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THE FREQUENCY OF LETHAL SECOND CHROMOSOMES OF DROSOPHILA MELANOGASTER FROM EASTERN TENNESSEE[1]

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INTRODUCTION

In their studies designed to obtain insights into the extent and role of genetic variability in natural populations, Drosophila workers have concentrated on the classes of lethal and semilethal chromosomes, which, for technical reasons, are among the most objectively and readily estimated of mutations in the genus Drosophila. The earlier work is reviewed by Dubinin (1946), Spencer (1947), and Dobzhansky (1951). References to some more recent work are contained in the papers of Goldschmidt *et al.* (1955), Paik (1960), and Dawood (1961).

The distribution of this genetic variability is poorly understood. For example, Dubinin (1946) reported a seasonal fluctuation in the frequency of lethal chromosomes in Russian populations of *D. melanogaster,* but Goldschmidt *et al.* (1955) failed to detect any such changes in Israeli populations of the same species. Although Pavan *et al.* (1951) found that there was a high, uniform distribution of lethal and semilethal chromosomes throughout many South American populations of *D. willistoni,* the marginal populations of Florida and Cuba (Townsend, 1952) and those of Argentina (Cordeiro *et al.*, 1958) were found to contain significantly fewer. Ives (1945, 1954) discovered that lethal second chromosomes in populations of *D. melanogaster* from northern parts of the United States were significantly less frequent than in populations from southern parts. He (1945) generalized that there was a north to south increase in frequencies of these lethals, possibly due to the increased favorable breeding season in the southernmost states.

If Ives' theroy is correct, one would expect a geographically intermediate population to have an intermediate frequency of lethal second chromosomes. The present study was undertaken mainly to provide such a test.

The frequency of allelism among the lethal and semilethal second chromosomes may be used to estimate the effective breeding size of the populations from which these chromosomes are derived (Dobzhansky and Wright, 1941; Ives, 1945). An estimate was obtained in the present study; and tests were made for the presence of segregation-distorter (S-D) factors, such as Sandler, Hiraizumi and Sandler (1959) found in the same species.

MATERIALS AND METHODS

Three samples of *Drosophila melanogaster* males were trapped on October 19 and November 3, 1960, at Wallace's Orchard, U. S. Route 25W, five miles northwest of Knoxville, Tennessee. These males were taken to the laboratory and individually mated to several *CY/cn bw* virgin females (*Cy*=Curly wing, with inversions in both arms of chromosome II and a recessive lethal effect; *cn* = cinnabar eye color; *bw* = brown eye color; but the double mutant homozygote, *cn bw/cn*, has white eyes). Among the descendants of each wild male only two F_1 males were used in further crosses: one male of the genotype *Cy/+* (phenotype, Curly) and one of the genotype *cn bw/+* (phenotype, wild type). The former was used in the lethal tests and the latter in the S-D tests.

In the lethal tests, a single F_1 *Cy/+* male was mated with several virgin *Cy/Pm* females (*Pm* = Plum eye color, with a recessive lethal effect). From the F_2, five pairs of *Cy/+* virgin sibs were mated, and the F_3 that resulted were counted. To insure a greater yield in the F_3, the parents were transferred to fresh bottles after five days. If the tested chromosome was normal, the expected proportion of non-curly (i.e., wild type) flies was one-third, because of the lethality of the homozygous Curly chromosome. Individual lines were classified according to the percentage of wild type flies. This classification, modified after Hiraizumi and Crow (1960) had four viability groups; those lines with no wild type (+/+) flies were scored as lethal; those showing from 0.0 to 16.7 per cent wild type, as semilethal; those showing from 16.7 to 25.0 per cent wild type, as deleterious; and those showing from 25.0 to 41.0 per cent wild type, as normal.

A minimum of 500 fiies was counted for each line. To be scored as lethal, a line had to produce no wild type flies in two consecutive generations of P_3 crosses. At least 200 offspring were counted in each of these tests. All of the proved lethal lines were outcrossed to each other to provide evidence concerning the presence of allelic lethals. Allelism was proved if only Curly flies were produced from such an outcross; if two lines contained nonidentical lethals, non-Curly flies appeared.

For the S-D tests, the F_1 *cn bw/+* males were mated individually to white-eyed (*cn bw/cn bw*) virgin females. The expected ratio is 1:1, red- to white-eyed individuals. If a significant excess of red-eyed flies (more than 80 per cent) appeared, an S-D factor may be suspected to be present. Additional tests can be used to verify this.

A temperature of 25° ± 1° C was used for all crosses except certain P_1 crosses, which were raised at 18° ± 1° C for reasons of convenience in scheduling. The medium used in all experiments was made of

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brewer's yeast, agar, maltose, sucrose, water and 0.5 per cent proprionic acid.

A homogeneity test disclosed no significant differences ($X^2=1.49$; $P>.05$) between the three different samples of *D. melanogaster* analyzed in these experiments. The samples were therefore pooled.

In Table 1 the 132 second chromosomes tested for their effects on viability are separated into four viability classes, based on the proportion of wild type (non-Curly) flies present in the F_3.

Table 1

Frequencies (in per cent) of Lethal, Semilethal, Deleterious and Normal Second Chromosomes in a Natural Population of *Drosophila melanogaster* from Eastern Tennessee. (For definition of viability classes, see text.)

Viability Class				Number Tested
Lethal	*Semilethal*	*Deleterious*	*Normal*	
27.27	16.67	19.70	36.36	132

So that these data may be compared with those obtained in most previous studies, all further references to lethal frequency, unless stated otherwise, combine the lethal and semilethal frequences. The eastern Tennessee sample so considered has a lethal frequency of 43.94±0.043 per cent. A majority of the chromosomes tested (about 64 per cent) were subnormal in viability. Most of the remaining were distributed at the lower end of the normal class range (25.1 to 41.6 per cent wild type). Only three chromosomes gave more than the expected 33.3 per cent wild type, the highest giving 35.1 per cent; several other chromosomes gave more than 30 per cent.

Two of the proven lethal lines were lost; therefore, only 34 lethal lines were available for outcrossing with each other in the allelism tests. Of 561 such outcrosses, 53 proved sterile. Of the remaining 508 outcrosses, two resulted in simple cases of allelic lethals: in lines 44 and 49 and in lines 9 and 226. A third case of allelism was more complicated: the line 1 lethal was allelic to lethals of lines 71, 101, 125 and 142; the line 71 lethal was allelic to those of lines 1 and 142, but not to those of lines 101, 125, and 232; the line 101 lethal was allelic to only the line 1 lethal; the line 125 lethal was allelic to those only of lines 1 and 142; while the line 142 lethal was allelic to those of lines 1, 125, 71 and 232. However, for reasons to be discussed, all lethals in this third case were grouped as the same lethal. The seven lines having allelic lethals thus gave a 1.37± 0.005 per cent allelism. This low proportion of allelism together with the high lethal frequency (43.94 per cent) indicates a large breeding population (Ives, 1945). The tests for the presence of S-D factor proved negative. A total of 171 chromosomes was tested.

Discussion

Ives (1945, 1954) examined the frequency of lethal second chromosomes in natural populations of *Drosophila melanogaster* from the northern states of Massachusetts, New York, Pennsylvania, Ohio, and Nebraska (35.9, 32.2, 28.2, 26.3 and 25.6 per cent, respectively), and compared these with the frequencies from the southern states of Virginia, Texas and Florida (43.0, 41.8 and 51.1 per cent, respectively). These observations led Ives to conclude that there existed a north-south cline of generally increasing second chromosomal lethal frequency in North American populations of this species. He further suggested that this trend might be an indirect result of climatic conditions, warmer climates favoring an extended breeding season that increases the period during which a southern population remains at maximum size thereby causing a greater accumulation of lethals.

Subsequent studies of North American populations of *D. melanogaster* have tended to support Ives' hypothesis; thus, Hiraizumi and Crow (1960) found that 29.0 per cent of the second chromosomes in a natural population from Wisconsin were lethal when homozygous. Band and Ives (1961) retested the Massachusetts population and found a lethal frequency of 36.9 per cent. The 43.94 per cent of lethal second chromosomes found in the eastern Tennessee population is about equal to the Virginia frequency (45.0 per cent) and clearly fits in the group of southern frequencies, thus contributing further support to the hypothesis.

At the present time it is not known whether such a north-south cline exists outside the United States. Local geographical conditions play an important role in any comparison of distant populations. For example, Dawood's (1961) examination of the second chromosome of *D. melanogaster* in lower Egypt (latitude 23°-25°N) revealed a median lethal percentage of 29.56 for three separate areas. This is almost exactly the same as the 29.0 per cent found by Hiraizumi and Crow (1960) in Wisconsin, at a latitude of 44°N. It would be of great interest if temperature has a world-wide relationship to lethal load.

There is no agreement on how populations are able to exist with such high saturations of lethals as those reported in natural populations of *D. melanogaster*. Dobzhansky and Wallace (1959) and their co-workers believe that heterosis may be the main force in survival. However, Prout (1952), Cordeiro (1952), and Hiraizumi and Crow (1960) have found that heterozygosity for lethal and semilethal chromosomes may produce deleterious effects. The last named workers found further that such heterozygous chromosomes produce highly detrimental effects not only on pre-adult viability, but also on the components of adult fitness (fertility, fecundity, etc.). Nonetheless, it is difficult to visualize the survival of populations containing many lethals without considerable heterosis. Indeed, Band and Ives (1962) have presented evidence that most of the genetic variability in the Amherst, Massachusetts, population is balanced, i.e., maintained by heterosis.

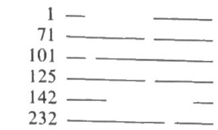
Ives (1954) concluded that the North American populations of *D. melanogaster* occur as large breeding units, continuous in time. Spencer (1941) found this to be true also of *D. hydei* in southern California. Such, however, is the converse of the condition reported for

Russian populations of *D. melanogaster* (Dubinin, 1946) and for North American populations of other *Drosophila* species, such as *D. pseudoobscura*, *D. immigrans* and *D. virilis* (Spencer, 1941). Dubinin (1946) indicated that *D. melanogaster* in Russia was subdivided into small breeding units, at least at the time of lowest numbers in the annual cycle of variation in size. *D. melanogaster* in the United States appears, thus, to be unique in population structure when compared with foreign populations of the same species or with domestic populations of most other *Drosophila* species.

The low allelic frequency (1.37±0.005 per cent) and the high percentage of lethality (43.94±0.043 per cent) in the eastern Tennessee population of *D. melanogaster* suggest that the breeding population is large. Casual observation failed to disclose any neighboring populations. Additional collections were attempted across the highway, about 100 yards distant, but no flies were seen there. The population sampled is perhaps a local breeding population similar to the one discovered by Ives (1945) in Massachusetts; in that population the cardinal eye color mutant gene persisted for at least eight years, during which time it was not found in any other nearby populations. This showed the existence of local units, continuous in time. In spite of the casual observations, however, it is possible that the eastern Tennessee population studied was, in reality, a unit of a larger breeding population. The absence of flies at the marginal areas may have been the result of seasonal fluctuation in numbers and of the lateness of the year. At the time the sample was taken the population may have concentrated its range to the area sampled in beginning the overwintering process. Very little is known of how the flies overwinter. Spencer (1941) demonstrated that the adults may survive in farm buildings, and the larvae in decaying fruit.

Some consideration needs to be given to the unusual results obtained in the allelism tests between the lethal second chromosomes in the eastern Tennessee lines 1, 71, 101, 125, 142, and 232; namely: the line 1 lethal was allelic to those of all lines except 232; the line 71, allelic to those of lines 1 and 142, but not to those of lines 101, 125, and 232; the line 101, to only that of line 1; the line 125, to only those of lines 1 and 142; while the line 142 lethal was allelic to those of all other lines except 101.

Two possible explanations are pseudoallelism (Lewis, 1951) and overlapping lethal segments (McClintoch, 1944). The former assumes that there exist subloci within the lethal locus, with different combinations of these subloci being viable. The latter explanation is based on the assumption that there are absent or inactivated segments of varying lengths on the lethal chromosomes. According to this explanation, an individual heterozygous for the two second chromosomes that do not share the absent or inactivated areas would be viable. The six unusual eastern Tennessee chromosomes can be represented as straight lines with the deficient or inactivated regions shown as blank spaces thus:



No attempt was made to distinguish between the two explanations.

The frequency of segregation-distorter factors in the second chromosomes of *D. melanogaster* from other natural populations is fairly low, about five per cent or less (Sandler, Hiraizumi and Sandler, 1959). Thus it is not surprising that no S-D factors were found in the rather small samples from eastern Tennessee, where their absence could be attributed to chance.

Summary

A total of 132 second chromosomes from a natural population of *D. melanogaster* in one area of eastern Tennessee was tested for their effect on viability when rendered homozygous. Of these, 58 (43.94 ± 0.043 per cent) proved to be lethal or semilethal. This frequency was higher than that known to occur in states north of Tennessee and lower than that in Florida. This study thus supports Ives' (1945) hypothesis that as one proceeds from north to south in the United States, an increase in the proportion of recessive lethal chromosomes is encountered.

The high frequency of lethal chromosomes and low frequency of allelism among them (1.37 ± 0.005 per cent) in the eastern Tennessee population suggest that the population is a large breeding unit. An unusual allelic relationship among six lethal lines is discussed.

No segregation distorter factors were found in a sample of 171 second chromosomes from the eastern Tennessee sample.

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NEWS OF TENNESSEE SCIENCE

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Two Tennessee scientists are among the newly elected members of the Board of Directors of the Oak Ridge Institute of Nuclear Studies. Elected to three-year terms are Robert T. Lagemann, Chairman, Department of Physics and Astronomy, Vanderbilt University, and John L. Wood, Chairman, Department of Biochemistry, University of Tennessee at Memphis.

Ralph T. Overman, Chairman of the ORINS Special Training Division, has been appointed to an advisory board of associate editors to help select subjects for American Chemical Society monographs.

New members of the faculty at Austin Peay State College include Floyd L. Brown, Assistant Professor of Biology, Harvey F. Blanck, Jr., Assistant Professor of Chemistry, Daniel D. Michalek, Assistant Professor of Geography, and Mildred E. White, Instructor in Mathematics.

A new department of psychology has been organized at ETSU under the chairmanship of Clayton Carpenter. Both undergraduate and graduate programs will be offered.

Richard R. Overman, Professor and Chairman of the Division of Radiation Biology at the University of Tennessee College of Medicine, has been named assistant dean for research affairs of the college.

Appointment of Associated Press newsman Joseph A. Sweat, Jr., to the public information staff of Vanderbilt University has been announced by the university. Mr. Sweat will fill the new position of science editor; his primary duties will be to assist news media in their reporting of the medical, scientific and engineering aspects of the university.

The new medical library at the Vanderbilt University School of Medicine will be dedicated, Thursday, Nov. 19 in ceremonies at the University's Science Center Lecture Hall. "For 56 years, since its founding in 1906, the medical library at Vanderbilt University has served as a resource both to medical teaching at the University and to the information needs of all physicians in this region," said Dr. Randolph Batson, director of medical affairs at Vanderbilt. "To assure that this facility remains adequate to the responsibilities assigned it, the University has invested major sums of money to relocate and enlarge the medical library."

Dr. Frederick Taylor Wolf, Professor of Biology at Vanderbilt University, has been named to the Gallery of Contemporary Noted Mycologists. He was so honored in *Mycopathologia et Mycologia Applicata,* the international journal for the field of mycopathology and applied mycology.

Dr. Mark M. Jones, Professor of Inorganic Chemistry at Vanderbilt University, has been awarded a two-year, $27,480 grant by the Air Force Office of Scientific Research for the study of coordination, ligand reactivity, and catalysis.

The goal of the study is a detailed understanding of the way in which coordination changes the kinetic parameters involved in the reactions of simple aliphatic ligands.

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