Colonization of the Americas by *Drosophila subobscura*: Lethal-Gene Allelism and Association With Chromosomal Arrangements

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ABSTRACT

Drosophila subobscura is a Palearctic species that has recently colonized the Americas. It was first found in 1978 in Puerto Montt, Chile, and in 1982 in Port Townsend, WA. The colonization and rapid expansion of the species in western South and North America provides distinctive opportunities for investigating the process of evolution in action. The inversion polymorphism in the O chromosome from populations of central California and northern Washington, separated by 1300 km, corresponds to a previously observed latitudinal cline, also observed in Europe. Recessive lethal genes are not randomly distributed among the chromosomal arrangements. The incidence of lethal allelism is high, yielding unrealistically low estimates of the effective size of these populations (on the order of 1000 individuals). The high incidence of lethal allelism is likely to be a consequence of the low number of the American colonizers (on the order of 10–100 individuals), but the persistence of the allelism over several years suggests that some lethal-carrying chromosomes may be heterotic owing to shared associations between lethal and other genes.

DROSOPHILA subobscura is a Palearctic species distributed all over Europe except central and northern Scandinavia, as well as in Northwest Africa and in the Atlantic islands, the Azores, Madeira, and the Canaries. In February 1978, *D. subobscura,* never before found in the Americas, was discovered in Puerto Montt, in southern Chile, where numerous collections had been made over many years. Subsequently, the species has spread in Chile to include a region from 29 to 53 deg latitude and eastward into Argentina. In many localities throughout that range, *D. subobscura* has become the most abundant Drosophila species. *D. subobscura* was discovered in North America in 1982, in Port Townsend (48 deg N), on the northern coast of Washington. Shortly thereafter, it was also collected in other localities of Washington, as well as toward the north in the vicinity of Vancouver, B.C., and toward the south in Oregon. Collections from the fall of 1984 to the present have shown that *D. subobscura* has become common through much of California west of Sierra Nevada, as far south as Ojai (34°28'N), 100 km northwest of Los Angeles. In many localities, *D. subobscura* has become quite common, on occasion the most abundant species of the *obscura* subgroup (AYALA *et al.* 1989; PASCUAL *et al.* 1993).

Investigation of the colonization of America by *D. subobscura* has shed light on a number of evolutionary issues, such as the adaptive character of the latitudinal clines of chromosomal arrangements (PREVOSTI *et al.* 1985, 1988, 1990); the effects of natural selection on different types of genetic variability (PEGUEROLES *et al.* 1995); the corroboration, by means of allozyme loci, of theoretical models predicting that polymorphism levels after a bottleneck depend on the growth rate of the population in addition to the strength of the bottleneck (BALANYÀ *et al.* 1994); the association between chromosomal inversions and lethal genes (MESTRES *et al.* 1990, 1992); the consequences of genetic drift at the beginning of the colonizing process (PREVOSTI *et al.* 1982, 1989; AYALA *et al.* 1989); and the size of the colonizing population (PREVOSTI *et al.* 1989; MESTRES *et al.* 1990). The success of this colonization has impacted the composition and other ecological characteristics of drosophilid communities in both North and South America (BRNCIC *et al.* 1985; AYALA *et al.* 1989; PASCUAL *et al.* 1993).

We report here the results of an investigation of the frequency and allelism of lethal genes and their specific association with chromosomal arrangements in populations separated in time, space, or both in an effort toward elucidating the genetic structure and population dynamics of the colonization.

MATERIALS AND METHODS

Populations: Two North American populations of *D. subobscura* have been sampled: Gilroy, in central California (collected in October 1990 and April 1991 and designated Gilroy II); and Bellingham, in Washington, ~1300 km to the north of Gilroy (collected in September 1991). These two populations are compared with a population herein designated Gil-

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TABLE 1

Percent frequency of the chromosomal arrangements in four natural *Drosophila subobscura* populations

Population	N	Chromosomal arrangement						
		O_{ST}	O_5	O_7	O_{3+4}	O_{3+4+2}	O_{3+4+7}	O_{3+4+8}
Gilroy I	142	14.9	0.7	0.7	7.8	39.7	19.9	16.3
Gilroy II	64	14.0	1.6	0.0	7.8	26.6	23.4	26.6
Bellingham	102	24.5	6.9	0.0	9.8	32.4	0.9	25.5
Arlington	178	31.5	5.6	0.0	2.8	28.1	8.4	23.6

N, number of chromosomes tested. Data for Gilroy I and Arlington are from PREVOSTI *et al.* (1988).

roy I, collected in 1984–1985 (MESTRES *et al.* 1990). The chromosomal polymorphism of Bellingham is compared to that of a previously studied population, sampled in 1986, in Arlington, state of Washington, located 70 km south from Bellingham. Comparisons will also be made with two Southern Hemisphere populations for which relevant data are available: Puerto Montt, Chile (MESTRES *et al.* 1990), and Santiago de Chile (MESTRES *et al.* 1992).

Chromosome extraction and viability tests: Chromosomes were made homozygous as follows. Wild-collected males were individually crossed with virgin females of the *chcu* strain, which is homokaryotypic for the chromosomal arrangement O_{3+4}/O_{3+4} and homozygous for the recessive alleles *cherry* (bright red eye) and *curled* (wings curled concave upward). One F_1 male was then mated with virgin females of the *Va/Ba* balanced-lethal strain, in which the *Va* chromosome also carries the *ch* and *cu* mutants (*Varicose* wing veins is a dominant mutant and recessive lethal; *Bare* has a dominant phenotype—absence or reduction of macrochaete number—and is also a recessive lethal). F_2 males and females exhibiting the *Va* phenotype were intercrossed and the viability of the wild-chromosome homozygotes was estimated from the phenotypic ratios in the offspring of these crosses. (For additional detail see, MESTRES *et al.* 1990, particularly Figure 4). Lethal-carrying chromosomes are maintained in heterozygous condition with the *Va* balancer chromosome.

Chromosomal polymorphism: The chromosomal arrangement of the wild chromosomes, made homozygous by the procedure just described, was identified by crossing homozygous males with females of the homokaryotypic strain *chcu* (O_{3+4}/O_{3+4}) and observing the polytene chromosomes of the offspring larvae. In the case of lethal chromosomes, males from the heterozygous *Va*-balancer lines were crossed to the *chcu* females (MESTRES *et al.* 1990).

Lethal-allelism tests: *Va* lines heterozygous for wild lethal-carrying chromosomes were intercrossed to ascertain whether the lethal genes were allelic. All possible crosses were carried out between Gilroy I, Gilroy II, and Bellingham, as well as within each of these populations. All lethal chromosomes from these populations were also tested against three lethal-chromosome lines available from each of the Chilean populations, Santiago and Puerto Montt.

The *Va* balancer chromosome carries two irradiation-induced overlapping inversions (referred to as VIII + 210) and the naturally occurring inversion arrangement O_{3+4} (see Figure 1 of MESTRES *et al.* 1990). About two-thirds (segment SII) of the O chromosome are covered by the VIII + 210 inversion; most of the rest (segment SI) is covered by the 3 + 4 inversion. Thus, if the wild chromosome carries the O_{3+4} arrangement without any overlapping inversions, crossing over becomes possible between the wild and the *Va* balancer chromosomes. We have tested this possibility and found it to be of nontrivial magnitude (see MESTRES *et al.* 1990). Thus, in the O_{3+4} wild chromosomes, lethal genes located on the SI segment can be lost through recombination, and therefore only those lethals present within the SII segment can be ascertained. However, the whole chromosome (segments SI + SII) can be assayed in wild chromosomes lacking the O_{3+4} arrangement. We have used LOUKAS *et al.*'s (1980) method, which converts the SI + SII lethal chromosomes into SII lethal "equivalents." (The method of LOUKAS *et al.* 1980 applied to the Bellingham population yields biologically impossible results; *i.e.*, an incidence of allelism greater than one. This problem can be corrected by using first a length-proportionality correction, such that the incidence of lethals observed in segments SII is extrapolated to the whole chromosome, based on the map lengths given by KUNZE-MÜHL and MÜLLER 1958. In this map, the O chromosome includes bands 75 to 99, whereas the SII segment includes bands 75 to 91 or, approximately, two-thirds of the chromosome.)

Statistical methods: The American populations studied are clearly not at equilibrium with respect to the incidence of lethal genes. However, for analysis purposes, we use mathematical models for estimating population genetic structure from lethal-allelism data that have been described elsewhere (DOBZHANSKY and WRIGHT 1941; WRIGHT *et al.* 1942; NEI 1968; WRIGHT 1978; BEGON *et al.* 1985). The effective population size of the populations is estimated according to NEI (1968) and BEGON *et al.*'s (1985) methods, which give very similar values. Only those obtained by NEI's method are reported below. Lethal heterozygote fitness reduction is estimated following NEI (1968) and CROW and TEMIN (1964). To compute these parameters, it is necessary to estimate the rate of allelism of independently arisen lethal genes (p_∞), the mean mutation-rate from nonlethal to lethal alleles per lethal-producing locus (μ), and the number of loci that mutate to recessive lethals in segment SII of the O chromosome (n). We have used previous estimates: $p_\infty = 0.41$, $\mu = 10^{-5}$ and two values for the number of lethal loci, $n = 238$ (based on the number of bands detected in segment SII of standard polytene O chromosomes) and $n = 307$ (estimated by using the number of bands detected within segment SII in stretched polytene chromosomes) (MESTRES *et al.* 1990; MESTRES and SERRA 1991).

All strains, stocks, and crosses are kept at 18° in standard Drosophila cultures.

RESULTS

Chromosomal polymorphism: Table 1 gives the frequency of the various chromosomal arrangements in the two populations sampled in 1990–1991 (Gilroy II and Bellingham), as well as in the two populations sampled 5 years earlier, Gilroy I and Arlington (PREVOSTI *et al.* 1988). The distribution of the chromosomal polymorphism is not different between the two Gilroy sam-

TABLE 2

Percent frequency of lethal and semilethal chromosomes and lethal load

Population	*N*	Fitness			Lethal load
		L	SL	L + SL	
Gilroy I	111	14.4 ± 3.3	2.7 ± 1.5	17.1 ± 3.6	0.156
Gilroy II	77	10.4 ± 3.5	6.5 ± 2.8	16.9 ± 4.3	0.110
Bellingham	108	12.0 ± 3.1	1.9 ± 1.3	13.9 ± 3.3	0.128
Santiago de Chile	20	15.0 ± 8.0	5.0 ± 4.9	20.0 ± 8.9	0.163

N, number of chromosomes tested. Data for Santiago de Chile are from MESTRES *et al.* (1992) for a collection made in December 1988. Values are means ± SE.

ples ($\chi^2 = 5.8$, d.f. = 6, $P > 0.10$; grouping the arrangements O_{ST}, O_5, and O_7, $\chi^2 = 3.7$, d.f. = 4, $P > 0.10$). The polymorphism distribution is, however, different between Bellingham and Arlington, located 70 km to the south ($\chi^2 = 14.0$, d.f. = 5, $P < 0.05$; grouping O_5 and O_{3+4+7}, $\chi^2 = 9.7$, d.f. = 4, $P < 0.05$). The polymorphism of the two Gilroy samples combined is significantly different from that of Bellingham, Arlington, or both populations combined ($\chi^2 = 27.8$, 24.8, and 40.2, respectively, d.f. = 5, $P < 0.01$). The chromosomal differences between the northern (Bellingham and Arlington) and southern samples (Gilroy I and II) are consistent with the latitudinal clines, previously observed, particularly a southward decrease in the frequencies of O_{ST} and O_5, and increase of O_{3+4+7} (PREVOSTI *et al.* 1985, 1988).

Viability tests: The viability distribution of flies homozygous for wild O chromosomes follows the typical bimodal pattern (DOBZHANSKY *et al.* 1977) with a lesser mode at 0 viability and a larger mode at quasi-normal viability, separated by a trough of semilethal and subvital chromosomes (between 0.05 and 0.50 of quasi-normal viability). Table 2 gives the frequency of lethal and semilethal chromosomes, as well as the lethal genetic load (GREENBERG and CROW 1960). In addition to the two Gilroy and Bellingham populations, the table includes data for a small sample from Santiago de Chile that had earlier been assayed for homozygous chromosomal viability (MESTRES *et al.* 1992). The frequencies of lethals, semilethals, or both combined are not significantly heterogeneous among the four populations ($\chi^2 = 0.73$, 3.9, and 0.63, respectively; d.f. = 3, $P > 0.10$).

The distribution of lethals among the various chromosomal arrangements is given in Table 3. In addition to the four samples assayed for homozygous viability (Table 2), we show data for the Chilean population of Puerto Montt, where the only chromosomes tested for viability were three carrying the O_5 arrangement, all of which proved to be homozygous lethals. The distribution of lethals among the chromosomal arrangements is not significantly different between the two Gilroy samples, nor between these two and Bellingham ($P > 0.10$ for every test). However, within any one population, lethals are not randomly distributed among all chromosomal arrangements ($\chi^2 = 14.6$ for Gilroy II, 54.4 for Bellingham; d.f. = 5; $P < 0.05$), an effect largely due to the high (complete) incidence of lethals among O_5 chromosomes.

Lethal allelism: We have carried out all possible crosses between the lethal chromosomes recorded in Table 3, within and between populations, in order to ascertain allelism. Table 4 summarizes the results. The complete data set is given in the APPENDIX (Tables A1–A4), except for the intrapopulational tests for Gilroy I, which have been published earlier (MESTRES *et al.* 1990), or the three lethals from Puerto Montt, which are all allelic, or the three from Santiago de Chile, which are not allelic to one another. The percent allelism (third column in Table 4) has been corrected (fourth column) using the method described in MATE-

TABLE 3

Chromosomal arrangements of lethal chromosomes

Population	Chromosomal arrangement						Total
	O_{ST}	O_5	O_{3+4}	O_{3+4+2}	O_{3+4+7}	O_{3+4+8}	
Gilroy I	2	4	2	5	3	—	16
Gilroy II	—	1	—	3	4	—	8
Bellingham	4	7	—	1	—	1	13
Santiago de Chile	—	1	—	1	1	—	3
Puerto Montt	—	3	—	—	—	—	3

The number of chromosomes tested and the frequency of lethals are those given in Table 2 for the top four populations. The data for Puerto Montt are from MESTRES *et al.* (1990), where lethality was ascertained only for O_5 chromosomes. The lethal-carrying chromosome lines are identified in the APPENDIX.

TABLE 4

Lethal allelism within and between three *Drosophila subobscura* populations

Population	Crosses N	Crosses Allelic	Percent allelism Uncorrected	Percent allelism Corrected
Gilroy I	120	9	7.5 ± 2.4	5.3 ± 2.1
Gilroy II	28	6	21.4 ± 7.8	13.5 ± 5.1
Bellingham	78	21	26.9 ± 5.0	34.1 ± 6.0
Gilroy I & II	104	13	12.5 ± 3.2	12.8 ± 3.3
Gilroy I & Bellingham	104	7	6.7 ± 2.5	6.7 ± 2.5
Gilroy II & Bellingham	169	28	16.6 ± 2.9	19.1 ± 3.2

Only 13 lethal-chromosome lines from Gilroy I are tested for allelism with Gilroy II and Bellingham because three lines (one each O_{ST}, O_{3+4}, and O_{3+4+7}) of the 16 listed in Table 3 were lost before performing the interpopulational allelism tests. Values are means ± SE.

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The intrapopulational lethal allelism in Gilroy II is entirely due to a lethal gene associated with the O_{3+4+7} (Table A1): all four lethal chromosomes with this arrangement (Table 3) are allelic, although no lethal genes occur in 11 other chromosomes with this arrangement. The intrapopulational allelism in Bellingham is entirely due to a lethal gene invariably associated with the O_5 arrangement (Table A2).

The interpopulational allelism is summarized in the three bottom rows of Table 4; the matrices with the individual crosses are given in the APPENDIX (Tables A3–A4). The allelism between the two Gilroy samples is mostly due to the O_5 and O_{3+4+7} arrangements: all O_5 chromosomes carry one and the same lethal gene, whereas all O_{3+4+7} chromosomes that carry a lethal gene are also interallelic. This is also the case for the one lethal O_{3+4+7} chromosome found in Santiago de Chile, which is interallelic with all other O_{3+4+7} lethals. Of the eight O_{3+4+2} lethal chromosomes found in either Gilroy I or Gilroy II, only two are allelic, one from each sample (moreover, a number of O_{3+4+2} chromosomes are not homozygous lethals). The interallelism between Gilroy I or Gilroy II and Bellingham is due exclusively to the O_5 chromosomes, all of which, as noted, are homozygous lethals and allelic to one another (including the three O_5 chromosomes from Puerto Montt and the one from Santiago de Chile).

Population parameters: Table 5 gives the estimate of the effective population size (N_e), based on the incidence of intrapopulational allelism, as well as estimates of fitness reduction caused in heterozygotes by lethal-carrying chromosomes. Two simply related N_e values are given for each population, based on two different estimates of the number of loci that may carry a lethal allele (n = 238 or 307). The N_e values are helpful primarily for purposes of comparison between samples. The absolute values obtained are biased owing to the high incidence of allelism, which in turn is a historical consequence of the small number of colonizers that have been estimated between 10 and 100 individuals on the basis of different sources of evidence (AYALA *et al.* 1989; PREVOSTI *et al.* 1989; MESTRES *et al.* 1990). The incidence of lethal genes, nevertheless, fits well with the expectations. Assuming n = 300 loci and standard values of mutation ($\mu = 10^{-5}$) and overdominance (h = 0.02), the expected frequency of lethal genes in the O chromosome is P = 0.15, consistent with the values observed in the American populations (Table 2).

DISCUSSION

The study of evolution in nature largely relies on the comparative study of organisms and the investigation of processes at equilibrium or quasi-equilibrium under the conditions that prevail in nature. The dynamics of adaptation and other population processes can be investigated in laboratory experiments that are limited in time and scope but are extrapolated to the scale of the evolutionary process. Occasionally, a natural (or anthropogenic) catastrophe or other drastic occurrence triggers a sequence of events that provide unique opportunities for investigating evolution in action. The colonization of western North America and South America by *D. subobscura* and its rapid expansion over the last two decades allows investigation of otherwise intractable problems.

An open question concerns whether or not clinal and

TABLE 5

Population parameters derived from lethal allelism

Population	n = 238 h	n = 238 h_e	n = 238 N_e	n = 307 h	n = 307 h_e	n = 307 N_e
Gilroy I	0.0166	0.0084	2145	0.0215	0.0132	1830
Gilroy II	0.0140	−0.0053	847	0.0181	−0.0012	651
Bellingham	0.1121	0.1051	212	0.1447	0.1376	164

n is the number of loci that can mutate to recessive lethals; h and h_e are the average fitness reduction in lethal heterozygotes according to NEI (1968) and CROW and TEMIN (1964), respectively. N_e is the effective population size.

other geographic patterns exhibited by the chromosomal polymorphisms of Drosophila species are adaptive. PREVOSTI (1964) and others (review in KRIMBAS and LOUKAS 1980; see AYALA *et al.* 1989; MESTRES *et al.* 1994) have determined that the frequencies of the chromosome arrangements of *D. subobscura* vary in clinal patterns that can be correlated with latitude. The adaptive character of these clines has been determined by showing that the same latitudinal clines found in the Old World have also evolved in the New World (PREVOSTI *et al.* 1985, 1988; AYALA *et al.* 1989). Moreover, the New World patterns have with time become increasingly similar to those in the Old World (PREVOSTI *et al.* 1990). Our results reflect the same patterns previously observed in both the New and the Old World: a decrease in the frequency of the arrangement O_{3+4+7}, and an increase in the frequency of O_{ST}, with increasing latitude. We have also encountered significantly higher frequencies of O_5 at higher latitudes, as previously observed in North America as well as in South America. In Old World populations, O_5 is absent or very rare, which precludes statistical analysis of clinal patterns (MESTRES *et al.* 1992). Nevertheless it is most often found in northern European populations, consistently with its higher frequency at higher latitudes in the Americas.

The frequency of lethal-carrying chromosomes in Gilroy and Bellingham (as well as other New World populations) is within the range, but toward the lower boundary, of the frequencies observed for the O chromosome in Old World populations of *D. subobscura* (SPERLICH *et al.* 1977; LOUKAS *et al.* 1980; PFRIEM and SPERLICH 1982; KOHONEN-CORISH *et al.* 1985; MESTRES *et al.* 1990). However, the incidence of lethal chromosomes is not randomly distributed throughout the genome but tend to be specifically associated with particular chromosome arrangements. The most notable association involves the O_5 arrangement, which is invariably associated with a recessive lethal gene that is, moreover, allelic throughout all the American populations (MESTRES *et al.* 1990, 1992). This invariant association is confirmed by the results presented in this paper. A newly detected association involves the O_{3+4+7} arrangement. About 20% of all O_{3+4+7} chromosomes carry a lethal gene, allelic in all of them. In contrast, there are no nonrandom associations between lethal genes and chromosomal arrangements in Bordils (100 km from Barcelona), a Palearctic population in the central area of the distribution of *D. subobscura* that was extensively investigated for the purpose (Mestres *et al.* 1990).

Nonrandom associations between lethal genes and chromosomal arrangements have also been described in *D. pseudoobscura,* for which a variety of explanations have been proposed (EPLING *et al.* 1961; MAYHEW *et al.* 1966). CRUMPACKER and SALCEDA (1969), for example, have proposed that selection for high average fitness would result in the association of deleterious recessive genes with rare chromosomal arrangements (see DOBZHANSKY *et al.* 1963). However, in the case of *D. subobscura* the nonrandom association between lethal genes and chromosomal arrangements is most parsimoniously explained as a consequence of a founder event, an explanation that also accounts for the high incidence of lethal allelism. Indeed, if the number of colonizers was <100, as indicated by various sources of evidence (AYALA *et al.* 1989), it seems likely that only one O_5 arrangement was included among them, because O_5 is quite rare in the Old World. This chromosome would have happened to carry a recessive lethal gene. Similarly, among the several O_{3+4+7} founder chromosomes (an arrangement quite frequent in many Old World populations), only one would have carried a lethal gene, which therefore would yield allelism among all New World lethal-carrying O_{3+4+7} chromosomes. This explanation depends, of course, on the persistence of specific associations between genes and chromosomal arrangements. This is indeed known to be the case in *D. subobscura,* where it has been attributed primarily to this species' rich chromosomal polymorphism, which handicaps recombination (KRIMBAS and ZOUROS 1969; SPERLICH and FEUERBACH-MARAVLAG 1974; KRIMBAS 1993). Moreover, specific linkage-disequilibrium associations between allozyme variants and chromosomal arrangements have been demonstrated in American populations (PREVOSTI *et al.* 1983; BALANYÀ 1989) as well as in the Old World (CHARLESWORTH *et al.* 1979; LOUKAS *et al.* 1979; GARCIA and PREVOSTI 1981; PINSKER and SPERLICH 1981).

The question arises of why recessive lethal genes occur at fairly high frequencies (10–30% per chromosome) in natural populations. The persistence of lethal genes has been attributed by WATANABE and OSHIMA (1970) to linkage association with adaptive gene complexes or heterotic inversions. Some authors have conjectured that lethal genes may increase the overall fitness of populations because they may be heterotic under particular recurrent conditions (SPIESS *et al.* 1963; IVES 1970; GOLUBOVSKY 1968; GOLUBOVSKY and VICTOROVA 1968). Some theoretical models have identified conditions that would account for the persistence of lethal genes in natural populations (PROUT 1967; ROBERTSON and NARAIN 1971). The persistence of lethal genes over the generations has been observed in natural populations, as well as changes in frequencies associated with the seasons and other climatic variables (DUBININ 1946; GOLDSCHMIDT *et al.* 1955; BAND and IVES 1961, 1968; BAND 1972; IVES and BAND 1986). It may be, however, that the high frequency of lethal genes may simply be a consequence of mutation/selection balance, which under standard assumptions (see RESULTS) yields an expected incidence of lethality of 0.15 for the O chromosome, which is approximately the average value observed in the American populations (Table 2).

With respect to these American populations of *D. subobscura*, the issue is not so much the persistence of lethal alleles over time, as observed in the Gilroy samples, but particularly the widespread occurrence of allelic lethals. WALLACE (1966) observed in populations linearly spaced at ~30-m intervals that there was a rapid decrease in the incidence of allelism with increasing distance. Other studies addressed to investigate the problem have similarly shown a rapid decrease in the rate of allelism over distance (PAIK and SUNG 1969; YAMAZAKI *et al.* 1986). Yet we find that O_5 lethal chromosomes are allelic in populations as distant as Gilroy and Bellingham and even between these and the South American populations of Puerto Montt and Santiago de Chile. Similarly, the O_{3+4+7} lethals found 5 years apart in Gilroy are allelic not only with each other, but also with the one found in Santiago de Chile. These allelisms are, no doubt, a consequence of the colonization process, which involved the successful multiplication and dispersal of the species from a small number of founders.

The colonization process explains why allelism occurs, but not why it persists with fairly high incidence. If allelic lethals occur at high frequency, homozygous lethals will also appear at fair frequencies, which would lead to a rapid decrease in lethal frequencies. Lethal allelism is indeed much higher in the American populations than in the Old World. In the well-studied population of Bordils (Barcelona, Spain), for example, the frequency of lethal genes is 29.0%, more than double the frequencies observed in North American populations, but the incidence of allelism is very low 0.7% (MESTRES *et al.* 1990). One possible explanation for the high lethal allelism of the American populations is, as we shall now argue, that heterotic effects due to linkage disequilibrium between the lethal recessives and other genes are responsible for the persistence of lethal allelism.

Table 5 shows that the effective size of the Gilroy population has ostensibly decreased in 6 years (1984–85 to 1990–91) to one-third of its original value. This reduction might be due to an actual reduction in population numbers, perhaps even a bottleneck, associated with ecological factors. In support of this conjecture, it might be noted that the frequency of *D. subobscura*, among 885 individuals collected of all the *obscura* species, was 14.1% in a 1985 sample; but only 7.5% in a sample of 893 individuals collected in 1991. This conjecture is, however, not supported as an account of the low effective population size estimated for Bellingham (~200 individuals), where *D. subobscura* is very abundant, in absolute numbers as well as in its proportion among other *obscura* species (94.7% in a 1991 sample of 720 *obscura* flies).

An alternative possibility is that the increase in lethal allelism (and, hence, the reduction in the apparent effective population size) observed in Gilroy may be due to natural selection favoring a frequency increase of some lethal-carrying chromosomes owing to heterotic effects associated with other loci. This would seem consistent with the heterosis detected in Table 5 by the *negative* average fitness reduction (h_e) in heterozygotes obtained by the method of CROW and TEMIN (1964). The fitness reduction is, however, positive when we use (h) the method of NEI (1968), who has argued that such negative fitness reductions may be statistical artifacts without biological significance (see MESTRES and SERRA 1991). The very low effective population size estimated for Bellingham in spite of the large abundance of *D. subobscura* flies might also be due to the small number of founders from which the *D. subobscura* American populations are derived. But this explanation is not satisfactory as an account of why Bellingham has a much smaller apparent N_e value than Gilroy. The evidence indicates that the North American colonization of *D. subobscura* occurred in the north (Washington or British Columbia) and spread gradually southward. Thus, small effective population sizes attributable to a small number of founders, should be smaller in southern than in northern populations, because of the derivative character of the southern populations—in addition to the noted observation that *D. subobscura* is conspicuously less abundant, in absolute as well as in relative numbers, in central California (Gilroy) than in northern Washington (Bellingham).

It seems that the final explanation for the increase in lethal allelism in Gilroy between 1984–1985 and 1990–1991, as well as the still higher lethal allelism observed in Bellingham must be left in abeyance, pending further investigations.

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APPENDIX

Lethal allelism tests within and between populations are presented as Tables A1–A4. The intrapopulation allelism for Gilroy I is given in MESTRES *et al.* (1990, Figure 6): nine tests are allelic, six among all four O_5 lines and three among all three O_{3+4+7} lines. Crosses made are indicated by +, allelism by A. Dummy numbers, 1–63, are used here to identify the lethal lines.

TABLE A1

Intrapopulation allelism in Gilroy II

		Line						
Line	Arrangement	2	3	4	5	6	7	8
1. GM4	5	+	+	+	+	+	+	+
2. GM24	3+4+2		+	+	+	+	+	+
3. GM107A	3+4+2			+	+	+	+	+
4. GF3I	3+4+2				+	+	+	+
5. FGF5	3+4+7					A	A	A
6. GM1BK	3+4+7						A	A
7. GM111A	3+4+7							A
8. GM121B	3+4+7							

TABLE A2

Intrapopulation allelism in Bellingham

		Line											
Line	Arrangement	12	13	14	15	16	17	18	19	20	21	22	23
11. BM1	ST	+	+	+	+	+	+	+	+	+	+	+	+
12. BM5	ST		+	+	+	+	+	+	+	+	+	+	+
13. BF20	ST			+	+	+	+	+	+	+	+	+	+
14. BF69	ST				+	+	+	+	+	+	+	+	+
15. BM2	5					A	A	A	A	A	A	+	+
16. BM42	5						A	A	A	A	A	+	+
17. BM63	5							A	A	A	A	+	+
18. BM79	5								A	A	A	+	+
19. BM90	5									A	A	+	+
20. BF8	5										A	+	+
21. BF30	5											+	+
22. BF66	3+4+2												+
23. BM41	3+4+8												

TABLE A3

Interpopulation allelism between Gilroy II and Bellingham

	Line												
Line	11	12	13	14	15	16	17	18	19	20	21	22	23
1	+	+	+	+	A	A	A	A	A	A	A	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A4

Interpopulational allelism between Gilroy II or Bellingham and Gilroy I, Santiago de Chile and Puerto Montt

			Line																				
Line	Arrangement		1	2	3	4	5	6	7	8	11	12	13	14	15	16	17	18	19	20	21	22	23
31	G301	ST	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	G7A	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
33	G61	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
34	G226	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
35	G240	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
36	G215	3+4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
37	G32	3+4+2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
38	G34	3+4+2	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39	G41	3+4+2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	G66	3+4+2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
41	FG6	3+4+2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
42	G39	3+4+7	+	+	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+
43	G209	3+4+7	+	+	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+
51	S47	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
52	S16	3+4+2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
53	S49	3+4+7	+	+	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+
61	P234	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
62	P269	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+
63	P282	5	A	+	+	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	+	+

Gilroy II, lines 1–8; Bellingham, lines 11–23; Gilroy I, lines 31–43; Santiago de Chile, lines 51–53; and Puerto Montt, lines 61–63.