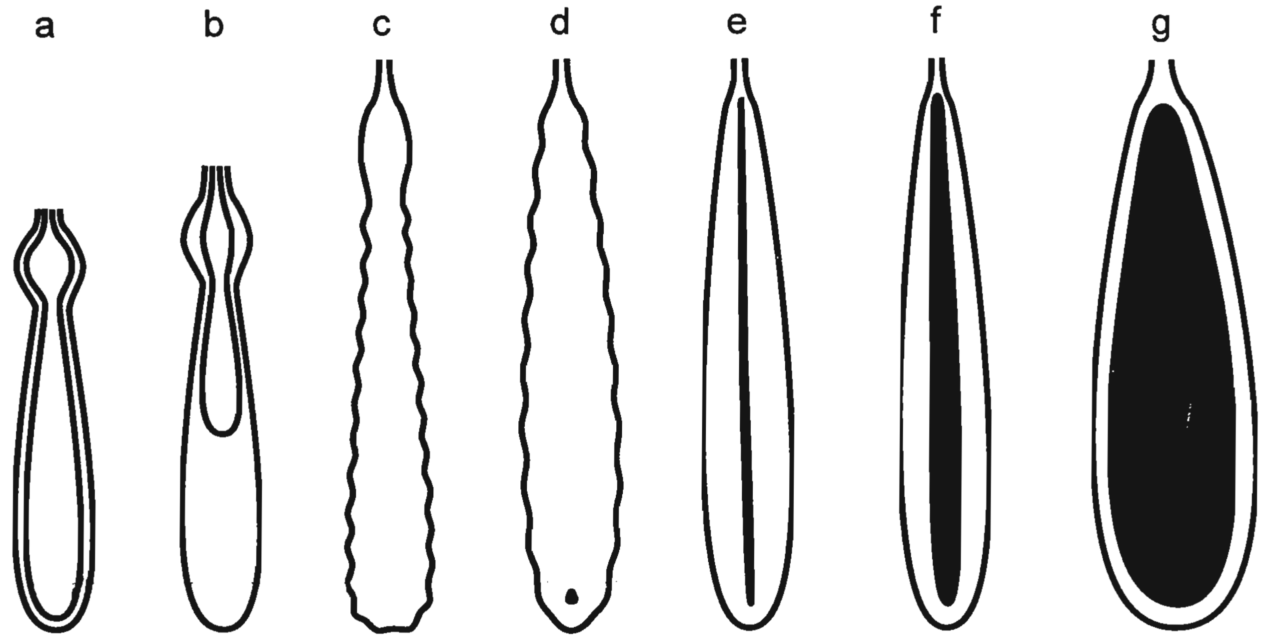

Figure 2. Changes of salivary gland morphology in the second half of third larval instar of *Drosophila melanogaster* (according to G. Richards, 1979, personal communication; Zhimulev *et al.*, 1981; Zhimulev, 1999).

a and *b* – the middle of third instar, *a* - the salivary gland cavity is open completely; *b* – the cells close up beginning from distal part, the cavity is seen only in proximal part of the gland, the cells do not contain glycoprotein granules.

c – the cells close up along the whole gland and they are filled with glycoprotein secretion granules, the puff pattern in chromosomes corresponds to PS1 stage.

d – a small cavity appears in the most distal part of gland at the expense of glycoproteins secretion, the set of ecdysone-induced puffs corresponds to the PS2-3 stage;

e – a thin bundle of secretion fills the whole gland, PS4-5;

f – the thickness of secretion bundle is about one third of gland thickness, PS6-7;

g – the gland looks like swollen bag filled by secretion, the developmental stage is the one just before extrusion of secretion in surroundings and spiracle eversion, PS10-11.

Detection of homokaryotype selection, especially for SC/SC (Santa Cruz) and rigid chromosomal pattern in marginal ecological conditions for the third chromosome rearrangements in the *Drosophila pseudoobscura* populations from the Colombian high plateau Andes.

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Five *Drosophila pseudoobscura* populations from the Colombia Cundiboyacense high plateau in the Andes (Torobarroso, Susa, Sutatausa, Potosí and Santillana) were systematically studied each week during a year (1997), to analyze comparatively the thrid chromosome rearrangements among these Colombian populations. The first chromosomal study of this species in Colombia, which had been discovered a few years earlier, was carried out by Dobzhansky *et al.* (1963). Only two different chromosomal rearrangements, Santa Cruz (SC) and Tree Line (TL), were found in that study. As in this previous work, the SC and TL were the predominant rearrangements in the Colombian populations studied by us. Nevertheless, for first time, other chromosomal rearrangements with low frequencies like Standard (ST), Olympic (OL), Cuernavaca (CU) and one highly similar to the Mexican endemism Amecameca (AM) were also detected. However, the first aim of the present study was the following: Can be detected any selective pressure in Colombia, like those reported in USA and Mexico that were associated with systematic changes in the chromosomal rearrangement frequencies during the year? Dobzhansky (1943, 1947a,b) determined that the natural selection was present in, at least, two of the three populations studied in St. Jacinto (California), because of the changes in chromosomal rearrangements detected month to month (the studies of Dubinin and Tiniakov, 1945, 1946 with *D. funebris* were also determinant). Lately, other studies also reported these cyclic changes in the following years (Dobzhansky *et al.*, 1964; Epling *et al.*, 1957). These changes cannot be caused by genetic drift, because they were cyclic and followed the seasonal changes every year. There were two demographic maximums in a year, for example in Piñon Flats or Andreas canyon, one in early spring, and other in autumn, exactly when the ST (Standard) frequencies were, with a high difference, the most elevated. In the hotter months (spring's end and almost summer) there was a demographic depression with an increasing of the AR (Arrowhead) and CH (Chiricaua) frequencies. In Mexico something similar was detected with the rearrangements CU and TL (Amecameca population). The former is more frequent in spring and autumn, while the latter is in summer. On the contrary, this last rearrangement (TL) did not show important changes with the season changes in the Californian populations (Levine *et al.*, 1995). However, the frequency variations are generally lighter in Mexico than in many of the reported Californian populations. Nevertheless, other Californian and North American populations did not show these annual cyclic changes (Powell, 1992). At 15 miles from the populations refereed, there is Keen Camp, with a different rain level but without cyclic variations during the year (Dobzhansky, 1943, 1947b; Epling *et al.*, 1957). For example, Crumpacker and Williams (1974) studied two groups of populations at North and South of Denver (Colorado). In the first one, the frequencies were constant during the year, while in the second one, the rearrangement frequencies were similar to the first, but they had seasonal cycles, with AR reaching high values on spring and early summer, with the lowest value finishing this season. In autumn, it reached a new maximum. This fact led us to formulate the following question: Have the Colombian populations a flexible genetic system like many of the American ones? Or contrarily, have they a rigid system? What kind of selection, if any, is present in Colombia?

Material and Methods

To establish the population dynamic of the two predominant rearrangements (SC and TL), the application of various population genetics procedures were performed. (1) To detect any bias of the Hardy-Weinberg equilibrium, the Wright'S F (1965), with the Rasmussen (1964)'s variance, and the Robertson and Hill's f (1984) statistics were used. The statistical significance of the hierarchical F statistics was obtained by means of the application of 500 jackknife permutations. In this way, confidence intervals (95 and 99 %) were generated for the three F statistics. (2) A correspondence factorial analysis (CFA) was performed to analyze the relationships between the populations and the chromosomal rearrangements studied simultaneously. The coordinate matrix from the factorial matrix, the absolute contribution matrix and the squared-correlation matrix were obtained both for the populations and for the chromosomal rearrangements. (3) To ratify, or not, the relationships found with the previous analysis, the Nei's (1978) and the Prevosti's (1974) genetic distance matrices among population-pairs were obtained. Two hierarchical tree algorithms were applied to these genetic distance matrices (UPGMA, Sokal and Michener, 1958; and WPGMC, Lance and Williams, 1967). (4) The last analysis used was a Mantel's (1967) test normalized with the Smouse *et al.*,'s (1986) procedure. The statistical significance of these results was carried out with a Monte Carlo simulation with 2000 permutations. The geographic distances between the populations were calculated both with the "Great Circle" method (Spuhler, 1972) and with aereal distances.

Results

The principal results obtained were the following: (1) The SC frequencies were very similar in the five populations studied (Table 1), being, in general, the SC frequencies slightly more elavated than those reported in past decades. This affirmation was specially significant for the Potosí population (0.782). (2) The five populations studied were not in Hardy-Weinberg equilibrium, individually and as a unique set, by an elevated excess of homokaryotypes, and the consequent defect, in proof, of heterokaryotypes (F = 0.94-0.99) (Table 2). (3) The genetic heterogeneity between the five populations studied was not significant (F_{ST} = 0.008), which puts in evidence a high genetic homogeneity at the chromosomal rearrangement level in Colombia, this being very different to those found in North America. (4) Changes in the chromosomal composition were not observed throughout a year in each one of the populations analyzed, although some week samples were little and they could be submitted to a strong sample error. (5) The WPGMC tree with the Nei's genetic distance (Figure 1) clearly showed that the Potosi population was the most differentiated of the five populations studied. (6) The Mantel's test results, with the Great Circle and with the aerial distances, showed in all cases non-significant negative correlations, which indicates that there was not a conspicuous relationship between the genetic distances and the geographical distances among the populations analyzed.

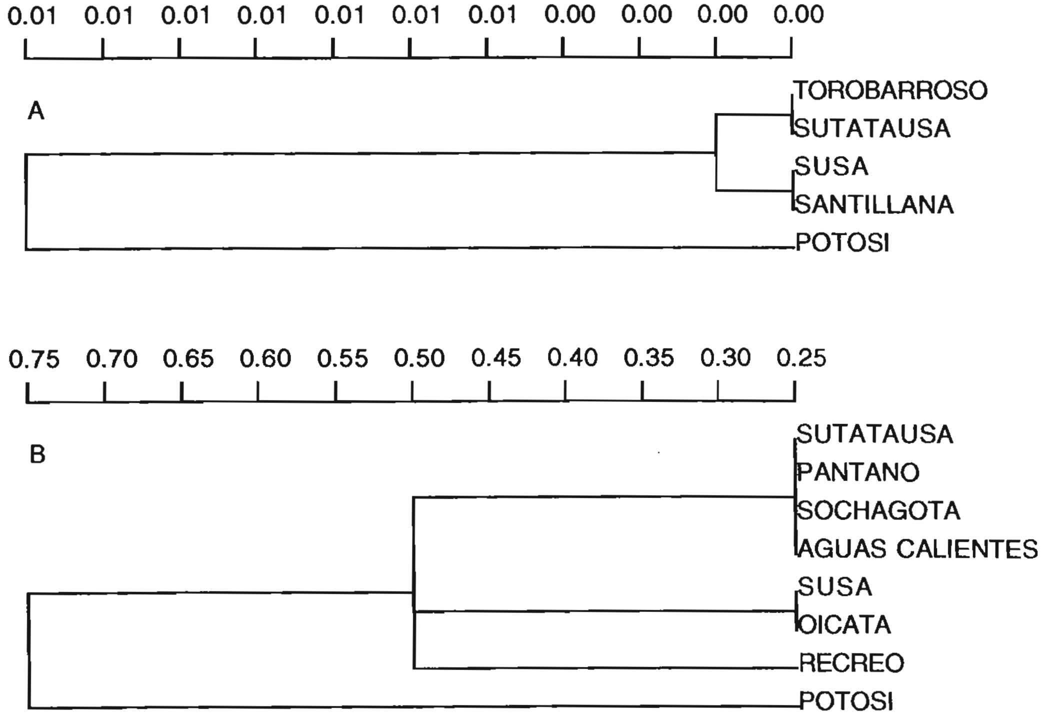

Figure 1. A, WPGMC tree with the Nei's genetic distance for the five Colombian *Drosophila pseudoobscura* populations studied for the third chromosomal rearrangement frequencies. B, Strict consensus tree using UPGMA and Single algorithms and the Manhattan and Canberra distances for eight Colombian *Drosophila pseudoobscura* populations sudied for 15 meristic morphological variables. See the strong difference of the Potosí population versus the other Colombian populations for both types of variables.

Discussion

Dobzhansky *et al.* (1963) showed that the *D. pseudoobscura* populations in Colombia were in contradiction with the Carson's theory, because a special heterotic system (excess of heterokaryotypes) was found in this population, isolated from the distribution central range in North America. In the 80's, Cárdenas (1988) found that this species in Colombia was basically in Hardy-Weinberg equilibrium for the third chromosomal rearrangements. In both studies it was affirmed that this species, although geographically restricted, was locally very common. On the contrary, this species was very scarce during our 1997 sampling. Only 96 isolines were established and 1672 chromosomes were analyzed. Our results are totally in contradiction with the results of previous authors and are in agreement with the Carson's theory. In all the populations studied the excess of homokarotypes was nearly to the fixation. This new result shows that certain selective parameters in favour of the homokaryotypes (specially for SC) are acting on these Colombian populations during the last years. These new changes could be related to the deforestation, the expansion of cattle fields and habitat destruction by the human beings and/or due to climatic changes motivated by "El niño" phenomenon that was very strong in the Neotropical area in 1997. Moreover, the genetic heterogeneity degree between the Colombian populations was non-significant (F_{ST} = 0.008), which puts in evidence the non-significant importance of genetic drift, inbreeding and isolation-by-distance for these chromosomal rearrangements. A lot of the results obtained are opposed to those found in the North American *D. pseudoobscura* populations, showing that the current Colombian population has a rigid genetic system. Another interesting difference between the central range and the Colombian populations was that in the latter the rearrangement frequencies were usually constant during the whole year. In this study, as in Cárdenas (1988), SC was more frequent than TL. Only in some few registers TL frequency was approximately equal to the SC frequency, but the oscillations were not substantial. On the other way, in the USA populations there were reported important frequency changes between seasons in a year, and between the following years. As it was commented before, Dobzhansky (1943) found in Piñon Flats that the ST rearrangement had its lower frequency on May-June, while AR had the higher, but the situation was the opposite on September-November when the temperature decreased. Strickberger and Wills (1966) reported an inverse situation in a population near to Berkeley. The ST frequency reached its maximum in summer, and its minimum in winter. CH showed the opposite behavior, while AR did not change. A similar phenomenon, but with other rearrangements, has been reported in Mexico. In Amecameca, CU decreased between May and August, while TL clearly increased and EP (Estes Park) had a slight enlargement during these months (Levine *et al.*, 1995). Because there are not seasons, at least temperature-related, in the Colombian plateau, it could exist a uniform selective pressure constancy during the whole year for many ecological conditions. This could explain why the rearrangement frequencies did not change in all the time that this study took.

As we commented before, the studied populations showed an important homokaryotype excess. We propose that these populations are affected by stabilizing selection favoring the homokaryotypes. In general, the homokaryotype with a higher adaptability would be SC/SC in the major part of the year. TL/TL would have a W (fitness) lower than SC/SC, while the heterokaryotype (SC/TL) would have the smallest W. With some non-temperature related microclimatic changes (wetness and the rain regimes or other variables, like food resources and the application of insecticides), TL/TL could increase its fitness to values near to unity, sometimes. This could allow the coexistence of both arrangements with an homozygous excess, and with the higher frequency for SC/SC. It could be argued that the homokaryotype excess could be caused by some stochastic process (genetic drift and founder effects), that usually are associated to consanguinity. If the sub-populations considered have a low effective number (Crawford, 1984), the endogamy should be favored, because of a limited availability of sexual partners. However, the presence of inbreeding and/or genetic drift in these populations could be discarded easily when this kind of marker is studied. If genetic drift was present, we should not find a low F_{ST} value (0.008) without statistical significance between the subpopulations. This genetic homogeneity, also detected with the genetic distances, shows that a process such as stabilizing selection is more probable than genetic drift. But this does not means that effective numbers of these populations are not low. At this moment, two of the authors (D. A. and M. R.-G.) are studying the same populations using molecular markers (nuclear DNA STRP's and mtDNA RFLP's). If using these markers, the differences between populations were higher, this could probe the action of stabilizing selection on the third chromosome rearrangements. Effectively, in our studies with five microsatellite loci (DPSX001, DPS2001, DPS3001, DPS3002 and DPS4001) with the Colombian *D. pseudoobscura* populations studied here, an F_{ST} of 0.042 was obtained (Alvarez *et al.*, 1999). This value is five times greater than that obtained here (0.008). Moreover, in one microsatellite locus like DPS4001, the F_{ST} value was 0.081 among the Colombian populations, that is, 10 times greater than the value for this statistic on the thrid chromosome rearrangements. Additionally, the major part of these microsatellite loci were in Hardy-Weinberg equilibrium in the analyzed populations. These data indirectly support the presence of some stabilizing selective agent currently acting on the third chromosome in the Colombian populations. Our explanation is in total agreement with Crumpacker *et al.* (1974). They showed that a bottle-neck occurred in the Rist Canyon population in 1967. There were a high AR (the most frequent) decrease and an increase of locally rare rearrangements such as TL, EP and ST. The frequency change of these arrangements is very difficult to explain by genetic drift. The presence of a strong selective pressure during an environmental stress period that caused a reduction in the population would be, as in our case, a good and parsimonious explanation. On the other hand, Strickberger and Wills (1966) in Berkeley sustained that CH was favored by high rain levels, while ST by an arid environment. Dobzhansky (1971) also showed the presence of increasing and decreasing in the ST and AT rearrangements during many years in Mather (California), related with the succession of wet and dry years, although in a lot of years the correlation was nonexistent. This could be a selective possibility in Colombia if the El Niño phenomenon has ecological consequences. However, in the Colombian plateau, contrary to the temperature ones, there are seasonal changes in the rain levels, although we did not find oscillations in the rearrangement frequencies associated to this fact.

The dispersion rates, at least, in the central distribution range are high. It has been detected values near to 170 m in the first day after reliberation (Crumpacker and Williams, 1973). Powell *et al.* (1976) concluded that the *D. pseudoobscura* dispersion is so elevated that they could homogenize populations in about one kilometer. However, the populations studied here were separated by more than these distances (more than 160 Km among the most distant populations). Gene flow seems to be a less parsimonious explanation than stabilizing selection for the chromosomal homogeneity found between the Colombian populations, because, moreover, the populations are not in Hardy-Weinberg equilibrium for these markers.

The fact that we could not detect clines in the Colombian plateau could suggest that there were no environmental gradients, which originated any significant spatial structure in the distribution of rearrangement frequencies (Endler, 1973, 1977). For this reason, we can sustain that in Colombia, there were not populations with flexible systems, nor separation zones among them like those found in North America, and there were not populations with rigid systems at a microgeographic level, like those found in Colorado by Crumpacker and Williams (1974), either. These authors detected two kind of patterns in Colorado, one population at Northern Denver, which lived in a place with wide macro and microflora, and had a rigid system of chromosomal polymorphisms, while another in Southern Denver, in a place with a poor flora, had a flexible pattern. They suggested that it would also occur in St. Jacinto, where there was a population with a rigid polymorphism scheme, that lived in a mature wood. In Colombia, this rule was broken, because there were populations with a constant chromosomal pattern, but in a possible marginal ecological situation. In USA some *D. pseudoobscura* populations with this last feature (rigid pattern in marginal conditions) have also been detected in the Yosemite region (Jacksonville).

We cannot discard either the possibility that insecticides, or another contaminant used, could have some selective impact on the chromosomal rearrangements. In fact, some of the Colombian populations studied are located close to flower farms where the use of these chemicals is very common (Potosí). Dobzhansky (1958) postulated that some Californian areas, where changes in chromosomal rearrangements were detected, have been submitted to the action of DDT and DDD. The PP appearance in California concurs with the use of DDT at high scale. This made some authors to think that PP was related with the use of this chemical, but during the 70's when DDT was still in use, the PP frequency began to decrease and the TL one to increase in some western USA areas (Anderson *et al.*, 1975). A population located near to the culture Riverside area was used to test if there was any relation between the insecticide use and the chromosomal anomalies in that region. No-anomalies were detected in that population (Dobzhansky *et al.*, 1964). Some authors, like L. Cory (in Dobzhansky, 1971), postulated that insecticides cannot be discarded as selective agents and experiments should be made in larvae and not in adults. It was sustained that some correlations, between the rearrangement changes and the place where the insecticides were used, could exist. However, the situation became complex when Anderson *et al.* (1968) introduced little DDT and dieldrine amounts in experimental populations with 4-5 arrangements. In the New York samples, there was not any fitness difference for the rearrangements in the colonies treated and untreated with insecticides. On the contrary, there were fitness differences between the two groups in the Japanese samples. The ST/ST homokaryotype had a better fitness than the ST/AR heterokaryotype in the insecticide presence, while in U. S. colonies, ST/AR always had a higher fitness independently of the environment. That means that little micro-habitat variances can be the cause of the differences in the susceptibility to the insecticide action as selective agent in *D. pseudoobscura*. These environmental particularities can be very diverse between populations and could make difficult the finding of general rules. In other species, such *D. willistoni*, differences have been found in some morphological traits between the flies that inhabit urban and rural zones in the Brazilian population of Porto Allegre (Lucchese *et al.*, 1994). In the urban areas, there were flies with higher thorax size, wing length and SO_2, Cd and Pb concentrations than in the rural zones. Related to this, Valente *et al.* (1989) and Regner and Valente (1993) showed that in this *Drosophila* species there was an increase in the average number of chromosome inversions in females and the number of inversions in the chromosome III was higher in the city center than in the peripheral populations. In *D. nebulosa*, this kind of clinal structure depending of the urban degree was restricted to the chromosome III. It cannot discarded that, for example, the Potosí population was the most differentiated because any influence of this nature.

Table 1. Santa Cruz (SC) and Tree Line (TL) rearrangement frequencies and standard deviations in five Colombian *Drosophila pseudoobscura* populations.

Populations	Frequencies	
	SC	TL
TOROBARROSO	0.712 ± 0.027	0.287 ± 0.027
SUSA	0.685 ± 0.026	0.314 ± 0.026
SUTATAUSA	0.721 ± 0.019	0.278 ± 0.025
POTOSI	0.781 ± 0.019	0.218 ± 0.019
SANTILLANA	0.676 ± 0.026	0.323 ± 0.026
TOTAL	0.720 ± 0.010	0.279 ± 0.010

Table 2. Wright F endogamy statistic and variance for each one of the five Colombian *Droosphila pseudoobscura* populations studied. d. f. = degree freedom.

Populations	Wright F	Variance	$\chi2$	d. f.
TOROBARROSO	0.9649	0.0006	129.413 *	1
SUSA	0.9833	0.0003	144.065*	1
SUTATAUSA	0.9846	0.0002	146.398*	1
POTOSI	0.9366	0.0008	214.061*	1
SANTILLANA	0.9462	0.0007	152.215*	1

*P < 0.0001

The comparison between the results of three of the populations studied here (Torobarroso, Potosí and Santillana) and also analyzed by Cárdenas (1988) shows that the differentiation dynamics are not the same in all of them. The SC frequency range between 0.60 and 0.80 (0.20 and 0.40 for TL) could be basically produced by stabilizing selection (Kimura, 1986). The Torobarroso population did not show significant differences in, at least, the last 220 generations (12 years). That means that the presence of genetic drift in this population has not been important, probably because it has maintained a big effective number in the last 12 years and the selective pressures had been constant. Moreover, during the sampling process this was the population where the capture of *D. pseudoobscura* was easier. On the contrary, the population from Potosí would be the one with the lower effective number, or where the selective pressures has changed more drastically. This fact is reinforced when we compared the results from Ruiz-Garcia and Alvarez (1997), who studied different morphological meristic features in eight Colombian and three American populations. Some of the populations analyzed here were included in that study (Potosí, Susa and Sutatausa) and they showed a very similar relationship in the dendrograms and in a principal coordinate analysis than the one presented here. Potosí was also the most divergent population (see Figure 2). This made us think that some morphological variations that have preferentially diverged in this population could be linked to some rearrangements of the third chromosome. That is, the genes (or polygenes) that codify for this kind of morphological variation (microchetae number in different structures) could be located on the third chromosome (Alvarez and Ruiz-Garcia, 1995).

The morphological, chromosomal, and molecular studies that are being currently made in some Colombian plateau *D. pseudoobscura* populations can profile, with a better precision, some of the evolutionary factors that rule the genetic structure of this insect.

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