Title
A grand experiment in evolution: the Drosophila subobscura colonization of the Americas

Journal
Genome, 31(1)

ISSN
0831-2796

Authors
Ayala, Francisco J
Serra, Luis
Prevosti, Antonio

Publication Date
1989

DOI
10.1139/g89-042

23.4.4 A grand experiment in evolution: the *Drosophila subobscura* colonization of the Americas

FRANCISCO J. AYALA[1]
Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A.

AND

LUIS SERRA AND ANTONIO PREVOSTI
Departamento de Genética, Universidad de Barcelona, Barcelona 08028, Spain

Symposium Editor: L. Levine

AYALA, F. J., SERRA, L., and PREVOSTI, A. 1989. A grand experiment in evolution: the *Drosophila subobscura* colonization of the Americas. Genome, **31**: 246–255.

Drosophila subobscura is a Palearctic species that has been extensively studied by population and evolutionary geneticists for nearly half a century. In 1978, it appeared in Puerto Montt, Chile; within a few years it extended over much of Chile and into Argentina and became the most common drosophilid in many places. In 1982, it appeared in the American northwest; shortly thereafter it was found extensively distributed from southern British Columbia, through Washington and Oregon, into southern California, west of Sierra Nevada. In North America also it has become a common drosophilid in many places. The source of the colonizers has been sought with four lines of research: sequence arrangement of the polytene chromosomes, allozyme polymorphisms, mitochondrial DNA restriction patterns, and frequency of lethal alleles. The origin of the colonizers remains uncertain, although all evidence indicates that both the North American and the South American colonizers derive from the same Palearctic population. The overall configuration of the chromosomal and allozyme frequencies suggests a western Mediterranean origin, which is consistent with the mtDNA data. The presence of a particular chromosome arrangement, O_5, suggests a northern European origin. Lethal allelism has opened up the possibility of discovering the precise origin of the colonizers: all O_5 chromosomes in the Americas carry a particular recessive lethal gene. There is strong evidence that the number of founders was not very small and not very large, perhaps between 10 individuals and several score. The chromosomal polymorphisms of *D. subobscura* exhibit well-defined latitudinal clines in the Old World. In the few years since the colonization, clines in every chromosome have evolved in the Americas that have identical latitudinal polarity with those in the Old World. This would seem strong evidence that the polymorphisms and the clines are adaptive.

Key words: chromosomal polymorphism, mitochondrial DNA evolution, allozyme polymorphism, lethal allelism, adaptation, geographic clines.

AYALA, F. J., SERRA, L., et PREVOSTI, A. 1989. A grand experiment in evolution: the *Drosophila subobscura* colonization of the Americas. Genome, **31** : 246–255.

Le *Drosophila subobscura* est une espèce paléarctique qui a fait l'objet d'études approfondies par les généticiens des populations et de l'évolution, durant près d'un demi siècle. En 1978, elle est apparue à Puerto Montt, au Chili; en peu d'années, elle s'est propagée sur presque tout le Chili jusqu'en Argentine et elle est devenue, en plus d'un endroit, l'espèce la plus répandue. En 1982, elle est apparue dans l'ouest de l'Amérique du Nord; peu après, sa distribution a été notée au sud de la Colombie-Britannique puis, à travers les états de Washington et de l'Orégon, dans le sud de la Californie et l'ouest de Sierra Nevada. En Amérique du Nord, elle est également devenue le drosophilidé le plus répandu en plus d'un endroit. La source des colonisateurs a été recherchée par quatre lignes d'études : l'arrangement séque ntiel chez les chromosomes polytènes, les polymorphismes d'allozymes, les profils de restriction de l'ADN mitochondrial t la fréquence des allèles létaux. L'origine des colonisateurs demeure incertaine, bien que toutes les évidences favorisent u ıe même population paléarctique comme colonisatrice des Amériques du Sud et du Nord. La configuration générale des fréquences chromosomiques et des allozymes suggèrent l'ouest de la Méditerranée comme origine, ce qui concorde avec les données issues de l'ADN_{mt}. La présence d'un arrangement particulier d'un chromosome, le O_5, suggère le nord de l'Europe comme origine. La létalité allélique a ouvert la possibilité de découvrir l'origine précise des colonisateurs : tous les chromosomes O_5 des Amériques sont porteurs d'un gène récessif létal particulier. Il existe une forte évidence que le nombre de fondateurs ne serait ni très petit ni très grand, peut-être entre dix ou plusieurs dizaines d'individus. Les polymorphismes chromosomiques présentent des gradients latitudinaux bien définis dans le Vieux Monde. En peu d'années depuis la colonisation, les gradients dans chaque chromosome ont évolué dans les Amériques qui ont des polarités latitudinales similaires à celles du Vieux Monde. Ceci serait une forte évidence que les polymorphismes et les gradients résultent d'adaptations.

Mots clés : polymorphismes chromosomiques, évolution de l'ADN mitochondrial, polymorphismes des allozymes, allélisme létal, adaptation, gradients géographiques.

[Traduit par la revue]

Drosophila subobscura is a Palearctic species of the *obscura* subgroup of *Drosophila*, which is endemic throughout Europe (except in central and northern Scandinavia) as well as in the Middle East, northern Africa, and in the Atlantic islands: the Azores, Madeira, and the Canaries (Fig. 1). The *obscura* subgroup consists of about 20 Old World species plus a few New World species, notably *D. pseudoobscura, D. persimilis*, and *D. miranda*, three common species in western North America (Lakovaara and Saura 1982). The distribution of *D. pseudoobscura* extends to Mexico and parts of Central America; a distinct subspecies, namely, *D. p. bogotana*, exists in the altiplano region near Bogotá, Colombia (Ayala and Dobzhansky 1974). No other *obscura* subgroup species had ever been found in South America before 1978.

Drosophila subobscura is a favorite species of European geneticists and evolutionists working with natural populations

[1]Author to whom all correspondence should be addressed.

Printed in Canada / Imprimé au Canada

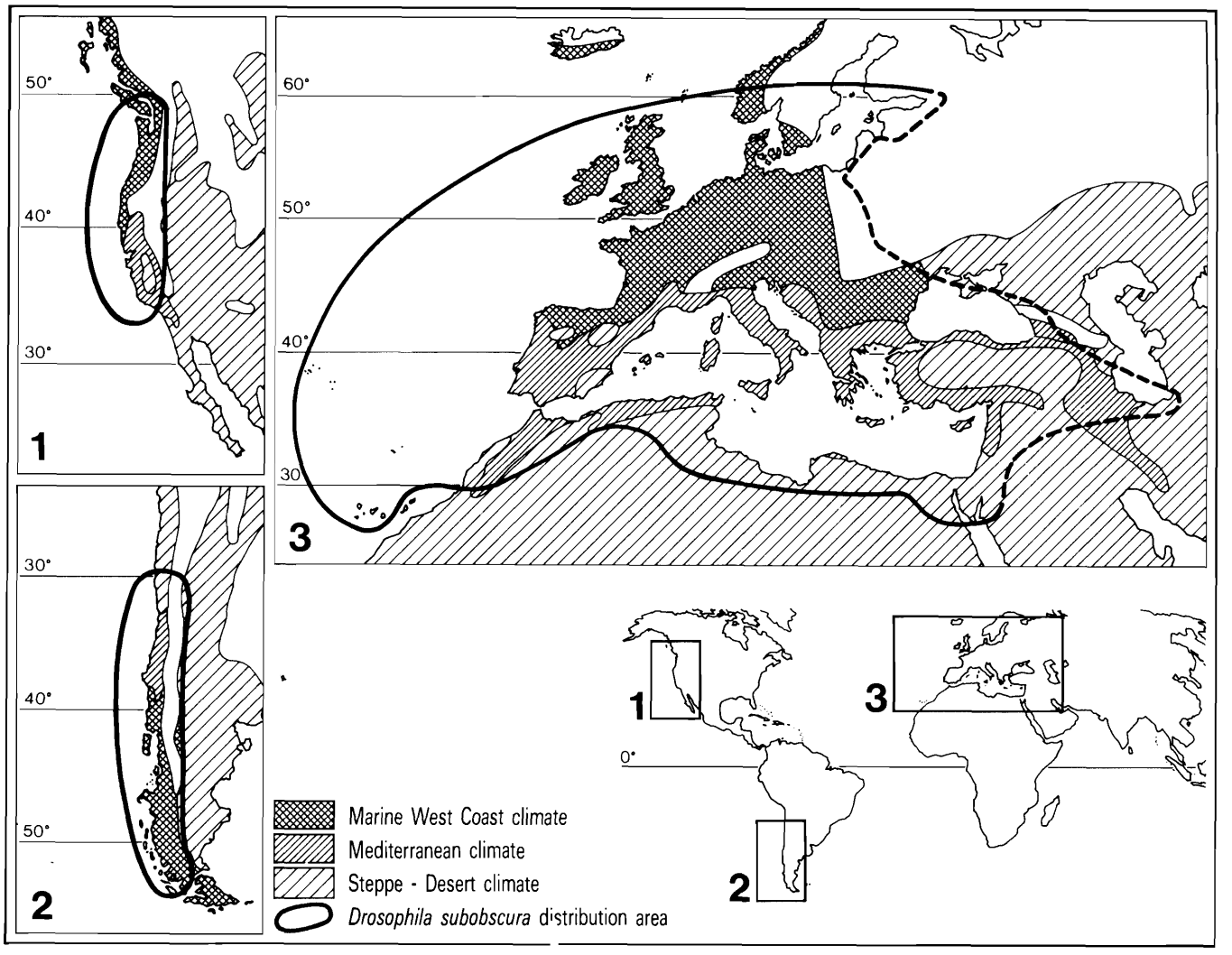

FIG. 1. Distribution areas of *Drosophila subobscura* on three continents. The prevailing climatic conditions in all three areas are Mediterranean and western maritime. With decreasing latitude, there is a transition from Mediterranean to steppe–desert conditions that overlaps one boundary of the species distribution. The eastern boundary of the Palearctic distribution of the species is uncertain and is indicated by a broken line marking the perimeter. From Prevosti et al. (1988).

of *Drosophila*. Half a century of research by scores of investigators in numerous laboratories has yielded considerable knowledge concerning the biogeography, ecology, behavior, cytogenetics, population genetics, and evolutionary biology of *D. subobscura*.

The colonization

In February 1978, *D. subobscura*, never before found in the Americas, was discovered in southern Chile, in Puerto Montt (41°28′S), in a place where numerous collections had previously been made over the years (Budnik and Brncic 1982; Prevosti et al. 1987). In the following summer, fall, and winter months, *Drosophila* collections in central Chile did not include the species, but by the next spring, towards the end of 1978, rich samples were obtained over a vast territory. In the summer of 1979, *D. subobscura* was found as far north as La Serena (29°55′S), although in low frequencies. In January 1981, it was collected in Punta Arenas (53°10′S) near the Magellan Straits. In November 1981, the species was discovered on the eastern side of the Andes, in San Carlos de Bariloche (41°11′S), Argentina, and in 1984 in Mar del Plata, on the Atlantic coast of Argentina, 400 km south of Buenos Aires. Later collections have failed to yield *D. subobscura* in the region of Buenos Aires (although it is very common in San Carlos de Bariloche) or in Punta Arenas and other localities somewhat northward. Thus, the range where *D. subobscura* has been found in South America extends over a broad region from 29 to 53°S and from the Pacific to the Atlantic, although it may not have become permanently established in the southernmost and easternmost reaches of that region (Fig. 2; Prevosti et al. 1987). Throughout much of that enormous expanse, *D. subobscura* has become very common, often the most abundant drosophilid.

Drosophila subobscura was discovered in North America in 1982, in Port Townsend (48°N), on the northern coast of Washington State. Shortly thereafter, it was also collected in other localities of Washington, as well as towards the north in the vicinity of Vancouver, B.C., and towards the south in Oregon (Beckenbach and Prevosti 1986). In the fall of 1983, the species was found in the Central Valley of California, in Davis, and in El Rio Vineyard, 25 km west and 75 km south-

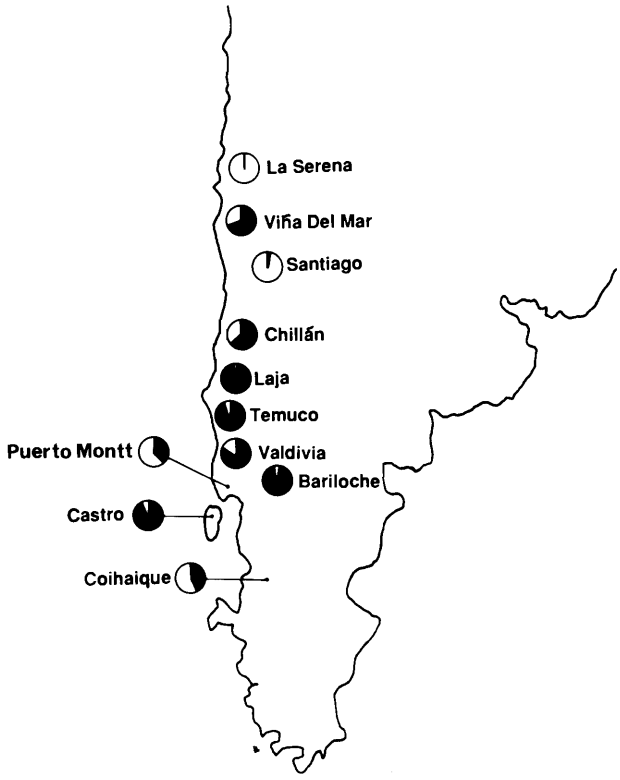

FIG. 2. Collection sites and frequency of *Drosophila subobscura* in South America. The closed sector of each circle represents the proportion of *D. subobscura* among all *Drosophila* species. Data from Prevosti et al. (1987).

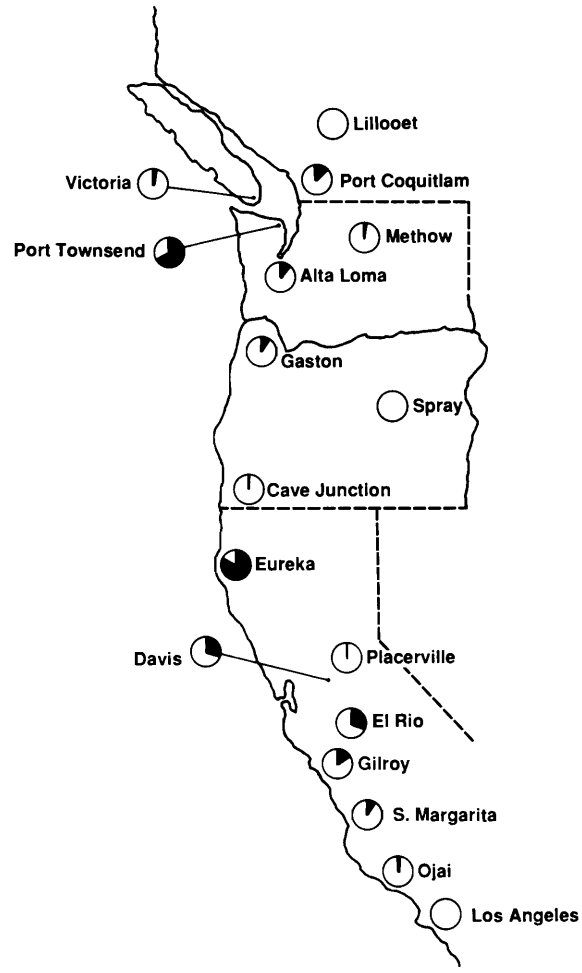

FIG. 3. Collection sites and frequency of *Drosophila subobscura* in North America. The closed sector of each circle represents the proportion of *D. subobscura* among all species of the *obscura* subgroup. Data from Beckenbach and Prevosti (1986); plus data from Prevosti et al. (1987).

east of Sacramento, respectively. 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 (Fig. 1; Prevosti et al. 1987). In many localities, *D. subobscura* has become quite common, on occasion the most abundant species of the *obscura* subgroup (Fig. 3). The frequency of *D. subobscura* relative to other *obscura* subgroup species, or to all *Drosophila* species, however, depends on the date of the collection (Fig. 4). In North America, *D. subobscura* is most abundant in the early spring. Extensive collections since the beginning of 1988 in Los Angeles, and particularly in Irvine and vicinity, have failed to yield any *D. subobscura* individuals, although endemic *obscura* subgroup species are present (M. Pascual, unpublished data). The southernmost limit of the North American distribution of *D. subobscura* appears to be north of Los Angeles, in the vicinity of Ojai.

There can be little doubt that the colonization of the Americas by *D. subobscura* is a recent event, which occurred probably not much earlier than 1978, when it was first discovered in Puerto Montt. Scientists of the Department of Genetics, University of Chile in Santiago, have for several decades collected *Drosophila* at frequent intervals throughout much of Chile. In Puerto Montt, at the very site where *D. subobscura* was first collected, *Drosophila* flies were collected every year for about 20 years. The *Drosophila* fauna in Chile does not include any species of the *obscura* subgroup, nor any other dark grey species that would resemble *D. subobscura* in overall appearance, and with which *D. subobscura* individuals might have been confused.

In western North America, however, there are a number of species that resemble *D. subobscura* in overall appearance and coloration, namely the three *obscura* siblings *D. pseudoobscura*, *D. persimilis*, and *D. miranda*, as well as species of the *affinis* subgroup, such as *D. azteca* and *D. athabasca*. *Drosophila subobscura* individuals might be confused with members of these species in casual classifications on the basis of overall appearance. Nevertheless, it seems likely that the colonization of western North America by *D. subobscura* is a fairly recent event. Professor Theodosius Dobzhansky and others have collected flies of the subgroup *obscura* in that region since the 1930s. The collections extended over the whole region where *D. subobscura* is now found, were repeated over the years, and typically included hundreds or thousands of individuals from each collecting site. A knowledgeable investigator concerned with the classification of *obscura*-like flies would not be likely to mistake *D. subobscura* for one of the North American endemic species. Moreover, many collections were subject to detailed chromosomal and other studies that unambiguously differentiate all the species at stake.

The colonization of Davis and nearby localities certainly occurred after 1975. From 1971 to 1975, Th. Dobzhansky,

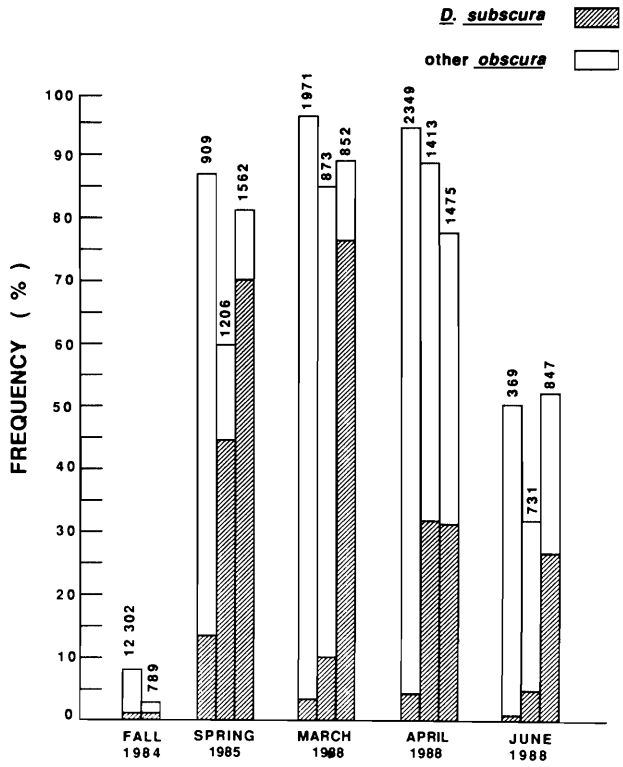

FIG. 4. Bar diagram showing the relative frequency of *Drosophila* species at different times in three different populations of California. The hatched part of each bar represents the frequency of *D. subobscura* and the open part the frequency of other *obscura* subgroup species, relative to all *Drosophila*. For each collection date, the left bar represents Gilroy, the middle bar Davis, and the right bar Eureka. (No collection was made in Eureka in the fall of 1984.) The number on top of each bar is the total number of *Drosophila* flies in the sample. Data for fall 1984 and spring 1985 are from Prevosti et al. (1987); other data are from M. Pascual (unpublished).

TABLE 1. Average frequency of various chromosome arrangements in populations from the Americas and Europe

Chromosome arrangement	South America	North America	Spain	Switzerland
A_{ST}	0.469	0.552	0.492	0.678
A_2	0.531	0.448	0.468	0.078
Other	—	—	0.036	0.244
J_{ST}	0.293	0.381	0.254	0.606
J_1	0.707	0.619	0.745	0.394
E_{ST}	0.588	0.622	0.372	0.874
E_{1+2}	0.002	0.022	0.204	0.061
E_{1+2+9}	0.122	0.159	0.145	0.017
$E_{1+2+9+3}$	0.192	0.116	0.054	0.003
$E_{1+2+9+12}$	0.093	0.080	0.195	0.005
E_{17}	0.002	—	—	—
Other	—	—	0.028	0.040
U_{ST}	0.506	0.404	0.045	0.635
U_{1+2}	0.318	0.354	0.514	0.265
U_{1+2+8}	0.176	0.242	0.430	0.042
Other	—	—	0.013	0.068
O_{ST}	0.244	0.229	0.240	0.833
O_5	0.067	0.059	—	0.007
O_7	0.001	0.001	0.027	—
O_{3+4}	0.031	0.038	0.212	0.051
O_{3+4+2}	0.323	0.352	0.035	0.004
O_{3+4+7}	0.118	0.123	0.337	0.001
O_{3+4+8}	0.217	0.198	0.068	0.071
Other	—	—	0.085	0.012
N	1626	1012	492	720
Populations	9	7	3	1

NOTE: *N* is the number of chromosomes sampled. Data are from Prevosti et al. (1982, 1985, 1987, 1988).

F. J. Ayala, and colleagues intensively collected in that area. In a particular location, McDonald Ranch, about 50 km west of Davis, monthly collections were consecutively made for 5 years. Tens of thousands of *obscura* subgroup flies were collected and examined. The enzyme polymorphisms at 8 to 30 loci were analyzed electrophoretically in each of many thousands of wild individuals and their progeny. The chromosome-arrangement polymorphisms also were studied in the progeny of thousands of wild individuals. Either one of these two types of study provides unambiguous discrimination between *D. subobscura* and the other *obscura* subgroup species (as well as among the latter ones). Not a single *D. subobscura* individual was ever found in those years. Nevertheless, since 1983 *D. subobscura* is readily found in Davis and nearby localities, at frequencies that range from a few percent to more than half of all *obscura*-like flies, depending on the particular site and time of the year.

Drosophila subobscura has been a successful colonizer of the Americas. In both North America and South America, it has rapidly spread and become very common over enormous regions. The two nearly simultaneous colonizations provide unique opportunities for evolutionary studies. Rarely, if ever, have two colonizations of this magnitude occurred that could be investigated almost from their start. The fact that much was already known about the population genetics and evolutionary biology of *D. subobscura* from the Old World contributes to the versatility and feasibility of the investigations that can be carried out.

The colonizers

Tracing the evolutionary changes of *D. subobscura* in the Americas would be enhanced if it were known where the colonizers came from and, hence, their genetic composition. Four lines of research have sought to resolve this issue: (*i*) sequence arrangement of the chromosomes, ascertained in the giant salivary chromosomes; (*ii*) allozyme polymorphisms; (*iii*) mitochondrial DNA sequence variations; and (*iv*) frequency of lethal genes and their allelism.

The karyotype of *D. subobscura* consists of five acrocentric chromosomes, named A, J, U, E, and O, plus a small dot-like chromosome. Variations in sequence arrangement have been found in every one of the five large chromosomes: more than 80 different arrangements in total. There are differences between localities, but in every population most or all five chromosomes are polymorphic.

The chromosomal polymorphisms have been studied in nine South American and seven North American populations. The average frequencies of the various arrangements found in each region are shown in Table 1. The capital letters represent the five chromosomes and the subscripts identify particular chromosome arrangements. Nineteen different arrangements have been found in North America and the same 19 plus 1 more in South America. The chromosome sequence found

only in South America is E_{17}, which has been found once in Santiago and three times in Chillán (see Fig. 2 for the location of these two populations) but nowhere else in the world. It seems likely that this arrangement may represent a chromosomal inversion that arose anew in Chile, after the colonization.

The frequencies of the various chromosomal arrangements are remarkably similar in South America and in North America. The presence of the same set of 19 sequences, of 80 known sequences, and in the same frequencies in both continents suggests that the two colonizations are not independent, that is, either the colonizers of one of the two continents may have originated from the other or both continents may have been colonized by individuals from the same Old World population. The set of chromosome frequencies suggests, according to Prevosti et al. (1987), that the colonizers may have originated in the western Mediterranean region. Table 1 shows the average chromosomal frequencies in three populations from Spain: Barcelona, Valencia, and Ibiza (Prevosti et al. 1982). These chromosomal frequencies are typical of the western Mediterranean region (and they are virtually identical in the three particular populations averaged in Table 1).

Some differences between the American and the Spanish populations are apparent in Table 1. Most notable is the presence in both American continents of O_5, an arrangement never found in the western Mediterranean region but present in the eastern Mediterranean and in northern Europe, where it occurs with appreciable frequencies (Prevosti et al. 1987). Other differences between the Spanish and American populations involve E_{1+2} and O_{3+4}, which are common (about 20%) in Spain but rare (3% or less) in the Americas; and U_{ST} and O_{3+4+2}, which exhibit the opposite frequency. These frequency differences could be explained by sampling accidents, but the presence of O_5 cannot be so explained, unless one were to assume (*i*) that O_5 does occur in the western Mediterranean but at such low frequencies that it has not yet been detected and (*ii*) that in spite of its rarity, O_5 was carried by one of the colonizers. Although not impossible, this explanation seems unlikely.

The arrangement O_5 has been found in one population of Israel and one of Greece as well as in a broad region that extends from Switzerland and Germany towards northwestern continental Europe. Its highest frequency (about 15%) occurs in southern Scandinavia (Krimbas and Loukas 1980). In central Europe, O_5 occurs at low frequencies in populations where all 20 arrangements found in the Americas are also present and where few others occur. Table 1 shows, in the last column, the arrangement frequencies found in a well-studied population from Zürich, Switzerland (Prevosti et al. 1982). The frequencies found in this population and in the Americas are fairly different for a number of chromosome arrangements, such as A_2, E_{1+2+9}, $E_{1+2+9+3}$, U_{1+2+8}, O_{3+4+2}, and others.

The extensive chromosomal polymorphisms found in the American populations establish that the number of colonizers was more than two individuals: seven different arrangements are present in the O chromosome and five (excluding E_{17}) in chromosome E. Brncic et al. (1981) argue that the number of colonizers must have been at least 10. The extensive enzymatic polymorphisms found in the Americas also indicate that the number of colonizers was not very small, but the same data as well as the lethal allelisms suggest that it was not very large either (see later).

TABLE 2. Average allele frequencies at seven loci coding for enzymes

Locus	Allele	South America (3 populations)	Spain (2 populations)
Aph	*46*	0.003	—
	73	0.362	0.476
	100	0.635	0.519
	Other	—	0.005
Est-3	*100*	0.508	0.711
	120	0.492	0.235
	Other	—	0.053
Est-7	*100*	1.000	0.952
	Other	—	0.048
Lap	*100*	0.560	0.716
	106	0.319	0.059
	111	0.121	0.150
	Other	—	0.075
Pept-1	*00*	0.177	—
	40	0.459	0.649
	100	0.363	0.324
	Other	—	0.027
Acph	*54*	0.007	0.074
	100	0.991	0.867
	188	0.002	0.027
	Other	—	0.033
Est-5	*90*	0.308	0.457
	100	0.439	0.495
	Other	—	0.047

NOTE: *Aph*, *Est-3*, and *Est-7* are on chromosome J; the other four loci are on chromosome O. For the J-chromosome loci, the sample sizes per locus are 396 and 200 for South America and Spain, respectively; for the O-chromosome loci, they are 540 and 491. Data are from Prevosti et al. (1982).

Table 2 summarizes the allele frequencies found at seven autosomal loci coding for enzymes. The frequencies found in South America (Chillán, Laja, and Valdivia; see Fig. 2 for their location) and in Spain (Barcelona and Valencia) are quite similar. The most important exception is Lap^{106}, which has a frequency of 0.288−0.346 in South America but a much lower frequency, on average, in Spain. However, its frequency is 0.118 in Barcelona (whereas it is absent in Valencia), which would not exclude the possibility that the colonizers came from this locality (or others in Spain or the western Mediterranean region).

The limited data available indicate that the American populations have fewer enzyme alleles than Old World populations, as a result of the absence in the former of nearly all alleles that are fairly rare in the latter. The alleles present in America are those that are common in the Old World and, indeed, present in virtually every Old World population, which deprives the allozyme data from any meaningful information about the precise origin of the colonizers. The absence of most rare alleles suggests, as pointed out, that the number of colonizers was not large.

Mitochondrial DNA (mtDNA) genotypes are clones maternally transmitted that do not recombine during sexual reproduction. They, therefore, evolve by accumulation of mutations and may provide definitive information about the female lineage from which an individual descends. The possibility of such discrimination depends, of course, on the extent and distribution of the mtDNA intraspecific polymorphisms, which are

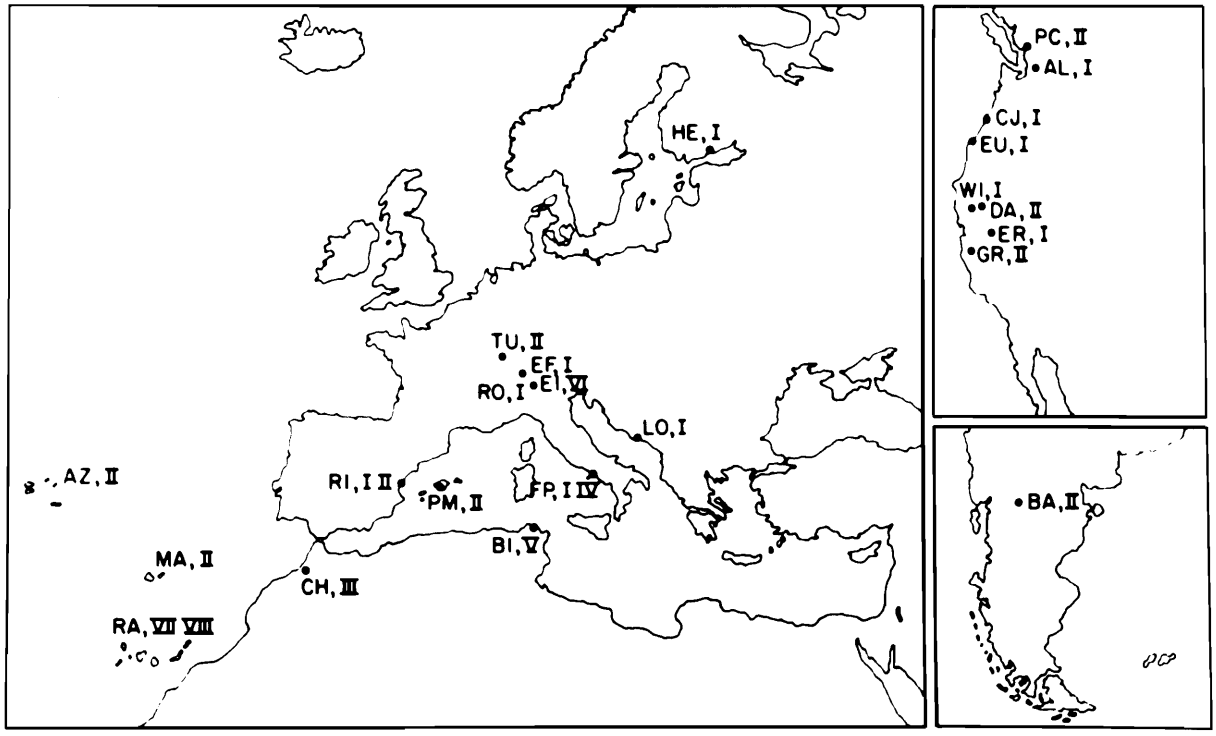

FIG. 5. Mitochondrial DNA patterns (I–VIII) found in 23 populations of *Drosophila subobscura*. Fourteen localities are in the Old World. In approximate north-to-south and east-to-west order, they are Helsinki (HE), Finland; Tübingen (TU), West Germany; Eierbrecht (EI, two strains), Effretikon (EF), and Rochefort (RO), Switzerland; Lokrum (LO), Yugoslavia; Formia-Ponza (FP, two strains), Italy; Palma de Mallorca (PM) and Ribarroja (RI, two strains), Spain; Bizerte (BI), Tunisia; Chechaouen (CH), Morocco; Azores (AZ, two strains); Madeira (MA); Raíces (RA, two strains), Canary Islands. The nine localities from the New World are Port Coquitlam (PC), British Columbia; Alta Loma State Park (AL, two strains), Washington; Cave Junction (CJ), Oregon; Eureka (EU), Davis (DA), Winters (WI, two strains), El Río Vineyard (ER, two strains), and Gilroy (GR, two strains), California; Bariloche (BA), Argentina. From Latorre et al. (1986).

TABLE 3. Genetic differentiation between the eight mtDNA haplotypes of *Drosophila subobscura*

	Haplotype							
Haplotype	I	II	III	IV	V	VI	VII	VIII
I	—	0.946	0.893	0.947	0.947	0.890	0.897	0.842
II	0.016	—	0.946	0.893	0.893	0.830	0.877	0.821
III	0.024	0.010	—	0.842	0.842	0.778	0.828	0.772
IV	0.015	0.035	0.042	—	0.897	0.836	0.848	0.828
V	0.009	0.024	0.033	0.023	—	0.873	0.848	0.793
VI	0.022	0.036	0.049	0.035	0.026	—	0.786	0.727
VII	0.023	0.029	0.038	0.040	0.032	0.047	—	0.949
VIII	0.042	0.050	0.058	0.047	0.050	0.064	0.015	—

NOTE: Above the diagonal: F, proportion of restriction fragments shared. Below the diagonal: p, estimated proportion of base differences between the morphs.

extensive in most animals studied (Avise and Lansman 1983; Boursot and Bonhomme 1986). The mtDNA restriction patterns of *D. subobscura* have been analyzed in 32 strains from 23 different local populations: 14 from the Old World, 1 from South America, and 8 from North America (Fig. 5; Latorre et al. 1986). The 10 endonucleases used yield a total of 42 restriction sites: 18 (43%) are shared by all strains, whereas 24 (57%) are polymorphic. Combination of the mtDNA patterns obtained with the various endonucleases yields eight different composite patterns or "haplotypes."

Table 3 shows the degree of differentiation between the haplotypes. The estimated numbers of nucleotide differences per site (given below the diagonal) range from 1 (between haplotypes I and V or between II and III) to about 6% (between VIII and III or VI). The "nucleotide diversity," which measures the probability that two mtDNAs randomly sampled from a population would differ at any given site (and, hence, is analogous to the "heterozygosity" used for diploid genomes, except that it measures variation per nucleotide site rather than per locus), is 0.83% for the species, which is within the typical range observed for many animals (Latorre et al. 1986). The nucleotide diversity is, however, greater for

the Old World than for the American populations: 1.10 vs. 0.45%, which reflects an impoverishment of genetic variation, as would be expected if the number of female colonizers would be small. Indeed, only two haplotypes (I and II) have been found in the American strains (Fig. 5). These are the two haplotypes most common in the European populations; also, one or the other is ancestral to all or most other haplotypes found in the species (Latorre et al. 1986).

It has been said that restriction analysis of mtDNA may be "the most sensitive technique available for reconstructing evolutionary relationships among conspecific populations" (Avise et al. 1979, p. 293). The presence of haplotypes I and II in the American populations demands that the colonizers of the New World comprise at least two fertile females, although there could be more; however, the extensive distribution of haplotypes I and II in Europe makes it impossible to identify the population whence the colonizers may have originated.

Mestres (1988) has studied the frequency of recessive lethal alleles in populations of *D. subobscura* from the Old and the New World. The population of Bordils, about 100 km northeast from Barcelona, Spain, may be taken as typical of the western Mediterranean populations whence the colonizers might have originated. The frequency of lethal-allele-carrying chromosomes in Bordils is 29.0%, and the frequency of lethal alleles is 0.69%. The fairly high value of lethal genes and the low incidence of allelism are characteristic of large central *Drosophila* populations. The Gilroy, CA, population, in contrast, has a frequency of 14.4% lethal chromosomes and 5.26% lethal alleles. These numbers indicate that the "effective" population size of the Gilroy population is small, no doubt as a result of the limited number of colonizers from which it is derived.

An important finding is that the chromosomes carrying the O_5 arrangement have a recessive lethal gene that is allelic in all chromosomes tested from Gilroy and is also allelic to the O_5 chromosomes from Puerto Montt in Chile. This supports the hypothesis that the colonizations of North and South America are related, either because one continent was colonized from the other or because both were colonized by individuals from the same Palearctic population. This finding opens, moreover, the way to identify the Old World source of the colonizers, by identifying the population(s) in which O_5 chromosomes carry a recessive lethal gene allelic to the one found in the New World. It may, of course, be the case that such an O_5 recessive lethal allele is widely distributed among O_5-carrying populations, which would not permit precise identification of the founder population without recourse to some other relevant methods, such as those just reviewed.

The frequency of the O_5 chromosome arrangement is fairly low (or absent) everywhere in the Old World, from less than 1 to about 15% in southern Scandinavia. On this basis, Mestres (1988) estimates that the number of *D. subobscura* colonizers of the Americas may be between 9 and 149, although the assumptions required make these estimates little more than educated guesses.

In conclusion, the precise source of the American colonizers remains unknown. The overall configuration of the chromosome-arrangement and allozyme frequencies suggests a western Mediterranean population, whereas the presence of chromosome O_5 suggests a central or northwestern European (or, possibly, eastern Mediterranean) source. The mtDNA restriction patterns suggest a European rather than North African population. The extensive chromosome and enzyme polymorphisms indicate that the number of colonizers was not very small, whereas the absence of rare enzyme alleles as well as the mtDNA patterns suggest that it was not very large, which is also corroborated by the lethal allelism study. The configuration of the chromosome-arrangement frequency (and to a lesser extent the allozyme and mtDNA patterns) suggests that the colonizers of both North and South America derive from the same original population. This is strongly corroborated by the lethal allelism of the O_5 chromosomes.

Chromosome polymorphism: geographic clines and adaptation

Prevosti (1964) and others (review in Krimbas and Loukas 1980) have determined that the frequencies of the chromosome arrangements of *D. subobscura* vary in clinal patterns that can be correlated with latitude. Correlations between genetic and environmental variables do not necessarily reflect causation. Nevertheless, the association between climatic variables and the *D. subobscura* chromosomal polymorphisms has been interpreted as the result of adaptation, although some authors have argued that historical factors can explain the correlations without invoking adaptive factors (Krimbas and Loukas 1980). The colonization of the Americas provides a unique opportunity to investigate this issue. If the chromosome polymorphisms are responding to environmental factors associated with latitude, one would expect that the clinal correlations observed in the Old World might also evolve in the New World. Given that the relevant climatic variables change in opposite north–south directions in the two Americas, one would expect that the chromosomal clines would have opposite polarity in the two Americas (although the same latitudinal direction in both). The reduced number of colonizers would further exclude historical factors as a likely explanation for the chromosome clines.

Table 4 gives the frequency of the chromosome arrangements in nine South American populations (Prevosti et al. 1985). Table 5 gives the frequencies in seven North American populations (Prevosti et al. 1988). Table 6 displays the correlation coefficients between latitude and chromosome frequency. The data provide a dramatic corroboration of the hypothesis that the chromosome polymorphisms are adaptive and that their frequencies are modulated by climatic and (or) other environmental conditions associated with latitude.

The correlation coefficients are statistically significant for six chromosome arrangements in South America and for six in North America. If we exclude the O_5 correlation (because it cannot be calculated in Europe, owing to its absence from most populations), all 10 correlations are of the same sign as in Europe, where all but three of them are also statistically significant. If we ignore the significance of individual correlations and consider only their sign, we notice that 12 of 16 correlations have the same sign in all three continents (and, indeed, 15 of the 16 correlations are of the same sign in South America as in Europe). Not all these correlations are independent. If we exclude one per chromosome, 9 of 12 correlations are of the same sign in all three continents. The probability of this coincidence being due to chance is <0.001.

Which phenotypic traits mediate the adaptive response of the chromosome polymorphisms to latitude is not known, although Prevosti (1967) has given evidence that body size may be one of them. What is surprising is the rapidity of the response: a few years have sufficed for the evolution of the

TABLE 4. Frequency of the chromosome arrangements in nine South American populations

Chromosome arrangement	Viña del Mar (32°02'S)	Santiago (33°30'S)	Chillán (36°37'S)	Laja (37°10'S)	Valdivia (39°46'S)	Bariloche (41°11'S)	Puerto Montt (41°28'S)	Castro (42°30'S)	Coihaique (45°35'S)
A_{ST}	0.444	0.397	0.459	0.517	0.500	0.500	0.411	0.431	0.559
A_2	0.556	0.603	0.541	0.483	0.500	0.500	0.589	0.569	0.441
J_{ST}	0.237	0.217	0.343	0.294	0.204	0.406	0.301	0.342	0.293
J_1	0.763	0.783	0.657	0.706	0.796	0.594	0.699	0.658	0.707
E_{ST}	0.474	0.500	0.615	0.538	0.629	0.669	0.568	0.644	0.659
E_{1+2}	—	—	0.005	0.010	0.005	—	—	—	—
E_{1+2+9}	0.237	0.144	0.073	0.186	0.113	0.038	0.089	0.110	0.106
$E_{1+2+9+3}$	0.237	0.234	0.183	0.130	0.145	0.218	0.219	0.203	0.163
$E_{1+2+9+12}$	0.053	0.115	0.110	0.136	0.108	0.075	0.123	0.042	0.073
E_{17}	—	0.005	0.014	—	—	—	—	—	—
U_{ST}	0.436	0.464	0.521	0.508	0.522	0.556	0.473	0.496	0.582
U_{1+2}	0.359	0.376	0.305	0.300	0.315	0.286	0.322	0.369	0.230
U_{1+2+8}	0.205	0.160	0.174	0.193	0.163	0.158	0.205	0.134	0.189
O_{ST}	0.263	0.240	0.275	0.280	0.153	0.241	0.247	0.227	0.341
O_5	—	0.026	0.055	0.051	0.095	0.090	0.130	0.101	0.057
O_7	—	0.005	—	0.003	—	—	—	—	—
O_{3+4}	0.026	0.021	0.050	0.044	0.053	0.030	0.021	0.017	0.016
O_{3+4+2}	0.342	0.359	0.326	0.338	0.384	0.293	0.315	0.303	0.244
O_{3+4+7}	0.158	0.156	0.106	0.133	0.074	0.105	0.130	0.168	0.033
O_{3+4+8}	0.211	0.193	0.188	0.222	0.242	0.241	0.158	0.185	0.309
N	38	192	218	301	186	133	146	199	213

NOTE: *N* is the number of chromosomes sampled. Data are from Prevosti et al. (1988).

TABLE 5. Frequency of the chromosome arrangements in seven North American populations

Chromosome arrangement	Arlington (48°12'N)	Centralia (46°43'N)	Woodburn (45°09'N)	Medford (42°20'N)	Eureka (40°49'N)	Davis (38°33'N)	Gilroy (37°00'N)
A_{ST}	0.531	0.561	0.579	0.595	0.537	0.537	0.521
A_2	0.469	0.439	0.421	0.405	0.463	0.463	0.479
J_{ST}	0.360	0.367	0.300	0.442	0.419	0.411	0.369
J_1	0.640	0.633	0.700	0.558	0.581	0.589	0.631
E_{ST}	0.668	0.741	0.634	0.660	0.621	0.517	0.514
E_{1+2}	0.006	0.018	0.056	0.021	0.012	0.022	0.021
E_{1+2+9}	0.112	0.082	0.099	0.160	0.186	0.250	0.225
$E_{1+2+9+3}$	0.152	0.129	0.113	0.085	0.093	0.099	0.141
$E_{1+2+9+12}$	0.062	0.029	0.099	0.074	0.087	0.112	0.099
U_{ST}	0.483	0.412	0.465	0.415	0.437	0.333	0.280
U_{1+2}	0.337	0.394	0.324	0.319	0.361	0.360	0.385
U_{1+2+8}	0.180	0.194	0.211	0.266	0.202	0.307	0.336
O_{ST}	0.315	0.300	0.243	0.149	0.269	0.176	0.149
O_5	0.056	0.106	0.086	0.064	0.077	0.018	0.007
O_7	—	—	—	—	—	—	0.007
O_{3+4}	0.028	0.035	0.043	0.021	0.032	0.031	0.078
O_{3+4+2}	0.281	0.288	0.329	0.457	0.340	0.370	0.397
O_{3+4+7}	0.084	0.023	0.171	0.074	0.096	0.211	0.199
O_{3+4+8}	0.236	0.247	0.129	0.234	0.186	0.194	0.163
N	178	170	70	94	128	230	142

NOTE: *N* is the number of chromosomes sampled. Data are from Prevosti et al. (1988).

latitudinal clines. The selective forces operating must be strong. It deserves notice, in any case, that the regions colonized in the two Americas occupy symmetrical locations relative to the equator and have climatic conditions that are similar, and change in parallel fashion, to those in the Old World region where the species is endemic (Fig. 1). California and Chile are the only two regions in the Americas where Mediterranean climatic conditions prevail. As pointed out by

TABLE 6. Correlation coefficient between latitude and frequency of the chromosome arrangements

Chromosome arrangement	South America	North America	Old World[a]
A_{ST}	0.492	0.347	0.880***
A_2	−0.492	−0.345	−0.864***
J_{ST}	0.457	−0.433	0.972***
J_1	−0.455	0.433	−0.973***
E_{ST}	0.838***	0.868**	0.973***
E_{1+2+9}	−0.575	−0.930***	−0.456
$E_{1+2+9+3}$	−0.306	0.348	−0.834***
$E_{1+2+9+12}$	−0.226	−0.696	−0.968***
U_{ST}	0.727**	0.850*	0.974***
U_{1+2}	−0.627*	−0.283	−0.387
U_{1+2+8}	−0.197	−0.881**	−0.944***
O_{ST}	0.219	0.793*	0.870***
O_5	0.730**	0.770*	—[b]
O_{3+4}	−0.325	−0.460	−0.870***
O_{3+4+2}	−0.718*	−0.690	−0.267
O_{3+4+7}	−0.618*	−0.674	−0.577*
O_{3+4+8}	0.412	0.385	−0.804***
N	9	7	9

NOTE: *N* is the number of populations used for each correlation. Data are from Prevosti et al. (1988). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[a]The populations used to calculate the correlations are, from north to south, Dröback (Sperlich 1964), Gröningen (Krimbas 1964), Fontainbleau (A. Prevosti, unpublished), Montpellier (Prevosti et al. 1984), Valencia (Prevosti et al. 1984), Malaga (Prevosti et al. 1984), Tangier (Götz 1965), and Essaouira and Agadir (Prevosti 1974).

[b]This correlation has not been calculated because O_5 is only present in the samples from Dröback and Gröningen. The presence of O_5 only in the higher latitudes of the Old World is consistent with its positive correlation with latitude in the new World.

Prevosti et al. (1988) "Southwards in Chile and northwards in North America, the Mediterranean climate merges into a western maritime climate; northwards in Chile and southwards in North America, it merges into semiarid and arid conditions. This latitudinal distribution of the climates is similar to that in the original area of distribution of *D. subobscura* in the Old World."

Coda

The colonization of the Americas by *D. subobscura* is a magnificent natural experiment in evolution. The studies reported here only scratch the surface of the body of research that is possible and the hypotheses that can be tested. Experiments at the University of Barcelona and at the University of California in Irvine are now in progress that explore the population dynamics, ecological preferences, and competitive ability of *D. subobscura* relative to *D. pseudoobscura* and other closely related species with which it has become sympatric in North America, as well as a number of population and evolutionary genetics questions. Other experiments are surely in progress elsewhere and many more are likely to follow. Evolutionists can only treasure this unique research opportunity.

Acknowledgments

Many investigators from three continents have participated in the study of the *Drosophila subobscura* colonization of the Americas. They include, in addition to several members of our laboratories, Drs. D. Brncic and M. Budnik in Santiago, Chile; A. T. Beckenbach of Simon Fraser University in British Columbia; A. Latorre and A. Moya in Valencia, Spain; as well as their collaborators. To all these we are grateful. This research is supported in our laboratories by a grant from the Comité Conjunto Hispano-Noreamericano para la Cooperación Científica y Técnica, Spain, and by grant 2844 from the Comisión Asesora para la Investigación Científica y Técnica to the Barcelona group.

AVISE, J. C., and LANSMAN, R. A. 1983. Polymorphism of mitochondrial DNA in populations of higher animals. *In* Evolution of genes and proteins. *Edited by* M. Nei and R. K. Koehn. Sinauer, Sunderland, MA. pp. 147–164.

AVISE, J. C., LANSMAN, R. A., and SHADE, R. O. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus *Peromyscus*. Genetics, **92**: 279–295.

AYALA, F. J., and DOBZHANSKY, T. 1974. A new subspecies of *Drosophila pseudoobscura*. Pan-Pac. Entomol. **50**: 211–219.

BECKENBACH, A. T., and PREVOSTI, A. 1986. Colonization of North America by the European species *Drosophila subobscura* and *D. ambigua*. Am. Midl. Nat. **115**(1): 10–18.

BOURSOT, P., and BONHOMME, F. 1986. Génétique et évolution du génome mitochondrial des metazoaires. Genet. Sel. Evol. **18**: 73–98.

BRNCIC, D., PREVOSTI, A., BUDNIK, M., MONCLUS, M., and OCANA, J. 1981. Colonization of *Drosophila subobscura* in Chile. I. First population and cytogenetic studies. Genetica, **56**: 3–9.

BUDNIK, M., and BRNCIC, D. 1982. Colonización de *Drosophila subobscura* Collin en Chile. Actas V. Congreso Latinoamericano de Genética. Universidad de Santiago, Chile. pp. 117–182.

GÖTZ, W. 1965. Chromosomaler Polymorphismus in einem Muster von *D. subobscura* aus Marokko, mit Darstellung der Heterozygotieverhältnisse als Heterozygotiediagramm. Z. Vererbungsl. **97**: 40–45.

KRIMBAS, C. B. 1964. The genetics of *D. subobscura* populations. I. Inversion polymorphism in populations of southern Greece. Evolution (Lawrence, Kans.), **18**: 541–552.

KRIMBAS, C. B., and LOUKAS, M. 1980. The inversion polymorphism of *Drosophila subobscura*. Evol. Biol. **12**: 163–234.

LAKOVAARA, S., and SAURA, A. 1982. Evolution and speciation in the *Drosophila obscura* group. *In* The genetics and biology of *Drosophila*. Vol. 3*b*. *Edited by* M. Ashburner, H. L. Carson, and J. N. Thompson. Academic Press, New York. pp. 1–59.

LATORRE, A., MOYA, A., and AYALA, F. J. 1986. Evolution of mitochondrial DNA in *Drosophila subobscura*. Proc. Natl. Acad. Sci. U.S.A. **83**: 8649–8653.

MESTRES, F. 1988. Estudio del alelismo de letales en poblaciones naturales de *Drosophila subobscura*. Doctoral dissertation, University of Barcelona, Spain.

PREVOSTI, A. 1964. Chromosomal polymorphism in *Drosophila subobscura* populations from Barcelona (Spain). Genet. Res. **5**: 27–38.

——— 1967. Inversion heterozygosity and selection for wing length in *Drosophila subobscura*. Genet. Res. **10**: 81–93.

——— 1974. Chromosomal inversion polymorphism in the southwestern range of *Drosophila subobscura* distribution area. Genetica, **45**: 111–124.

PREVOSTI, A., RIBÓ, G., GARCÍA, M. P., SAGARRA, E., AGUADÉ, M., SERRA, L., and MONCLÚS, M. 1982. Los polymorfismos cromosómico y aloenzimático en las poblaciones de *Drosophila subobscura* colonizadoras de Chile. Actas V. Congreso Latinoamericano de Genética. Universidad de Santiago, Chile. pp. 189–197.

PREVOSTI, A., DE FRUTOS, R., ALONSO, G., LATORRE, A., MONCLÚS, M., and MARTÍNEZ, M. J. 1984. Genetic differentiation between natural populations of *Drosophila subobscura* in the western Mediterranean area with respect to chromosomal variation. Genet. Sel. Evol. **16**: 143–156.

PREVOSTI, A., SERRA, L., RIBÓ, G., AGUADÉ, M., SAGARRA, E., MONCLÚS, M., and GARCIA, M. P. 1985. The colonization of *Drosophila subobscura* in Chile. II. Clines in the chromosomal arrangements. Evolution (Lawrence, Kans.), **39**: 838–844.

PREVOSTI, A., SERRA, L., MONCLÚS, M., MESTRES, F., LATORRE, A., RIBÓ, G., and AGUADÉ, M. 1987. Colonización de America por *Drosophila subobscura*. Evolución Biológica,a **1**: 1–24.

PREVOSTI, A., RIBÓ, G., SERRA, L., AGUADÉ, M., BALAÑA, J., MONCLÚS, M., and MESTRES, F. 1988. Colonization of America by *Drosophila subobscura*: experiment in natural populations that supports the adaptive role of chromosomal-inversion polymorphism. Proc. Natl. Acad. Sci. U.S.A. **85**: 5597–5600.

SPERLICH, D. 1964. Chromosomale Strukturanalysen und Fertilitätsprüfung an einer Marginalpopulation von *Drosophila subobscura*. Z. Vererbungsl. **95**: 73–81.