Recessive Mutants in a Wild Drosophila melanogaster Population[1]

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INTRODUCTION

Mutations in wild populations are the raw materials of evolution. They may result from spontaneous mistakes in gene replication, environmental conditions such as radiation, and the effect of chemical mutagens or mutation-producing agents, many of which are organic products of the

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organism's own body. Most *Drosophila* in nature are phenotypically wild-type. The genotype, however, often includes recessive genes masked by the wild-type allele. Dominant and sex-linked mutations are recovered less frequently because of increased pressures of selection against them, i.e., there is little buffer between them and the environment. Recessive mutants, even if detrimental in a homozygous condition, are retained for a long time in the gene pool of the species, since the probability of obtaining a homozygous recessive individual is normally low and selection, therefore, slow. In addition, heterozygosity itself is frequently advantageous to the individuals, producing an effect called "hybrid vigor."

In the laboratory, series of crosses, such as will be described later, are used to analyze the chromosomes and determine the frequency with which a certain recessive mutant is carried. Such an analysis may eventually be useful in investigating the rate of mutation in the population.

Several population genetics studies of this type have been done with *Drosophila* species. Tschetwerikoff, for example, collected 239 wild *Drosophila* and, in analyzing them, found 32 different recessive mutants carried in a heterozygous condition (Gardner, 1960). Dubinin found that the number of mutant genes carried varies with the season (Gardner, 1960). In a study of 130,000 wild *D. melanogaster* from several locations in southern Russia, Dubinin and his co-workers found more than 2,800 "aberrant" individuals, many of which carried mutant abnormalities (Dobzhansky, 1941). Dobzhansky (1937), studying *D. pseudoobscura,* found that approximately 25% of all tested autosomes carried a recessive lethal or semilethal mutant, while 4-14% of the chromosomes carried a sterility mutant.

The present study represents an attempt to analyze the mutants causing morphological changes carried in the wild populations of *D. melanogaster* in and near Marshall County, Oklahoma. Special attention was given to mutants affecting the wings, bristles, and color of body and eye.

Methods

Wild *D. melanogaster* were collected in adapted funnel traps (Thompson, in press). *Drosophila,* attracted by the banana bait, enter a small funnel opening into a glass jar. Because the opening is small, finding it again is difficult, and the flies are trapped. Collections were made in the evenings at four major areas, concentrated collections being made on the campus of the Oklahoma University Biological Station, Willis, Oklahoma. Other collection sites were near the Willis Bridge, Texas, five miles southwest of the station; Madill, Oklahoma, approximately 20 miles north; and Nocona, Texas, approximately 60 miles southwest. Collection sites on the station grounds were centered around the kitchen area. Grocery stores at the other locations served as trapping sites. Collections were made from 22 June to 16 July 1966.

The present series of crosses was divided into two sections. One section utilized wild females, and the other utilized wild males and virgin females gathered as larvae at collection sites. The first section was made up of 77 tested females, while the second section comprised 16 male-female crosses.

Wild females were placed singly in culture vials as they were collected. The food used in their vials was a Cream-of-Wheat and molasses medium (Demerec and Kaufmann, 1964). Females were allowed to lay eggs and their offspring to develop. Ten virgin females were collected from each vial as they eclosed and were placed singly in 10 fresh culture vials. To each vial a single brother was added. The offspring of each of these crosses were collected, classified, and recorded. By following

this method, a recessive mutant carried by the original female or the wild inseminating male would have a high probability of becoming homozygous in the second generation and being identified in approximately one-fourth of the offspring of one-fourth of the second generation.

The second series of crosses, utilizing wild males, was carried on in a similar manner. The main difference was that instead of using already inseminated females, pair matings were made between wild males from the traps and females collected as larvae and gathered as they eclosed in prepared culture vials. Offspring of these crosses were mated in 10 pairs, as described above, and the second generation was collected and classified.

Bridges and Brehme (1944) was used, insofar as possible, as a reference of mutant types. However, further tests must be run to definitely identify the mutants recovered in these crosses. Thus, mutants in the results section are listed by a short morphological description, instead of by name. All mutants reported are only "presumptive mutants" subject to confirmation by later tests.

RESULTS

In section 1, involving trapped wild females, 77 females were tested. Of these, 67 tests were successfully completed; the others were terminated by mold. For each female 10 vials were made to recover any recessive mutants present on the chromosomes. From this total of 670 vials, 26,597 second generation offspring were collected and examined. The results of the examination are presented in Table I. Presumptive mutants are listed under four categories: bristle mutants, color mutants, wing mutants, and miscellaneous. Wild-type (normal) flies accounted for 20,353 of the offspring, as was to be expected, for the vast majority of flies should carry at least one set of normal genes. Of the now homozygous mutant individuals, the most prevalent phenotype was a variably expressed bristle mutant which shortened and thickened bristles at random, affecting all or part of the total number of bristles. This was present in 65 (97%) of the females tested.

In section 2, involving wild males, 16 tests, including an unsuccessful one, were attempted. From the 150 vials, 3708 second generation offspring were collected and classified. The results are summarized in Table II. Wild-type flies again accounted for the great majority, 2678 individuals. The same bristle mutant encountered in section 1 was again found in 14 of the 15 sets and was identified in a total of 927 offspring. Three mutants were identified in the second section of crosses which were not present in section 1. These were brown eye color, rudimentary-like wings, and nicked wing tips.

In the stocks which were made of the mutants recovered in these tests, three additional mutants were later discovered. These were delta-like wings, erected wings, and wings bent at two places over the abdomen. Since these were not recovered as a definite number of individuals from a known number of original vials, they can not be included in the tables.

Although collections were made at several different locations, the results of such collections were not greatly different from the main collections on the biological station grounds. All collections are thus reported together, with no notation as to location.

DISCUSSION AND CONCLUSIONS

In the 93 attempted tests reported here, a total of 38 distinguishable mutants were recovered from 82 successful tests. As mentioned before, 11 sets were killed by mold growth. Most sets contained more than one mutant, and some carried as many as eight. Counting each different

TABLE I. OCCURRENCE OF MUTANTS: SECTION 1

Mutant		No. of sets involved	No. of indiv. involved
A.	Wild-type (normal)	77	20,353
B.	Bristles		
	1. Irregularly stubbled	65	4957
	2. "Stubble"-like	2	327
	3. "Singed"-like	1	1
C.	Color		
	1. Orange eye	5	55
	2. Brown-streaked thorax	5	52
	3. Yellow body	3	143
	4. Brown body	3	61
	5. Light-red eye	2	20
	6. Red-brown eye	1	46
	7. "Plum"-like eye	1	35
D.	Wings		
	1. Bent wing base	19	138
	2. Fragments of extra veins present	12	106
	3. "Dichaete"-like	8	88
	4. Notched	5	22
	5. Vein V not touching the wing margin	4	15
	6. U-shaped wing notch	3	8
	7. Wavy	3	indet.
	8. Small, almost opaque, wrinkled	2	35
	9. Vein LII broken	1	10
	10. Folded	1	7
	11. Thickened veins	1	14
	12. Eave-like	1	6
	13. Held out with rough veins	1	14
	14. Vein LII forked	1	13
	15. "Miniature"-like	1	20
	16. Posterior crossvein half present	1	32
	17. Added crossvein	1	4
	18. Ballooned	1	5
E.	Miscellaneous		
	1. Abnormal abdominal segmentation	9	87
	2. Sterility mutant	2	— *
	3. Low viability factor	1	— *
	4. Rough eye	1	4
F.	Others (unsuccessful)	10	—

* These mutants are functions of the set, not of individuals.

occurrence of a mutant separately, 249% of the tested females in section 1 carried a recessive mutant, and 206% of the original parents in section 2 carried a mutant. It should be noted that this only includes morphological mutations, with the exception of two affecting fertility and viability. It is also important to remember that the morphological mutants are concentrated in only three main areas. Although these are very representative and important characters, other mutations could have occurred which, because of their nature, were not recognized. Two nonmorphological mutants were tentatively identified. This is unusual, since the crosses were not intended to pick up this type of mutation. Mutants affecting viability and fertility must normally be detected using a completely dif-

TABLE II. OCCURRENCE OF MUTANTS: SECTION 2

Mutant	No. of sets involved	No. of indiv. involved
A. Wild-type (normal)	16	2678
B. Bristles		
1. Irregularly stubbled	14	927
C. Color		
1. Brown eye color *	1	21
2. Orange eye color	1	12
D. Wings		
1. Bent wing base	4	12
2. Fragments of extra veins present	3	20
3. Vein LII broken	1	15
4. Ballooned	1	8
5. "Rudimentary"-like *	1	6
6. Nicked wing tips *	1	5
7. Vein V not touching wing margin	1	4
E. Miscellaneous		
1. Abnormal abdominal segmentation	3	10
F. Others (unsuccessful)	1	—

* These mutants did not occur in Section 1.

ferent system of matings. Thus, the number of mutants reported here, though rather large, is almost certainly only a small fraction of the actual existing mutants carried by the original parents and by the population at large.

The significance of these data can best be evaluated by comparing them with data from earlier similar studies. The study by Tschetwerikoff (Gardner, 1960), though carried on under different conditions, affords such a comparison. Tschetwerikoff collected 239 wild *Drosophila* and obtained 32 different recessive mutants. Thus far this series has tested 82 wild *Drosophila melanogaster* and recovered what appears to be 35 different mutants plus three appearing later in the stocks. It should also be noted that Tschetwerikoff studied all body parts while this series concentrated on wing, color, and bristle mutants. Thus, it can be seen that the results of this latest series are strikingly different from the earlier ones. The number of mutants carried in the population now under study appears to be significantly greater. Possible reasons for such an increase are very important to investigate, especially if this same increase in gene pool mutants is occurring in other animals, including man, for an increase in the mutants carried in a population increases the number of mutant individuals that will occur.

There are several possible explanations for the increase in recessive mutants. One concerns the area in which the tests were carried on. It has been shown (Muller, 1928) that high temperatures can increase the mutation rate under certain conditions. The present study was made in the temperate climate of Oklahoma, while that of Tschetwerikoff was done in the much colder Ural Mountains. There is also the possibility of different selection pressures under these varied environmental conditions. Radiation is yet another possibility; its mutation-inducing effects are well known. Radiation from fallout, however, has been estimated to be from 1/10 to 1/30 the level of natural background radiation, and conse-

quently will only account for a small percentage of the observed spontaneous mutants (Glasstone, 1962). Additional investigations will need to be planned to establish the cause, if possible.

A certain bristle mutant was observed in the offspring of 97% of the original wild parents. Although this at first seems unusual, it can be fairly easily explained. In a hypothetical scheme, the production of normal bristles proceeds as a chain of reactions each of which is catalyzed by a different enzyme: A—>B—>C—>D—>bristle. Each enzyme is produced by a different gene or genes. Thus, a mutation at any one of the several loci involved in bristle production would result in a mutant bristle phenotype. In a population carrying several different bristle-affecting mutations, the frequency of their occurrence would likely be high enough so that a very large percentage of the population would carry one or more of them, especially if the heterozygous condition were beneficial as in hybrid vigor. Several mutations of this type could also explain the variability in the expression of this mutant. The other mutants obtained in this study differ, in that they represent mutations at a single gene locus. Likewise, one further possibility is that the bristle mutant represents a single mutation which, due to hybrid vigor, is carried in a heterozygous condition in a large number of individuals. It is hoped that future studies will clarify this phenomenon.

From the above discussion it can be seen that the number of mutants carried in a heterozygous condition is much greater than reported by earlier investigators, indicating an increased rate of mutation and/or a reduction in selection pressure against the mutant alleles. Further study must be made, however, to determine the cause of this phenomenon.

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