GENE FLOW AND THE GEOGRAPHICAL DISTRIBUTION OF A MOLECULAR POLYMORPHISM IN *DROSOPHILA PSEUDOOBSCURA*[1]

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ABSTRACT

This paper discusses the relation between the geographical distribution of an enzyme polymorphism and population structure in *Drosophila pseudoobscura*. California populations of this species living in very different montane and lowland habitats separated by several kilometers are similar to each other in the frequency of an esterase allele. Previous estimates suggest that gene flow is too limited to account for this homogeneity of genetic structure, so that it must reflect some balancing force of natural selection. We show, however, that dispersal over unfavorable habitats is much greater than earlier supposed. Isolated populations of *D. pseudoobscura* separated by 15 km from other populations are subject to large amounts of immigration. This is shown by changes in the seasonal abundance of this species and in the annual pattern of lethal alleles in such populations. The genetic structure of an experimentally perturbed isolated population in an oasis returned to normal within a single year, suggesting that such populations are ephemeral and that the oasis is subject to annual recolonization by distant migrants. Direct assessment of marked flies shows that they can move at least 10 km in 24 hours over a desert. Such extensive gene flow may help explain the distribution of the esterase allele, and is relevant to the high level of molecular polymorphism and its general lack of geographic differentiation throughout the range of *D. pseudoobscura*.

IT has been recognized since the publication of WRIGHT's (1931) synthetic theory of evolution in Mendelian populations that the proper study of genetical evolution must include not only the forces of natural selection, but also the effects of breeding structure. DOBZHANSKY, in his early work on inversion polymorphism in *Drosophila pseudoobscura*, recognized the importance of population structure by studying the allelism of lethals in an attempt to estimate effective population size (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY and HOVANITZ 1942) and by measuring the rate of migration of individuals and genes (DOBZHANSKY and WRIGHT 1943; DOBZHANSKY and WRIGHT 1947). When it became clear that natural selection indeed acted on inversions (DOB-

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ZHANSKY 1947), the study of population structure in *D. pseudoobscura* was abandoned for almost thirty years (DOBZHANSKY and POWELL 1974).

Much of the recent work in theoretical and experimental population genetics has been directed towards explaining the large amount of genetic polymorphism that exists for structural genes. The competing theories (especially the "neutralist theory") depend strongly on assumptions about the breeding structures of populations. KIMURA and OHTA (1971) and MARUYAMA and KIMURA (1974) pointed out that the observed distribution of molecular polymorphisms might arise from the random origin of new mutations and their diffusion by migration through a population. The exchange of an average of one or more migrant individuals between populations per generation will lead to the populations evolving together, unless selection actively operates to drive them apart (SPIETH 1974; SLATKIN 1977). Although some of these models (KIMURA 1979; OHTA 1976; OHTA and KIMURA 1975) incorporate the action of weak purifying selection in removing deleterious alleles from large populations, they do not invoke the necessity of balancing natural selection to preserve molecular polymorphism. However, most recent experimental research in evolutionary genetics has been devoted to attempts to demonstrate the action of balancing selection (CLARKE 1975). We felt it important to return to the problems of population structure and migration in species whose molecular polymorphism has been studied in order to determine whether the theoretical models are biologically realistic.

In this paper, we describe work on allele frequency, migration and population structure in *Drosophila pseudoobscura* that may throw some light on its genetic polymorphism. Initially, we consider the frequency of alleles at a structural locus detected by electrophoresis in different populations of this species. We then discuss the allelism of lethals in a population physically isolated by a large area of desert, in an attempt to determine whether it shows genetic evidence of isolation. Finally, we describe experiments on migration of flies across unfavorable habitats, which attempt to relate migratory behavior to the data on allelism and on the genetic differentiation of populations. If *D. pseudoobscura* consists of a series of "islands" of population, with essentially no migration among them, then historical events and accidents of sampling will cause gene-frequency differences that will persist over long periods. Similarities in the frequencies of molecular polymorphisms among geographically separated populations could then be ascribed to the balancing effect of some selective force operating over a large area. If, on the other hand, populations are connected by extensive migration, similarities in allele frequencies are to be expected in the absence of uniform selection. Large geographical differences in gene frequencies could then plausibly be attributed to locally differentiated selective forces. Information on gene flow may in some circumstances provide evidence relevant to the competing theories of molecular polymorphism.

Previous research on migration in *D. pseudoobscura* suggested that movement is in general rather restricted. The classic work of DOBZHANSKY and WRIGHT (1943, 1947), who measured the rate of diffusion of a visible mutant from a

central release point, showed that in montane habitats *D. pseudoobscura* is sedentary, with little genetic interchange between populations living more than a few kilometers apart. Thus, after one year, 99% of the progeny of the mutants were found within 2.2 km from the release point. This finding reinforced the observation of WRIGHT, DOBZHANSKY and HOVANITZ (1942) that in the forests of Mount San Jacinto, California, recessive lethals from populations 16 km apart are no more likely to be allelic than are those from populations 320 km apart (in which any allelism, they suggest, can safely be presumed to have arisen from independent mutation). More recent observations on the movement of this species in favorable habitats, such as coniferous forests, have given rather higher estimates of dispersal than those obtained by DOBZHANSKY and WRIGHT. For example, DOBZHANSKY and POWELL (1974), using wild flies marked with fluorescent dust (rather than mutant flies), found that the rate of dispersal was about three times higher than that previously measured by DOBZHANSKY and WRIGHT in the same locality. The mean distance dispersed was about 170 m per day (POWELL *et al.* 1976). A similar figure was reached by CRUMPACKER and WILLIAMS (1973), who studied populations of *D. pseudoobscura* in coniferous forests in Colorado. These authors agree that, although this degree of gene flow is enough to lead to homogeneity of gene frequencies over short distances (not much exceeding one km), it will not suffice to cause more widely separated populations to evolve together as a result of the exchange of genes.

Previous work on the distribution of alleles detected by electrophoresis in various species of Drosophila has often shown that populations separated by great distances have rather homogeneous gene frequencies. The existence of similar gene frequencies in populations that appear from movement experiments to be genetically isolated from each other has, therefore, been used as evidence in favor of the thesis that balancing natural selection, must actively maintain molecular polymorphisms (AYALA *et al.* 1974). However, there is a dilemma here, as conditions that would allow the maintenance of such large amounts of variation by balancing mechanisms, such as heterosis, are extremely restrictive; if biologically realistic assumptions are used, heterosis can maintain only a small number of of alleles or a very narrow range of allele frequencies (LEWONTIN, GINZBURG and TULJAPURKAR 1978). Therefore, it is necessary to investigate more closely patterns of gene frequency in relation to gene flow and population structure.

We have studied the relationship between gene flow and the geographical distribution of an esterase allele in California populations of *D. pseudoobscura*. This is a center of abundance for this species, which is found from sea level to an altitude of 10,000 feet (DOBZHANSKY and EPLING 1944). The *Est-5*$^{0.85}$ allele was chosen as a representative molecular polymorphism. The esterase locus is polymorphic throughout *D. pseudoobscura*'s range, and has 13 alleles that can be detected by the conventional methods of gel electrophoresis (PRAKASH, LEWONTIN and HUBBY 1969; LEWONTIN 1974). *Est-5*$^{0.85}$ has a low mobility on an electrophoretic gel, so that its frequency can rapidly be assessed when carrying out population surveys.

METHODS

Population sampling of wild flies was by the usual banana-bait method. Flies were returned to the laboratory and electrophoresed; the isofemale line technique was not used.

Electrophoresis was carried out on vertical slabs of 5% acrylamide gel at pH 8.9 in the apparatus of ROBERTS and JONES (1972). Techniques were similar to those described by PRAKASH, LEWONTIN and HUBBY (1969).

Marking of flies for movement experiments was by fluorescent dusts (CRUMPACKER 1974). Trap lines for recapturing flies were made up of half-gallon containers baited with fermenting banana.

Allelism of lethals was estimated by using a standard crossing scheme in which a wild-caught male was mated to a virgin female who had a dominant visible marker, lethal in double dose, on each of her second chromosomes. The Delta/Bare^{Inv} balancer stock was utilized (SVED and AYALA 1970), and the frequency of lethals was estimated by using the breeding program described by BRYANT (1976).

RESULTS

Geographic patterns of allele frequency: In many parts of its range, *D. pseudoobscura* is distributed in a series of large islands of suitable habitat separated by deserts or by grasslands. In southern California, for example, it is largely restricted to forested or scrub-covered mountain ranges separated from each other by extensive deserts (DOBZHANSKY and QUEAL 1938). It can, however, be collected at certain times of year in the oases that are scattered through the low deserts (WRIGHT, DOBZHANSKY and HOVANITZ 1942) and in some other lowland habitats. Pairs of samples were collected from contrasting montane and lowland habitats in several parts of central and southern California to investigate whether gene frequency was related primarily to local topography (and hence to environment) or, rather, to the geographical position of each pair.

A total of 16 samples was collected (Figure 1). *D. pseudoobscura* was common in piñon pine-juniper scrub (*Pinus monophylla* and *Juniperus otabensis*) up to about 1600 m, and above this in coniferous forest (largely *Pinus Jeffereyi*, *P. Lambertiana*, *Quercus Kelloggi* and *Abies concolor* with shrubs such as Arctostaphylos and Salix). Although this species is in general absent from the arid low deserts (whose vegetation consists primarily of scattered Atriplex, Larrea and Prosopis shrubs), it can sometimes be found in oases. Many of these have stands of Tamarix trees, together with Washingtonia palms or groves of dates (*Phoenix dactylifera*). Various bushes (Nerium and other species) are also found. In the higher deserts, *D. pseudoobscura* was collected in shrub vegetation (Palos Verdes, Cercidium sp., iron wood *Olynea tesota*) in arroyos, although it was rare in the open desert. Other collections were made at low elevations near the coast, mainly in planted citrus groves. *D. pseudoobscura* occupies a great diversity of habitats in this region.

Figure 1 shows the frequency of *Est-5*$^{0.85}$ in 22 California samples, 16 of which are paired between mountain and lowland. The distance between the upper and lower samples in a pair varies from 10 km (Desert Center—Corn Spring) to 30 km (China Ranch–Charleston Park). These distances are considerable in relation to the dispersal distances previously recorded in *D. pseudoobscura*. *Est-*

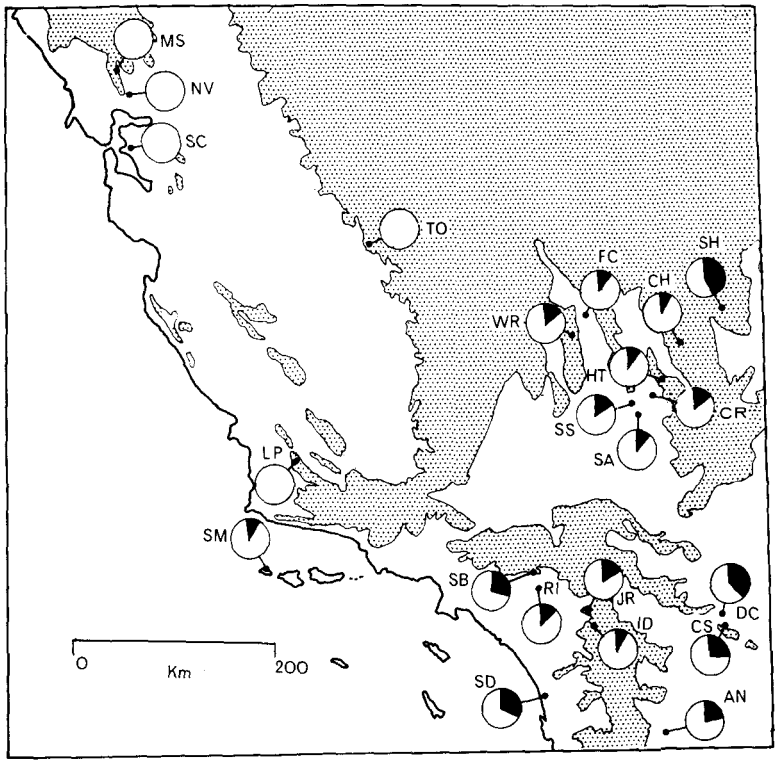

FIGURE 1.—Frequency of the *Est-5*$^{0.85}$ allele (black sector; full circle = 10% *Est-5*$^{0.85}$) in Californian samples of *Drosophila pseudoobscura*. Land above 3000 feet stippled. Localities (lowland samples first in paired samples) and frequencies (*Est-5*$^{0.85}$ followed by total number of *Est-5* alleles in sample): SD = San Diego (6/210)/ID = Idyllwild (2/308). RI = Riverside (4/297)/JR = James Reserve (6/308.) DC = Desert Center (20/610)/CS = Corn Spring (4/162). SS = Saratoga Spring (3/174)/HT = Horse Thief Spring (2/212). Cr = China Ranch (5/330)/CH = Charleston Park (1/135). FC = Furnace Creek (2/258)/WR = Wild Rose (1/74). SM = San Miguel Island (7/660)/LP = Los Padres (0/122). NV = Napa Valley (0/23). MS = Mount Saint Helena (0/77) (PRAKASH 1974). SB = San Bernardino (2/91) (PRAKASH and MERRITT 1972). AN = Anza Borrego Desert (2/108). SA = Salt Creek (3/208). SH Sheep Mountains (2/49). TO = Tollhouse (0/118). SC = Strawberry Canyon (0/212).

$5^{0.85}$ has a center of abundance in the southeastern part of the survey area (and reaches a frequency as high as 7.6% at Portal, Arizona: PRAKASH 1974). It is less common to the north and west of this, and is absent from the northern part of California. There is significant geographical heterogeneity in the frequency of *Est-5*$^{0.85}$ both within mountain ($\chi^2 = 11.08$; $p < 0.05$) and within lowland ($\chi^2 = 18.39$; $p < 0.02$ samples. There are however, no significant differences in the frequency of this allele between mountain samples andthe lowland member of the same pair ($\chi^2 = 1.67$; $p = 0.97$). Adjacent populations living in very dissimilar habitats have similar frequencies of this allele, while samples that are geographically distant from each other, but that live in rather similar habitats, have different frequencies of *Est-5*$^{0.85}$.

These observations might be interpreted as showing that natural selection by obvious environmental differences associated with topography has little effect on the geographical distribution of this allele. However, if the *Est-5*$^{0.85}$ allele is selectively neutral and the population "islands" sampled are virtually isolated (as is implied by previous estimates of gene flow), they should then be randomly differentiated from each other. Either there are strong and uniform forces of balancing selection acting on the *Est-5* locus that are not reflected in topography and that operate over large areas, or there is considerable migration from population to population. Previous work on migration in *D. pseudoobscura* has been concerned only with rather favorable environments for this species; the possibility of long-range dispersal over unfavorable habitats has not been explored. This possibility was investigated by examining the patterns of persistence of local populations of *D. pseudoobscura* and by direct and indirect measures of the amount of long-distance dispersal among populations of this species.

Ephemeral populations of Drosophila pseudoobscura: The existence of small *D. pseudoobscura* populations in desert oases, orange groves and the like that are separated by large tracts of unfavorable habitat from neighboring populations provides an excellent opportunity to investigate the possibility of long-distance dispersal. If it could be shown that such populations are periodically extinguished and recolonized from a distant source each year, this would be evidence of a degree of gene flow sufficient to have a profound effect on the genetic structure of *D. pseudoobscura* populations because, if flies can migrate long distances to the oases, they can also be presumed to migrate between permanent populations.

We have studied a habitat that might be expected to support ephemeral populations. Oases in the Mojave Desert are small in size, isolated from one another by many miles of hostile environment, and more than 1,000 m below any possible montane population sources. Therefore, they might support ephemeral *D. pseudoobscura* populations because of the very high temperatures reached in summer. In citrus groves, *D. pseudoobscura*, although common during the winter, is nearly absent during the summer months (BRYANT, unpublished). In the desert oases, trapping in three Death Valley localities (Salt Creek, China Ranch and Furnace Creek; Figures 1 and 2), suggests that flies are present from March until June. Attempts to trap in these localities in August and November yielded no *D. pseudoobscura*, and usually no Drosophila at all.

These patterns of annual population change could arise either from the few *D. pseudoobscura* that might survive the summer being sufficient to found the next year's abundant fall and winter population (although this is scarcely possible in desert oases), or from an effective extinction of the local population in the summer followed by a recolonization in the fall from montane populations. The desert populations experience summer temperatures above 44°. As far as is known, *D. pseudoobscura* has no mechanism of diapause that would allow it to survive such temperatures. However, it might be that eggs, larvae or pupae occupy some protected microhabitat that allows them to survive during periods of heat stress, so that these oasis populations could be genetically continuous and maintain themselves without annual long-range immigration from a distant

source. The degree of genetic persistence of oasis populations was tested by examining the degree of genetic identity of the recessive lethals present in such a population on two sampling dates, one year apart, and also by perturbing gene frequencies at the Esterase-5 locus during one spring and investigating whether this perturbation persisted to the following spring.

Annual changes in the allelism of lethals: All *D. pseudoobscura* populations contain genetic variants in heterozygous condition that are lethal when homozygous (DOBZHANSKY and WRIGHT 1941; WRIGHT, DOBZHANSKY and HOVANITZ 1942; DOBZHANSKY and SPASSKY 1953, 1963). The degree of genetic identity of populations can be estimated by the amount of allelism among their recessive lethals, and this parameter has been used to give one measure of the amount of genetic exchange between populations. WRIGHT, DOBZHANSKY and HOVANITZ (1942) showed that in coniferous forests there is enough migration between populations up to 3.5 km apart to increase the allelism to a level greater than that of populations 100 km apart, but that populations 9 km apart are no more likely to share allelic lethals than are widely separated populations. The level of lethal allelism among desert oases hundreds of kilometers apart in southern California is similar to that existing among widely scattered forest populations (BRYANT 1976). These results have been interpreted as showing that, while (in coniferous forests at least) migration is sufficient to lead to the exchange of lethals between populations up to 3.5 km apart, it does not exert a significant effect over distances greater than this.

Allelism of lethals in the same locality over a period of time can provide evidence on the degree of genetic continuity and isolation of a population. In a continuous population, the same individual lethals will persist for many generations. Differences in the identity of lethal alleles over a period of a few generations, therefore, may indicate that the population has been subject to immigration from other populations that possess a different set of lethal alleles. Any population that has been subject to extinction and recolonization during the period of observation might be expected to show considerable temporal changes in lethal constitution that reflect the genetic structure of the various sources from which the ephemeral population has been founded.

Between-year allelism of lethals in the desert oasis population at Furnace Creek, Death Valley was measured, using the Delta/Bare-balancer technique (BRYANT 1976). This population lives at an altitude of 58 m below sea level in about 1 km^2 of habitat suitable for Drosophila. The oasis is artificially watered and consists largely of a grove of date palms together with planted Tamarix trees. It is surrounded by arid desert consisting of barren salt pans and scattered Atriplex, Prosopis and Larrea shrubs. No Drosophila were found in the surrounding desert. The nearest habitat likely to be able to support *D. pseudoobscura* is on the slopes of the Panamint mountains, 25 km away. The Wild Rose population is at 2400 m on the western slope of the Panamints (PRAKASH, LEWONTIN and HUBBY 1969).

A sample of *D. pseudoobscura* was collected at Furnace Creek on April 22 and 23, 1974, and another on April 27, 1975. The relationship between lethal alleles

at Furnace Creek and those in other populations in 1974 has been described previously (BRYANT 1976). Second chromosomes of the males were made homozygous, using the crossing scheme of BRYANT (1976). In 1974, 528 successful crosses were made, and 424 in 1975. In 1974, 17% of the chromosomes tested were lethal in double dose. The same proportion of the 1975 chromosomes (70 out of 424) was lethal when made homozygous. Although the frequency of lethals is not significantly different between years, there is a significantly lower degree of allelism between years than within years. Of 657 tests among the 1974 lethals, 21 (3.2%) proved to be allelic. In 1975, the figure was 12 out of 596 (2%). Six (or 1%) of the 577 between-year allelism tests were allelic. The allelism rate of the 1975 test is not significantly different from that of the 1974 test ($p = 0.2$) or that of the between-year test ($p = 0.2$), but the between-year test has a significantly lower allelism rate than the 1974 test ($p = 0.01$). This might suggest that new lethals appeared at an appreciable frequency in the population (perhaps as a result of immigration) between the two sampling dates. Tests of the identity of individual lethals in each year confirm this.

In 1974, three different lethals were present six times in the chromosome samples (hence, each had a frequency of about 1% in the population). These were designated FC7, FC25 and FC9 (a combination of the FC9 and FC38 of BRYANT (1976), which have since been found to be allelic). None of the three lethals is allelic. Since only 16% of the possible tests for allelism were carried out, the six appearances of each of these lethals is a minimum estimate of their frequency in the 1974 sample.

Nine % of all possible between-year allelism tests have been undertaken, 26 on FC7, 37 on FC9 and 50 on FC25. Both FC7 and FC25 were found twice in the between-year allelism tests, but FC9 was not detected. However, two new lethals appeared in relatively high frequency in 1975. FC75/2 was present four times and FC75/18 three times among 424 chromosomes tested. Again, these are minimum estimates, since the 596 allelism tests carried out represent only 25% of those possible. In 1974, the allelism of lethals taken from Furnace Creek with lethals from three montane populations hundreds of kilometers away was approximately 0.7% (BRYANT 1976). This between-population figure is not significantly different from the 1.0% between-year figure for Furnace Creek given above. On the basis of the statistical power available, the populations in two different years in Furnace Creek appear to be as different from each other as is the Furnace Creek population from distant populations. The probable disappearance in a subsequent year of a lethal (FC7) common in the first year of sampling, and the appearance of two new lethals (FC75/2 and FC75/18) on several chromosomes on the second sampling occasion might indicate that the Furnace Creek population went through a considerable bottleneck between the two sampling dates, and also that there might have been immigration from another population, despite the great distance separating Furnace Creek from possible sources of new lethal alleles. This oasis population may not persist as a large and isolated pool of genes, but may become nearly or completely extinct during the heat of the summer, only to be re-established in the fall by flies from a montane source

population at least 25 km away, perhaps with the assistance of a few oversummering flies. As the mean temperature in August at this locality is 44°, extinction of all Drosophila is likely. An attempt to collect in apparently favorable conditions on August 9 and 10, 1976, yielded no Drosophila.

There remains the possibility that the reduced between-year allelism rate reflects a high turnover in lethal composition in a continuous, but small, population rather than an ephemeral one. Another possibility, which is difficult to test experimentally, is that the annual changes in the identity of frequent lethals arise from their transient association with heterotic blocks of loci (FRYDENBERG 1963) within a continuous population, and not from the annual extinction of the population. Much more extensive evidence over several years is required in order to obtain strong evidence on these points and to make use of the available theory on allelism through time (PROUT 1967; ROBERTSON and NARAIN 1971). Also, it should be noted that surveys of the pattern of change of lethal allelism over time cannot in themselves assess the extent of long-distance migration. It is possible that an identity of lethals between years could arise from an annual colonization from the same distant population, while a complete lack of identity could arise from the same degree of migration, but from different source populations each year.

Furnace Creek is an unusually large oasis that is artificially watered. Therefore, its population might be more persistent than are those living in smaller natural oases, and its genetic constitution might depend less on annual long-distance migration than does that of populations that experience the unmitigated rigours of the desert summer. For these reasons, the genetic continuity of natural oasis populations was investigated further by examining the persistence of an experimental change in gene frequency in such a population.

Gene frequency manipulation of an isolated population: Perturbations of allele frequency at the *Est*-5 locus were carried out in two small oases at the south end of Death Valley; Salt Creek (alt. 160 m) and Saratoga Spring (alt. 60 m) (Figure 2). Each oasis consists of a number of Tamarix and Prosopis trees with other vegetation. Salt Creek is a brackish creek that flows for about 1 km in the spring, but is dry for most of the year. Saratoga Spring has a more extensive area of permanent open water surrounded by reeds. Neither population was as abundant as that of Furnace Creek; a maximum of about 30 flies per day could be collected in March and April. Each oasis is surrounded by desert, in which no *D. pseudoobscura* is ever found. Although the bed of the Amargosa River (which sometimes contains a large amount of water) passes near Salt Creek, this does not support a population of Drosophila. The nearest large area of potential Drosophila habitat to the two oases is found near the summits of the Avawatz mountains, some 15 km away and 1500 m above the desert floor. These support piñon pine and juniper.

The frequency of the *Est*-$5^{0.85}$ allele in the native populations of each oasis was approximately 0.02 (Figure 1). Gene frequency perturbation was carried out by releasing large numbers of laboratory-bred flies homozygous for this allele. In the laboratory, at least, there is evidence that selection has little effect on gene

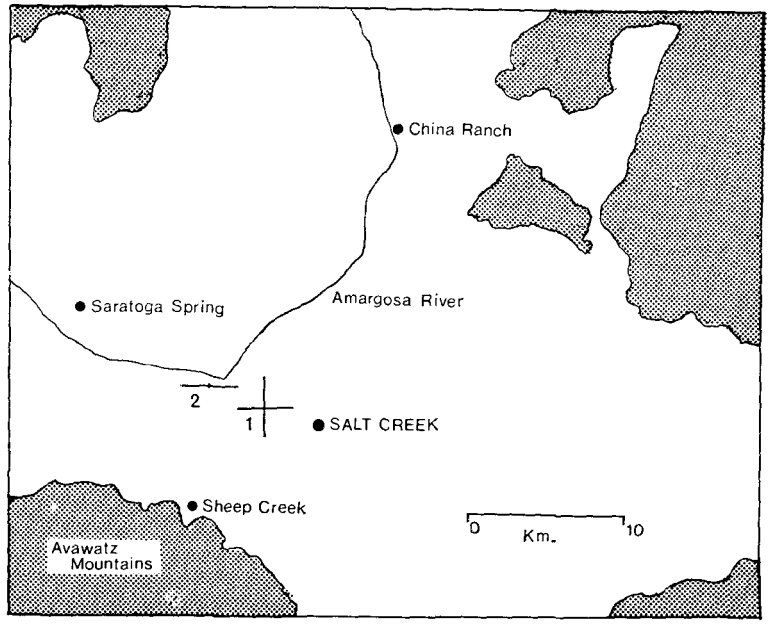

FIGURE 2.—Collection and release localities for *Drosophila pseudoobscura* in Death Valley. 1 = Historical Marker release site. 2 = Sheep Creek Junction release site. Land over 2500 feet stippled.

frequencies at the *Est-5* locus, provided that there is no linkage disequilibrium between this locus and those surrounding it (YAMAZAKI 1971). Experiments using varying numbers of *Est-5* lines of independent origin also show little evidence of selection, provided that sufficient lines are used to minimize the possibility of disequilibrium (JONES and YAMAZAKI 1974). To reduce the chance of confounding any changes in the frequency of *Est-5*$^{0.85}$ that are due to extinction and recolonization from these resulting from such linkage effects, the released population was made up of a large number of independently derived chromosomes carrying this allele. As there were insufficient flies at either of the release points to allow the collection of enough chromosomes carrying *Est-5*$^{0.85}$, the experimental population was derived from another desert population of *D. pseudoobscura.* This was at Desert Center, 200 km south of Salt Creek. In March 1971, 610 females were collected from a field of rotting watermelons; the frequency of *Est-5*$^{0.85}$ was 3.28%. The progeny of females heterozygous for this allele were used to produce 20 independent lines homozygous for *Est-5*$^{0.85}$. These lines were then combined to produce a release stock homozygous at the esterase locus, but having at other loci an approximation of the genetic background of a natural desert population.

This stock was expanded to produce about 60,000 adult flies. On the evening of March 22, 1973, half of these were released at Salt Creek and half at Saratoga Spring. Few flies could be collected at Saratoga Spring in the weeks following the experimental release. At Salt Creek, immediately after the release, the frequency of the *0.85* allele was more than 90% (Table 1). This frequency declined

TABLE 1

Frequency of the Est-$5^{0.85}$ *allele in an experimental population of* Drosophila pseudoobscura

Days after release	Females			Males		Proportion *Est-*$5^{0.85}$
	0.85/0.85	*0.85/X*	*X/X*	*0.85*	*X*	
1	45	0	0	31	1	0.992
7	40	0	3	22	4	0.911
16	31	0	9	31	5	0.802
22	1	3	21	4	30	0.107
27	4	4	35	1	29	0.112
33	14	0	30	9	15	0.338
37	30	0	7	41	13	0.780
44	9	0	14	10	11	0.431
53	0	1	2	1	9	0.06

as released flies died and local flies emerged. It reached a low point 22 days after the release. By day 38, the frequency of *Est-*$5^{0.85}$ had reached 0.78. The cohort of offspring of released flies began to hatch at 22 days and reached its peak about 5 weeks after the release. This gives an estimate of the development time of *D. pseudoobscura* under spring desert conditions. The cohort then began to be replaced by the hatching of native flies, and the frequency of *Est-*$5^{0.85}$ in the adult population reached a low point of 6% at 53 days. Its frequency among eggs, larvae and pupae (most of which will have been produced by the released flies), of course, will be much higher than this. The population of adults decreased as the days became hotter, and no Drosophila of any species were trapped after the 58th day.

Does the disappearance of the adult population in mid-May mean that there is no biological continuity from year to year at these oases, or are adults and immature stages aestivating, only to reappear in the following spring? If the former is true, then a year later, the frequency of *Est-*$5^{0.85}$ would be expected to be the same as that of the surrounding populations. If there is aestivation and biological continuity, however, the aestivating flies (and, in particular, the immature stages) would have been sampled from the experimentally perturbed population, and the subsequent year's sample would have a much higher frequency of the allele.

The populations were resampled one year later on March 24–26, 1974. Only three flies were found at Saratoga Spring (where the vegetation had been greatly reduced by tree felling); 28 females and 17 males were collected at Salt Creek. None of these possessed the *Est-*$5^{0.85}$ allele. Therefore, the frequency is very close to that before the experimental perturbation.

It is scarcely possible that this drop in frequency resulted from natural selection against this allele; even assuming that the population breeds throughout the year, this would represent an intensity of selection greater than that previously recorded against any enzyme allele. It is much more likely that this oasis population is an ephemeral one; that flies do not survive the summer in significant numbers, and that the local population is refounded each year by immigrants.

As the nearest possible source of such immigrants is 15 km away, this finding reinforces the suggestion from the work on allelism of lethals in the Furnace Creek oasis that there must be a degree of migration far greater than that previously described for *D. pseudoobscura*, and that diffusion of genes between populations living in different habitats has a large effect on the genetic structure of at least some populations of this species. This degree of gene flow must imply that flies can disperse for considerable distances across a hostile desert. Therefore, we examined this possibility directly by observing the movement of marked flies across such habitats.

Dispersal of D. pseudoobscura *across unfavorable habitats*: Drosophila movement was studied at the southern end of Death Valley (Figure 2). The area used for release was a dry desert (altitude 120 m) whose vegetation consisted primarily of scattered Atriplex and other shrubs. There was no native Drosophila population. Wild flies were collected at Furnace Creek and transported within 48 hours to the release point. Here, they were marked with fluorescent dusts (CRUMPACKER 1974) and released at one of two localities: the historical marker 60 km north of Baker on California Route no. 127 or the junction of the track to Sheep Creek on the road from this marker into Death Valley. The Furnace Creek population contained a proportion of "yellow flies" (*D. melanogaster* and *D. simulans*). These survived transport to the release point and the conditions in the release area much less well than did *D. pseudoobscura*, and few were recaptured. Only the data on *D. pseudoobscura* were used in this work.

Initial experiments involved a "cross" of traps around a central release point similar to those employed by DOBZHANSKY and WRIGHT (1943), DOBZHANSKY and POWELL (1974) and POWELL *et al.* (1976). Small vessels containing fermenting banana bait were placed 160 m apart in four lines approximately north, south, east and west from the release point. Each line extended for 1600 m from the point of release. Three such experiments were carried out. In each case, several thousand marked flies were released in the late evening, and the pattern of dispersal over the trap lines examined in the morning and evening activity periods of the following day. The bait containers were kept closed except during the time of recapture to minimize the "funnelling" effect of lines of highly attractive bait (JOHNSTON and HEED 1975). Recapture frequencies and other data are shown in Tables 2, 3 and 4 and Figures 2 and 3.

The rate of movement across the desert is much greater than that previously recorded in coniferous forest, so much so that flies reached the ends of the trap lines within 12 hours of release. Within a day of release, very few flies could be found within the experimental area. For example, in experiment 2, only 7 marked flies were collected after 36 hours. Figure 3 compares our 15-hour recapture rate with the 24-hour recapture in a forest at Mather (DOBZHANSKY and POWELL 1974). In spite of the shorter period available for movement, the desert flies can be seen to have dispersed to a considerably greater extent than did those in the forest. There is little merit in attempting to calculate precise dispersal parameters from these data, as subsequent experiments showed that our desert trap lines greatly underestimated the real extent of dispersal. Some very general comparisons with earlier work are, however, possible.

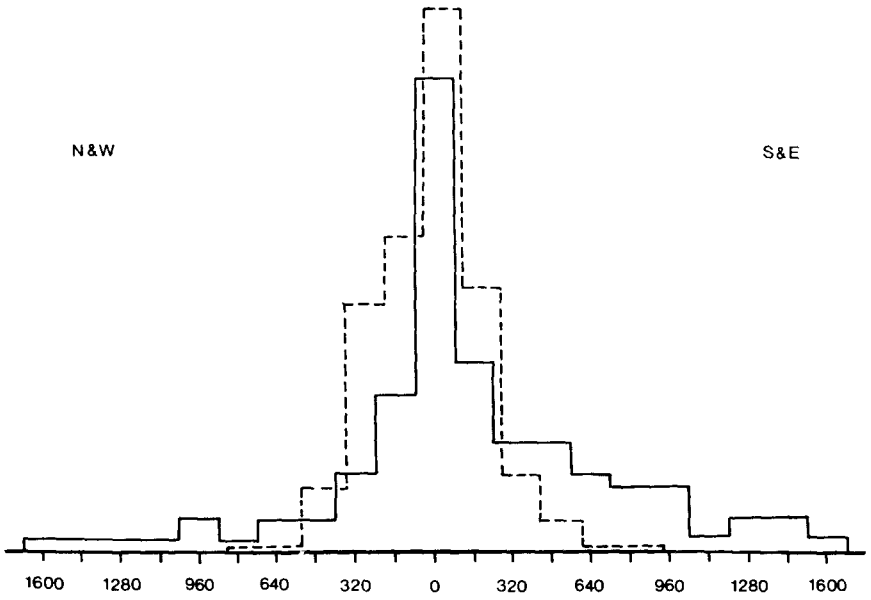

FIGURE 3.—Recaptures of *Drosophila pseudoobscura* in release experiments. Continuous line: 15 hour recapture in Death Valley (all experiments); dotted line: 24 hour recapture at Mather (DOBZHANSKY and POWELL 1974).

The mean distance moved (calculated as $\Sigma rf/n$, where r = distance of trap from release point, f = the number of flies collected at that trap and n = the total number of flies recaptured) in the three experiments was 405 m, 509 m and 392 m in 15 hours, as compared with an average movement of 167 m in 24 hours found at Mather.

Flies are active only for a brief period in the morning and another in the evening (DOBZHANSKY and EPLING 1944). In each desert experiment, the traps were uncovered after dark when flies were no longer moving, but had dispersed during the evening activity period. On the next morning flies near the traps will enter them at the beginning of the morning activity period and will remain there. Our trap lines are therefore mainly a sample of a single dispersal event rather than the two dispersal periods included in each observation by POWELL and DOBZHANSKY. The dispersal rate in the desert as measured by these experiments is probably at least 5 times higher per activity period than at Mather.

There are no consistent effects of wind on dispersal. It is not the case that the flies are dispersed only by wind, or that they fail to disperse when it is windy (although, in the laboratory, flies cling to surfaces when there is strong air movement).

Long-distance movement was investigated by using the oases themselves as "natural Drosophila traps" for flies released in the desert some distance away. Three oases were used: Salt Creek (a small stream, lined with tamarisk trees, whose nearest point is 2 km from the first release site); Sheep Creek (a patch of submontane vegetation at 540 m in a shrubby canyon; the oasis itself is 8 km from the first release site, but the flies may be funnelled into the oasis by the

TABLE 2

Release experiments for Drosophila pseudoobscura *in Death Valley*

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
Location	Historical Marker (Site 1)	Historical Marker (Site 1)	Sheep Creek Junction (Site 2)
Date, time and temperature of release	April 3, 1975, 1745 PDT, 28°	April 4, 1975, 1730 PDT, 26°	March 22, 1976, 1730 PST, 29°
Wind at release	SSW, 12–14 mph, gusty	NW, 15 mph, gusty	Calm

canyon, which opens 6 km from this site); and Saratoga Spring (an area of open water and reeds, 10 km from our release site). At Salt Creek, 12 bait traps were placed at 180 m intervals along the creek. In addition, funnel traps were placed at 5 m and 10 m above the ground in tamarisk trees. At Sheep Creek and Saratoga Spring, bait traps were scattered among reeds and other vegetation at the edge of open water. On April 3, 1975, at 17.45 hours, 3,000 marked flies (collected 24 hours previously at Furnace Creek) were released at the historical-marker site (Figure 2). The wind was gusting to 10 mph with an air temperature of 29°C. At 1800 hours on April 4, 1975, a second release of 4,000 flies marked with dust of a different color was made (air temperature 25°; wind to 15 mph). On the morning of April 5, 64 marked flies from both releases were collected at Salt Creek (Table 4). Collections made at Sheep Creek later that day yielded 9 marked flies released on April 3 and 5 marked flies released on April 4.

A similar experiment was carried out in March 1976; 4,000 marked flies (collected at Furnace Creek) were released on the evening of March 22 at Sheep Creek Junction. There was little wind, and the air temperature was 29°. Collections were made on the following day in three oases at varying distances from

TABLE 3

Recaptures of Drosophila pseudoobscura *in Death Valley*

	Trap no. (160 m intervals)									
	1	2	3	4	5	6	7	8	9	10
Experiment 1 (38 at Release trap)										
West	8	9	1	1	1	7	4	—	3	2
East	5	4	0	1	6	1	0	0	0	1
North	9	2	0	5	1	4	—	1	1	0
South	4	0	0	0	0	0	0	0	0	0
Experiment 2 (45 at Release trap)										
West	5	2	2	0	0	0	1	0	0	0
East	6	6	4	10	8	5	1	3	6	—
North	3	0	2	1	0	1	1	1	0	0
South	4	4	2	0	1	0	0	1	0	—
Experiment 3 (112 at Release trap)										
West	48	23	8	8	1	4	2	4	1	2
East	64	33	45	23	15	19	9	8	11	7

TABLE 4

Yield of flies from traps at Salt Creek on the morning after release experiment 2

Trap	Distance from release point (meters)	Flies trapped Release #2	Release #1	Unmarked
F	500	3	0	0
E	680	1	0	0
D	860	1	0	0
C	1040	2	0	0
B	1220	2	0	1
A	1400	4	0	0
1	1580	1	0	0
2	1760	0	0	0
3	1940	2	0	1
4	2120	1	0	1
5	1200	0	0	0
6	2480	0	0	1
Base camp	1500			
Ground level		13	4	5
5 meters up		12		0
10 meters up		18		0

Flies from experiment 2, experiment 1 and unmarked flies appearing in the traps are shown separately.

the point of release: 15 marked flies were recovered at Salt Creek (7 km), 6 at Sheep Creek (8 km) and 29 at Saratoga Spring (10 km). Eight more marked flies were collected at Saratoga Spring on the morning of March 24. There is no possibility that the marked flies had been accidentally transported to the oases, as there was no communication between the release points and the recapture sites during the course of the experiment. It is also unlikely that flies covered these great distances simply by being blown by the wind; there was little breeze on either of the release dates, and movement took place in three different directions from the release point.

Several points emerge from these experiments. First, a rather large number of flies will disperse as much as 2 km in a day over inhospitable habitat. Second, some flies will disperse as far as 10km in a single day. Third, the number of flies covering 10 km in a day cannot be small. The angle subtended by Saratoga Spring from the release point is about 3°. Using a very simple model of dispersal, it can be calculated that over the whole of the experimental area, $29 \times 360/3$, or about 3,500 flies travelled at least 10 km in a day. Although this figure is a very crude estimate of movement, it suggests a migration rate far higher than that previously recorded.

It is very likely that, having reached a suitable habitat, the flies tend to stay in it rather than proceeding further. Thus, a collection at Sheep Creek on April 14, 1975 yielded three marked flies released on April 5. In Salt Creek, marked flies were found dispersed throughout the oasis for at least three days after each release, and could be collected in traps placed 10 m above ground in the trees. A similar tendency for flies to remain in suitable habitats was found by WALLACE

(1970) in *D. melanogaster*, by JOHNSON and HEED (1976) in *D. nigrospiracula* and by DOBZHANSKY and POWELL (1974) and DOBZHANSKY *et al.* (1979) in *D. pseudoobscura.* Many of the flies estimated to have travelled 10 km in our experiment may have migrated farther than this in one day, as they did not encounter a favorable habitat. On the other hand, oases might have a considerable attractive radius, so that the large number recorded from Saratoga Spring were drawn from a wider sector of the experimental area than the angle subtended by the Spring itself. Also, flies may be funnelled into Sheep Creek by the canyon in which it is located. Our dispersal estimates are therefore approximate.

There is need for further experiments on, for example, the attraction of oases to flies in the desert, and the extent to which flies in an oasis or other suitable habitat leave it to traverse hostile habitats at the high speeds that we have observed. Nevertheless, the observation that *D. pseudoobscura* can migrate 10 km in less than 24 hours is in itself remarkable. It suggests that dispersal between mountain and oasis populations is feasible, as indeed is gene exchange between the main montane centers of abundance of this species. This result is relevant to our finding that the frequency of an esterase allele in California *D. pseudoobscura* is more a function of the location of a population than of the habitat (mountain or lowland) that it experiences.

DISCUSSION

The experiments described in this paper show that flies can disperse over unfavorable habitats, that this can lead to migration between distant populations and that such migration might help explain similarities in allele frequency in populations living in very different habitats. Some aspects of the seasonal patterns of abundance of *D. pseudoobscura* in different habitats remain unclear. In montane coniferous forests, flies appear about June, and disappear about September (COOPER and DOBZHANSKY 1956; DOBZHANSKY and EPLING 1944). Lowland populations such as those discussed in this paper have received less attention, but our observations, together with those of DOBZHANSKY and EPLING (1944), show that flies do not become abundant until after the montane populations have entered their inactive winter state. For example, in the Riverside citrus grove, *D. pseudoobscura* does not become abundant until October or November; in the oases, flies are absent until early March (when the high mountain populations are beneath the snow). The montane populations therefore appear to be inactive at the times when they might be supposed to be supplying founders for those in the deserts. More extensive trapping might resolve this paradox. There are remarkably few records of seasonal abundance in the Great Basin area, and nothing has been published on the seasonal patterns of change in *D. pseudoobscura* at intermediate altitudes. Further work is needed here, and it is important to publish data concerning the absence (as well as the presence) of *D. pseudoobscura* in a particular locality at a particular time.

The patterns of dispersal discussed here do suggest that, as *D. pseudoobscura* throughout most of its North American range lives in patches of favorable habitat separated by deserts or grasslands, there is the opportunity for substantial amounts of gene flow between populations many kilometers apart. A tendency

for flies to move rapidly when faced with an unfavorable environment has also been found in other species of Drosophila. In *D. melanogaster*, flies hatching into an optimal environment (such as a tropical greenhouse) travel only a few meters during their lifetime. In an arid environment, such as a "desert" greenhouse, the rate of movement is so great that the flies fill the available space in a few minutes (WALLACE 1970). The desert species *D. nigrospiracula* is closely associated with its host plant, the Saguaro cactus *Cereus giganteus*. It frequently migrates between cacti as much as one km apart (JOHNSTON and HEED 1976). Other cactophilic species are sometimes found in large numbers as much as 15 km from their host plants (HEED and HEED 1972).

The potential for flight of tethered Drosophila in the laboratory has been estimated to be as much as 75 km in a fly's lifetime (HOCKING 1963), and individual drosophilids have been collected in the aeroplankton 550 km from shore (JOHNSON 1969). There is also evidence that passive transport of members of the *D. obscura* group by winds and by human agency has led to great range extensions by several species in the past 40 years (DOBZHANSKY 1973). Although records of exceptional dispersal by occasional individuals are of less significance than are the large-scale movements of many flies that we have found, and although much remains to be done on the details of its dispersal patterns, it is probable that the genetic system of *D. pseudoobscura* involves genetic exchange among widely separated populations within a period that is short in evolutionary terms.

This finding may help explain the patterns of geographical distribution of the esterase allele described here. Although populations separated by distances previously thought to be too great to allow significant exchange of genes share a common allele frequency, it is no longer necessary to invoke uniform forces of balancing selection in very different montane or lowland habitats to explain their similarity. Gene flow acts to promote genetic homogeneity over relatively long distances. There are differences in the frequency of *Est-5*$^{0.85}$ between northern and southern California; these may reflect either some selective differences between these regions or the progress of a selectively neutral allele by gene flow through these populations. The extent of individual dispersal shown by our experiments does suggest that gene flow must play some part in the pattern of distribution of *Est-5*$^{0.85}$ in California.

Extensive movement over unfavorable habitats may also be relevant to the high level of enzyme polymorphism and its general lack of association with habitat throughout the range of *D. pseudoobscura*. Electrophoresis of a series of soluble enzymes on 5% polyacrylamide gels at pH 8.9 reveals extensive protein polymorphism in this species; 13 such loci are polymorphic in at least one of thirteen North American populations (PRAKASH, LEWONTIN and HUBBY 1969; LEWONTIN 1974). In general, there is rather little divergence among North American populations for polymorphisms detected in this way; although there are a few cases in which particular alleles are confined to a single population, and several large scale clines similar to the Esterase cline discussed in this paper, the general picture that emerges is one of relative genetic homogeneity in the face of ecological diversity. More refined analyses involving improvement in electrophoretic techniques have shown that there is further genetic variation at some of the

loci controlling soluble enzymes (so that, for example, the xanthine dehydrogenase locus is now known to have 27 alleles rather than the six previously detected (SINGH, LEWONTIN and FELTON 1976), while proteins that have a structural role (and that are far more abundant in the cell than are soluble enzymes) possess almost no genetic polymorphism (BROWN and LANGLEY 1979). The geographical distributions of gene frequency for the newly discovered enzyme polymorphisms are generally similar to those of the alleles detected by simple electrophoresis; for some loci (such as xanthine dehydrogenase), there is almost no geographical differentiation, while for others (including Esterase-5) there is more evidence of differentiation among populations hundreds of kilometers apart (SINGH 1979). In general, there is far less geographic heterogeneity throughout North America for polymorphism at loci controlling soluble enzymes, however assessed, than for chromosome inversions. Our findings in California may help explain these patterns of variation.

Previous estimates of gene flow in *D. pseudoobscura* led to the conclusion that it would take many years for a newly arisen allele to diffuse even a few kilometers from its point of origin. Models that depend on the passive diffusion of randomly originating alleles over great distances and their maintenance in large numbers because of the large effective population size of a species in which dispersal is not restricted (*e.g.*, KIMURA and OHTA 1971; MARUYAMA and KIMURA 1974) therefore seemed biologically unreasonable. The same criticism could be applied to models that allow selection to act only by removing alleles that deviate from the norm in such large populations (OHTA and KIMURA 1975; OHTA 1976), rather than by the active promotion of genetic diversity. This difficulty is reduced by our observations. It has been calculated that the global population of *D. pseudoobscura* might be as great as 10^{14} (JOHNSTON and HEED 1976), and if gene flow is of the extent suggested by our experiments, the effective size of many populations may be only a few orders of magnitude less than this. Such large populations would allow the maintenance of many neutral or nearly neutral alleles at a polymorphic locus without their loss through drift. The probable existence of scores of alleles at, for example, the *Xdh* locus in this species might therefore be more easily accommodated in a neutral hypothesis or a hypothesis of weak purifying selection than was the case when the effective size of *D. pseudoobscura* populations appeared to be low because of restricted migration. Genetic uniformity at the molecular level between populations living in very different environments might also reflect the relatively rapid spread of neutral or nearly neutral alleles by long-distance dispersal, rather than a general uniformity of selective forces responsible for the active maintenance of protein polymorphism over a large area.

The importance of long-distance gene flow in reducing genetic divergence within a continuous population is emphasized by the molecular polymorphism of the isolated population of *D. pseudoobscura* at Bogotá, Colombia. This is separated from the main body of the species' range by a gap of 2000 km (DOBZHANSKY *et al.* 1963). The protein polymorphism of the Bogotá population differs considerably from that of the North American population. At the *Xdh* locus, three quarters of the Bogotá lines carry alleles unique to that population (SINGH,

LEWONTIN and FELTON 1976), and there is also considerable differentiation at the alcohol-dehydrogenase-6 locus (COYNE and FELTON 1977). This contrasts with the relative uniformity of these polymorphisms in populations separated by 2000 km within North America and further emphasizes the probable role of gene flow in affecting the geographical distribution of protein polymorphism in this species; Bogotá is isolated from the source of new alleles that diffuse by means of long-distance migration through the main body of the species' range and, as a result, has accumulated a different set of molecular polymorphisms.

The influence of gene flow on the distribution of protein polymorphisms is reinforced by the patterns of molecular variation in species in which dispersal is much more restricted than in *D. pseudoobscura*. The Hawaiian species *D. mimica* (which is restricted in its movement) shows much greater geographical differentiation in its protein polymorphism than does *D. pseudoobscura* (JOHNSTON and HEED 1976; RICHARDSON and JOHNSTON 1975; ROCKWOOD 1969). Molluscs, which are notoriously sluggish, show quite striking local differences in the frequencies of protein polymorphism. The genetic divergence among *Helix aspersa* populations within a single city block in Texas (as assessed using simple one-step electrophoresis) is as great as that of the whole North American *D. pseudoobscura* population (SELANDER and KAUFMAN 1975). Although this may be an overestimate of differentiation at equilibrium because of the recent origin of these populations, the degree of molecular divergence between natural populations of *Cepaea nemoralis* in Europe separated by a few kilometers is also of this magnitude (JONES, SELANDER and SCHNELL 1980). Comparison of closely related species of intertidal molluscs that have a planktonic larva (and hence, large amounts of gene flow in each generation) with those in which there is very limited dispersal shows that there is considerably greater geographical heterogeneity in the distribution of protein polymorphism in the latter (BERGER 1973).

The existence of geographical homogeneity of molecular variation promoted by gene flow does not deny the possible action of natural selection in affecting polymorphism. Recent theoretical work by KIMURA and others shows that weak purifying selection can sustain very many alleles, if population sizes are large enough and suggests that structural proteins are highly invariant as they are closely interlocked with each other and have a low tolerance to mutations that change their shape. Many mutants are removed by natural selection. Soluble enzymes, such as xanthine dehydrogenase, are subject to few such constraints; most of the changes in amino acid sequence that result from mutation have no effect on the molecule's function and persist in the population for a period that is determined largely by the accidents of sampling (and hence by population size). The relative abundance of various electrophoretic alleles in *D. pseudoobscura* may be constrained by purifying selection to a degree that depends on their deviation from a functional norm (COYNE and FELTON 1977). The biological nature of most of these constraints remains a mystery, although there are a few cases of laboratory populations of *D. melanogaster* (which are insulated from the effects of gene flow) in which an environmental stress has led to a predictable change in the frequency of an enzyme variant (CLARKE 1975). The distribution of genes must always depend on a balance between evolutionary forces—includ-

ing natural selection—that lead to local population differentiation and gene flow that promotes the diffusion of alleles among populations and, hence, their genetic homogeneity. We have shown that *D. pseudoobscura* is far less sedentary than formerly believed. The geographical differentiation of inversion frequency and of body size (SOKOLOFF 1966; ANDERSON 1968) most probably reflect selective pressures great enough to overcome the unifying force of migration. However, in the case of the large-scale distribution of the *Est-5*$^{0.85}$ allele in California, and by extension perhaps also of the majority of molecular polymorphisms in North American populations of this species, it seems that the effects of gene flow may prevail.

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