

THE FREQUENCY AND ALLELISM OF LETHAL CHROMOSOMES
IN ISOLATED DESERT POPULATIONS OF
DROSOPHILA PSEUDOOBSCURA^{1,2}

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ABSTRACT

Second-chromosome lethals were extracted from four populations of *Drosophila pseudoobscura* in Southern California. Two of the populations were from desert oases and two from the classic habitat on Mt. San Jacinto, previously studied by DOBZHANSKY. Allelism tests were made on the lethals within and between all locations. The frequency of lethal second-chromosomes in each location was 0.18, and this was not different from the results of other workers for samples throughout the species range. Interpopulational allelism rates were about 0.005, and not different from earlier results of DOBZHANSKY. Intrapopulational rates in this study were, with one exception, the same as the interpopulational rates, and significantly lower than DOBZHANSKY found using the third chromosome. This may be due to lethals being linked with heterotic third-chromosome inversions. The allelism rate of the exceptional population (about 0.03 and equal to DOBZHANSKY's intrapopulational results) may be due to heterotic lethals, or a founder effect. Two lethals were found in three populations each, possibly due to migration among these populations, which are up to 334 km apart.

MIGRATION in natural populations of *Drosophila* is a subject of interest to population biologists, for it is through migration that gene flow takes place. Recently, interest in the amount of gene flow among populations has increased because of the development of the neutralist theory to explain the maintenance of electrophoretic variation. (KIMURA and OHTA 1971, and references therein.)

Direct studies of migration with *Drosophila pseudoobscura* have until recently been carried out in pine forests. These studies showed that most flies do not move farther than about 350 m in a day (CRUMPACKER and WILLIAMS 1973), and that a gene does not often travel farther than 2-3 km in a year (DOBZHANSKY and WRIGHT 1947). The frequency of allelism of lethal chromosomes, an indirect technique, has been used to study migration occurring at slow rates over long distances, and I show here that there is an appreciable amount of this kind of migration in Southern California. This has been shown to some extent by earlier

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work on rates of allelism in *D. pseudoobscura* living in pine forests. (WRIGHT, DOBZHANSKY and HOVANITZ 1942.) I extend it to consider desert populations and the actual lethal genes involved.

Desert oasis populations of *Drosophila pseudoobscura* were chosen for the experimental work because it was believed that they were contained within the boundaries of the oases. It was thought they might have different population structures than the classic mountain populations which were used for controls. I sought answers to two main questions. First, what are the population sizes, and second, are the desert populations really isolated?

Locations

Drosophila species were collected from four locations in Southern California. Each collection was made from coffee cans using mashed bananas as bait. The flies were collected in plastic bags and transferred to food bottles for transportation to the laboratory (BRYANT and JONES 1976).

Furnace Creek

Location: Furnace Creek Ranch, Death Valley, California.

Elevation: -58 m (-190 feet).

Coordinates: 116° 52' 04" W, 36° 27' 30" N.

Furnace Creek Ranch is a resort in the middle of Death Valley. The structures and irrigated area comprise about 1 km², and are surrounded by dry desert. The flies used in the experiment were collected by a date orchard on 22 and 23 April 1974, each trap yielding several hundred flies from each of several samplings. Over 3000 *Drosophila* were collected. About 95% of these were members of the *obscura* group; the rest were mostly *D. melanogaster* and *D. simulans*. Several completely unsuccessful attempts were made to trap *D. pseudoobscura* away from the ranch.

Desert Center

Location: 6 km northwest of Desert Center, California.

Elevation: 183 m (600 feet).

Coordinates: 115° 21' 23" W, 33° 44' 58" N.

The Brown Ranch on Rice Road includes several trailers, a small irrigation pond, a grapefruit orchard, and a vineyard. The trailers and irrigated area comprise about 0.2 km². The desert surrounding the Brown Ranch is not nearly so desolate as that in Death Valley, but is liberally sprinkled with *Larrea*, *Olynea*, *Cercidium*, and *Prosopis* shrubs.

Collections were made in the grapefruit orchard on 15 and 16 April 1974. Over 5000 *Drosophila* were collected and somewhat less than half were of the *obscura* group. Most of the rest were *D. simulans* and *D. melanogaster*; a few individuals of several other species were also found.

James Reserve

Location: James Reserve, Lake Fulmor, Mt. San Jacinto, California.

Elevation: 1646 m (5400 feet).

Coordinates: 116° 46' 28" W, 33° 48' 34" N.

The James Reserve is located on Indian Creek. During June and July, the creek dries, and flows only intermittently through the surrounding lower yellow pine forest. Collections were made on 27 and 28 June and 02 and 03 July 1974, and combined to provide the James Reserve sample. About 3500 *obscura* group flies were trapped, along with several hundred *D. occidentalis*, *D. simulans*, and *D. melanogaster*, as well as smaller numbers of several other *Drosophila* species.

Idyllwild

Location: Idyllwild, Mt. San Jacinto, California.

Elevation: 1841 m (6040 feet).

Coordinates: 116° 41' 36" W, 33° 45' 46" N.

This collecting site, about 9 km from James Reserve, is in an area of open upper yellow pine forest. Eight evening collections were made from 12 July to 11 August, 1974, and combined to provide the Idyllwild sample. About 7000 *obscura* group flies were collected, along with several hundred *D. immigrans* and *D. occidentalis*, as well as smaller numbers of *D. melanogaster*, *D. simulans*, *D. hydei*, and several other species.

MATERIALS AND METHODS

Extraction of lethal chromosomes

Drosophila pseudoobscura and sibling species were sorted from the collections and the males used in the standard crossing scheme with the Delta/Bare^{Inv} balancer stock to make lines homozygous for the second chromosome (SVED and AYALA 1970). The crossing scheme automatically sorts *D. pseudoobscura* from its sibling species, since F₁ hybrid males are sterile (DOBZHANSKY and EPLING 1944, pp. 5-11). Thirty-six percent of the lines failed to produce enough offspring to survive the extraction process, many of these because the male used apparently contained the "sex-ratio" X chromosome. All semi-lethal lines became either fully lethal or quasi-normal (DOBZHANSKY and SPASSKY 1953) after a few generations in the laboratory.

Cultures were raised under constant light in an incubator at 10° or 22°, and 85% relative humidity on a cornmeal-yeast-sugar food (LEWIS 1960). The lower temperature was used irregularly to lengthen development time and permit an orderly work schedule.

Allelism tests

To test whether two independently extracted lethal chromosomes carry the same lethal, an allelism (complementation) test was done. A male heterozygous for Bare and a natural chromosome (*Ba*¹/*lethal*₁) is crossed to a virgin heterozygous for Bare and a different natural chromosome (*Ba*¹/*lethal*₂). No wild-type offspring indicates that lethals 1 and 2 are allelic.

Allelism tests were made among the lethal lines extracted from each location and between lines extracted from each pair of locations. This is a total of 10 allelism tests, the four intrapopulation tests and the 6 interpopulation tests. Each test involved over 500 crosses of the type described in the preceding paragraph.

The chromosomes *lethal*₁ and *lethal*₂ were considered allelic if no wild-type progeny were produced in two or more replicate crosses of at least 15 progeny each, and if no replicate produced more than 10% wildtype. The cultures were raised in constant light at 22° and 85% relative humidity on the cornmeal-yeast-sugar food.

RESULTS

Frequency of lethal chromosomes

The frequencies of lethal chromosomes extracted from the four locations are as follows. For Furnace Creek, 17% (91 of 528) were lethal; for Desert Center,

17% (91 of 547); for James Reserve, 20% (86 of 436), and for Idyllwild, 19% (80 of 417). In all, 1928 chromosomes were tested and 348 (18%) were lethal in double dose. There is no significant difference ($p = 0.6$) in the frequencies of lethals among locations. These data are consistent ($p = 0.1$) with other results for the same chromosome over a wide geographical range (DOBZHANSKY and SPASSKY 1953, 1963; MARINKOVIC 1967).

Allelism of lethal chromosomes

Table 1 lists, for each of the 10 allelism tests, the number of crosses completed, the number of crosses showing allelism, and the frequency of allelic crosses. In all, there were 49 allelic crosses (0.0086) among the 5690 crosses made.

Three different heterogeneity χ^2 calculations for the number of allelic crosses per test are shown. Except for the Furnace Creek test, the data are quite homogeneous, and the three most common lethals there can account for its higher rate of allelism. Lethals that appear three or more times in the allelism tests are termed "chain lethals" (IVES 1970).

Sixty-six separately extracted lethals (of the 348) showed allelism to at least one other lethal. These 66 comprised 20 different lethals, for many extracted

TABLE 1
Frequency of allelic crosses

Locations crossed	Number of crosses completed	Number of allelic crosses	Frequency of allelic crosses
FC × FC	657	21	0.032
DC × DC	632	3	0.005
JR × JR	616	3	0.005
ID × ID	523	2	0.004
FC × DC	504	7	0.014
FC × JR	528	2	0.004
FC × ID	553	2	0.004
DC × JR	536	3	0.006
DC × ID	581	3	0.005
JR × ID	560	3	0.005
Totals	5690	49	0.009
FC = Furnace Creek			
DC = Desert Center			
JR = James Reserve			
ID = Idyllwild			
			$\chi^2 = 54$ with 9 d.f. $p = 10^{-8}$
Totals excluding the three high frequency chain lethals in the FC × FC test:	5447	34	0.006 $\chi^2 = 11$ with 9 d.f. $p = 0.2$
Totals excluding the FC × FC test entirely:	5033	27	0.005 $\chi^2 = 8.3$ with 8 d.f. $p = 0.4$

TABLE 2

Observed numbers of extracted lethals, by location, allelic to listed lethals

Lethal identification	Number in FC	Number in DC	Number in JR	Number in ID	Total	Frequency
FC 1	2	3	3	2	10	0.1515
FC 7	6	1	1	0	8	0.1212
FC 9	2	2	0	0	4	0.0606
FC 13	2	0	0	0	2	0.0303
FC 14	2	0	0	0	2	0.0303
FC 25	6	0	0	0	6	0.0909
FC 26	3	0	0	0	3	0.0455
FC 35	2	0	0	0	2	0.0303
FC 38	4	0	0	0	4	0.0606
FC 65	1	0	1	0	2	0.0303
FC 79	2	1	0	0	3	0.0455
DC 12	0	1	0	1	2	0.0303
DC 13	0	2	0	0	2	0.0303
DC 16	0	2	0	0	2	0.0303
DC 23	0	1	0	2	3	0.0455
DC 39	0	1	1	0	2	0.0303
DC 47	0	1	2	0	3	0.0455
JR 15	0	0	2	0	2	0.0303
JR 51	0	0	2	0	2	0.0303
ID 3	0	0	0	2	2	0.0303
Totals: 20	32	15	12	7	66	
Frequencies:	0.4848	0.2273	0.1818	0.1061		1.000

lethals were allelic to several other extracted lethals. Table 2 shows these 20 different lethals, and for each one, how many times it appeared in each location. The data from all 10 allelism tests were combined to make the table.

The analysis of variance for Table 2 reveals that there is no significant difference in the number of times the lethals showing allelism appeared in the experiment, that is, no difference in the row sums in Table 2 ($p = 0.72$). There is, however, a significant difference among the numbers of lethals per location, that is, among the column sums ($p = 0.02$). If the three high-frequency Furnace Creek chain lethals (FC 7, 25, 38) are left out, the probability among columns is 0.26, and if the Furnace Creek test is left out entirely, $p = 0.35$. There is no other test that can be left out to make the column sums homogeneous at the 5% level.

There are no significant deficiencies in Table 2 in what are expected to be the most common types of allelic lethals in the experiment. The differences observed are due to the much higher allelism rate in the Furnace Creek intrapopulation test when compared to the other nine tests. This high allelism rate requires explanation.

DISCUSSION

I suggested previously that data would be sought to determine whether gene flow takes place between natural populations of *D. pseudoobscura* hundreds of

kilometers apart. The results presented show that at least two lethals (FC 1, FC 7) are widespread in the area studied. It is likely that these lethals are heterotic and have been spread by migration from their points of origin in the same way as have the third-chromosome inversions in this species. If they are heterotic, they should reappear at Furnace Creek in succeeding seasons. Allelism tests between samples collected in 1974 and 1975 are underway. IVES (1970) failed to find significant interyear allelism of chain lethals in a Massachusetts population of *D. melanogaster*.

Identity by descent and identity by mutation

WRIGHT (WRIGHT, DOBZHANSKY and HOVANITZ 1942) and WALLACE (1966) have estimated the number of allelic lethals arising independently and those which are the descendants of a single mutation. These are the two sources of allelism. Experiments of HOCHMAN (1971), IVES (1945), and WALLACE (1950) indicate that there are between 400 and 600 genes capable of lethal mutation in the second chromosome of *D. melanogaster*. Since the second chromosome of *D. pseudoobscura* is about half the length of the second chromosome in *D. melanogaster*, an estimate of the number of genes capable of lethal mutation is 200–300. Using WALLACE'S (1966) formula, an allelism frequency of 0.004–0.006 in this study can be explained solely on the basis of independent mutation of genes to lethality. Thus, only the Furnace Creek population has an allelism rate significantly higher than can be explained by mutation, suggesting that some of the allelic lethals from this location are identical by reason of descent from a common ancestor. This is supported by the fact that the high allelism rate is due to a small number of chain lethals.

Population size

In 1942, WRIGHT first gave equations for calculating the effective population size based on the allelism of lethal chromosomes. NEI (1968) extended WRIGHT'S work and found the frequency distributions of lethal chromosomes for several values of h , the dominance coefficient, in equilibrium populations. However, neither NEI'S estimators, nor WRIGHT'S, should be regarded as more than very rough indicators of population size using allelism data, for they either require data not obtainable from allelism tests, or assume properties not consistent with the biology of *D. pseudoobscura* lethal chromosomes.

The effective sizes of three of the populations studied are probably large, for their allelism rates do not indicate any allelism by reason of common descent. If the chain lethals in the Furnace Creek population are in high frequency because they are heterotic, the effective size of the Furnace Creek population may be large; if they are in high frequency because of common descent, the effective population size would be smaller than in the other locations.

Gene flow

As RODELL (1972) has pointed out, if the lethals involved in gene flow among populations tend to belong to the group of heterotic lethals, the number of lethals showing allelism in the interpopulational tests should be less than the number

showing allelism in the intrapopulation tests. This is because the newly arisen, and on the average slightly dominant, lethals have a shorter lifetime in large populations than neutral or heterotic lethals (ROBERTSON and NARAIN 1971), and therefore would not spread as widely through the species.

The number of different lethals per cross for the interpopulation tests is 0.0039, and for the intrapopulation tests is 0.0066. These figures are not significantly different ($p = 0.16$). In addition, the same fraction (0.7) of the allelic lethals was part of chains in the inter- as the intrapopulation tests. These data indicate that the chain lethals do not migrate more frequently than the uncommon lethals.

Lethals carried by a few flies founding a new population will increase in numbers with the population size for several generations irrespective of whether they are dominant or heterotic; only afterwards will the effect of selection on the dominant lethal cause its frequency to decline with respect to that of the heterotic lethal (ROBERTSON and NARAIN 1971).

The lethals showing allelism to several other lethals in different locations are likely to be heterotic, for selection against dominant lethals had time to act, since the populations were (except possibly for Furnace Creek) large in size. Those lethals showing a high frequency in Furnace Creek only are probably the result of a founder effect. The rest of the allelic lethals can be accounted for by independent mutation.

ROBERTSON and NARAIN conjecture that the mean lifetime for a completely recessive lethal is $1.27 N_e$; for a heterotic lethal it would be appreciably longer. Therefore, the lethals FC 1 and FC 7 may fall into about the same class of genetic variations as chromosome inversions. They might be of almost any age, and old enough that the same migration which has carried chromosome inversions over the species range also could have widely spread these lethals. Thus, there appears to be some migration among populations far apart, but not enough to widely spread most lethals.

Comparison with other results

WRIGHT, DOBZHANSKY and HOVANITZ (1942) compared the allelism of third-chromosome lethals from mountain ranges in the desert with the allelism from different areas on Mt. San Jacinto. They found an allelism rate among collecting sites at least 15 km apart of 0.004, which is not different ($p = 0.26$) from my interpopulation rates, and can be explained by mutation. They also found an allelism rate of 0.013 among collecting sites 200 m–3.5 km apart, while I found a rate of 0.005 for sites 9 km apart. These rates are not significantly ($p = 0.21$) different, and are consistent with other data (POWELL and DOBZHANSKY 1976) that flies do not move far in a pine forest.

The most interesting differences between my results and those of WRIGHT, DOBZHANSKY and HOVANITZ are the allelism rates within populations. They found intrapopulation rates of 0.025 on Mt. San Jacinto, and 0.005 and 0.03 for samples from desert mountain ranges. The mean of these rates is significantly ($p = 10^{-4}$) higher than the rates I found, except for the Furnace Creek test. Dif-

ferent environmental conditions between my work and that of over 30 years ago may account for the different rates, for they found a significant ($p = 0.01$) difference in tests of the same locality between 1937 and 1939.

Another possibility is that the structure of the second and third chromosomes accounts for the different rates. The chromosomes are about the same length, the second perhaps a little shorter. The major difference is that the third chromosome possesses a rich inversion polymorphism, while the second is virtually monotypic (DOBZHANSKY and EPLING 1944). If a lethal arose in an uncommon type of third chromosome, it would be subject to those forces which are important in small populations, since it could not easily escape from the inverted chromosome by recombination (STURTEVANT and MATHER 1938). However, allelic lethals are found in different inversion types, though they appear to be commoner in the same type (DOBZHANSKY, SPASSKY and TIDWELL 1963).

DOBZHANSKY believes (1970, Chapter 5) that there is heterosis among the third-chromosome inversion types, and that the relative fitness of the types changes with the seasons. If a lethal arose in an inversion type which was being favorably selected, its frequency, and therefore the allelism frequency, would rise. There is evidence (LEWONTIN 1974, p. 135 ff.) that alleles of three electrophoretic loci on the third chromosome are closely associated with specific inversion types. These associations cause linkage disequilibrium among the alleles at these loci.

EPLING, TINDERHOLT and MATTONI (1961) found nonrandom associations of lethals and inversion types, with lethals concentrated in the rare inversion type. DOBZHANSKY, SPASSKY and TIDWELL (1963) show that, for heterotic lethals, the lethal allele should associate with the rarer gene arrangements for the population to have the highest average fitness. Their data are inconclusive on this, as are data from WRIGHT, DOBZHANSKY and HOVANITZ (1942) on the frequencies of lethals in Standard and non-Standard gene arrangements. In the latter paper, WRIGHT, DOBZHANSKY and HOVANITZ found that the frequencies of lethals were higher in non-Standard than in Standard chromosomes, but the difference was barely significant ($p = 0.05$) in the grand totals. In these populations, Standard, Arrowhead, and Chiricahua inversions each composed about 30% of the population (DOBZHANSKY and EPLING 1944).

Conclusions

All observed allelism rates can be accounted for by independent mutation of genes to lethality, except for the Furnace Creek intrapopulation test. There are three lethals in a frequency of about 1% each in this population, and that can account for the high rate of allelism. Two of these lethals may be heterotic. These two are present in two other locations, and they may have been spread by migration. My data, as well as those of DOBZHANSKY, indicate that flies do not disperse far in a pine forest.

The situation in the desert may be quite different. Oasis populations may be founded each winter by migrating flies, and oases close to each other may receive flies from the same source, though this could not be tested. Certainly there is

enough migration to keep the species coherent in the area studied, for as SPIETH (1974) said “. . . In terms of gene flow, the distinction between absolutely none and almost none is enormous.”

The difference in allelism rates between the second and third chromosomes may be due to lethals accumulating in rare third-chromosome inversion types, but the evidence is inconclusive.

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