occurred in the latter, they could only have appeared by chance, or as a correlated response. It might be expected that random changes in sexual behavior would be slow, even though they arose as a secondary response to an adaptive change in the population. Mating involves the cooperation of the two sexes and it seems unlikely that a genetic shift in the population causing a change of sexual behaviour in the female, perhaps by a modification of the pattern by which a male recognises an animal of his own species, would also change male behaviour in a compensating manner (although an exception to this might be in habitat preference). An individual with aberrant sexual behaviour is not likely to leave many progeny. A population gradually changing its genetic situation could only change its pattern of mating by the selection of males capable of responding to the altered female behaviour. This must constitute a brake on the change of mating behaviour either by chance changes in the genetic situation or even as a correlated response to an adaptive change. This will be particularly true of inbred lines in which selection between potential mates is small or non-existent. Reproductive behaviour, excepting perhaps choice of habitat, would therefore be more stable than other physiological systems to genetic changes.

Both the hypotheses that we have discussed demand the development of a previous geographic isolation before sexual isolation can be established. In this sense, the selection hypothesis is perhaps clumsy, since in the formation of a new species showing sexual isolation with the parent species it demands first a geographic isolation and then an overlapping of the species range so that members of the two species can be selected for refusal to crossbreed. It seems to us that sexual isolation instead of being a consequence of geographic isolation, may be a contributory factor in its establishment. The spread of a population into new territory will often involve the occurrence of genotypes with new hereditary habitat preferences. The existence of such preferences amongst Drosophila stocks has recently been shown by Waddington, Woolf and Perry (1954). In organisms such as birds, in which rather sudden changes in geographical or ecological range are well-known, learning may play a part, but this may also have an important genetic component. In the genetic constitution of a sub-population which has broken out of the original species boundaries and is spreading into new territory, one must expect to find that a number of adjustments are occurring simultaneously. There is most likely to be, in the first place, an evolution of a new system of habitat-preferences and/or of general activity; in the second, the adaptive characters and general fitness of the migrating group will be attuned to the new circumstances which it has to meet. Both these necessary modifications of the gene pool will be made more easily if the genetic constitution of the sub-population is prevented from continual intermingling with that of the original stay-at-home group. Thus any tendency for preferential mating within the migrating group, and sexual isolation between it and the main population, will acquire selective value. It seems rather probable that a species may be able to spread into new territory even if no sexual isolation develops between the main population and the migrating one; but if the increase in species-range demands considerable adjustment of the genotype to fit the new

environment, the evolution of some degree of mating-barrier will undoubtedly be of considerable advantage. Our experiments show that the necessary genetic variability is likely to be present in a population; and the fact that the change of environment involves alterations to the behaviour pattern of the migrating animals makes it more likely that their preferences for sexual partners as well as for habitats, will exhibit evolutionary flexibility. Thus species-spread and sexual isolation will tend to act synergistically.

Summary

1. Partial sexual isolation (between two stocks of *D. melanogaster* differing only in marker genes) has been established by selection of the offspring of flies mating with their own type. This has been demonstrated by a reduction in the number of cross-bred offspring found and also by examination of the progeny of individual females.

2. In a small series of tests, no tendencies towards preferential mating were found to have arisen by chance in a sample of lines which had been inbred or selected for number of abdominal chaetae, although there were differences in intensity of sexual drive.

3. Changes in reproductive behaviour brought about by selection are more likely to affect female than male behaviour. Willingness to cross breed, in a male, will not materially reduce the number of his pure-bred offspring but in a female it usually will.

4. It is argued that selection pressure against cross-breeding of two partially separated populations, although probably effective when it occurs, is not likely to be the only mechanism by which sexual isolation between taxonomic groups develops in nature. It is suggested that an important part in the origin of such isolation may be played by the factors (e.g. changes in hereditarily controlled behaviour patterns) which bring about the spread of an initial panmictic population into new geographical or ecological situations.

Literature Cited

Dobzhansky, T. 1937. Genetics and the Origin of Species. Columbia Univ. Press, New York.

Dobzhansky, T., and P. C. Koller. 1938. An experimental study of sexual isolation in Drosophila. Biol. Zentral., **58**: 589–607.

King, J. C. 1947. Interspecific relationships within the guarani group of Drosophila. Evolution, **1**: 143–153.

Koopman, K. F. 1950. Natural selection for reproductive isolation between Drosophila pseudoobscura and Drosophila persimilis. Evolution, **4**: 135–148.

Merrell, D. J. 1954. Sexual isolation between Drosophila persimilis and Drosophila pseudoobscura. Am. Nat., **88**: 93–100.

Muller, H. J. 1939. Reversibility in evolution considered from the standpoint of genetics. Biol. Rev., **14**: 261–280.

Rendel, J. M. 1951. Mating of ebony, vestigial and wild-type Drosophila melanogaster in light and dark. Evolution, **5**: 226–230.

Wallace, B. 1950. An experiment on sexual isolation. D.I.S., **24**: 94–96.

Waddington, C. H., B. Woolf, and M. Perry. 1954. Environment selection by Drosophila mutants. Evolution, **8**: 89–96.