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MICRO-GEOGRAPHIC VARIATION OF INVERSIONS IN NATURAL POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA*

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Chromosomal polymorphism for the third chromosome in *Drosophila pseudoobscura* has been widely studied for genetic variation in different environments or locations far apart. However, there is less information regarding sites geographically near one another. Targeting on possible micro-geographic variation in the species, a serial study was done on 12 Mexican populations grouped in four regions, including locations in Durango (DU), the border area of Guanajuato-San Luis Potosí states (GP), Chiapas (CH) and Saltillo (SA). Flies were trapped in their natural habitats using fermenting bananas as bait. They were individually cultivated in the laboratory for larvae production. In a given population sample several flies were cultivated together but only one larva per culture provided salivary tissue to observe polytene chromosomes. Gene arrangements or inversions were identified and frequencies were calculated. A total of 767 third chromosomes were studied and 11 different inversions detected. The type and relative frequencies of the arrangements varied among regions. From the 11

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inversions observed, 10 were found in Durango, five in Guanajuato-San Luis Potosí and Chiapas, and eight in Saltillo. The inversion frequencies in the Durango locations varied in four cases, but only one in Chiapas, three cases in Guanajuato-San Luis Potosí, and five in Saltillo. It was also observed that there were geographical gradients for inversions within regions as follows: four arrangements in Saltillo and Durango, three in Guanajuato-San Luis Potosí and one in Chiapas. Moreover, all four regions studied showed evidences for micro-geographical variation. Nevertheless, more studies are needed to elucidate the relevance of the inversion frequencies changes in neighboring populations and also any seasonal-annual frequencies observed in the locations studied here.

Key words: inversion polymorphism, clines, *Drosophila pseudoobscura*

INTRODUCTION

Studies on several species of the genus *Drosophila* during the past 75 years have found a considerable amount of variability in the form of chromosomal arrangements or inversions. In natural populations of *Drosophila pseudoobscura* the third chromosome is more variable than any of the others (DOBZHANSKY and EPLING, 1944). They consist of 40 different gene arrangements related to each other, mainly as overlapped inversions (OLVERA *et al.* 1979).

The distribution of each inversion of this species is geographically restricted to a portion of the species distribution area, although a particular location may contain several arrangements in the same population.

The populations can differ in both a quantitative and qualitative manner. The former refers only to differences in relative frequencies of inversion types, whereas the second targets the presence or absence of particular types. Whenever sample populations have been studies in a series of widely separated geographical locations, it was found that the relative frequencies of different chromosomal arrangements might increase or diminish in a regular manner following a given geographical direction. For example, the Santa Cruz (SC) inversion has not been detected in the states of Oregon and Washington and it is rare in the San Francisco area, but it is very common in the south of California; all of this in the United States. The same SC inversion is very common in the Mexican states of Nuevo León, Durango, Zacatecas, Guanajuato, Jalisco and Chiapas, as well as in several locations of Guatemala and around Bogotá in Colombia (DOBZHANSKY, 1939a; DOBZHANSKY *et al.*, 1963, GUZMAN *et al.*, 1975 and OLVERA *et al.*, 1982). This type of geographical gradient is very familiar to researchers on variation in both plants and animals.

On the other hand, differences among populations inhabiting neighboring location might present some different kind of variation, as the type found by DOBZHANSKY (1939b) in seven populations closely located in Texas. In this context, the objective of this work is to look for evidence of micro-geographical variation in several adjacent locations, grouped in four distant and different regions in Mexican territory.

MATERIAL AND METHODS

Specimens of *D. pseudoobscura* were collected in 12 locations grouped in four regions. Locations were very close to each other, in distances no more than 38 Km apart. Region Canatlán in Durango (DU) had three locations; region GP is situated in the states boundary of Guanajuato and San Luis Potosí (GP) and has two sites both very close to Santa María del Río; region Chiapas (CH) also has two locations, San Cristóbal las Casas and the road to Ocosingo; and region Saltillo with five locations surrounding clockwise East-South-West the city of Saltillo capital of the state of Coahuila.

As can be seen the regions are scattered along the Mexican boundary and include the Northwest with DU, Northeast with SA, North Mexican Plateau with GP, and Southeast with region CH; each one is far from the others hundreds of kilometers.

Towns, distances and directions among localities in each area as follows: DU, around Canatlán. Heading Northeast to Southwest, the place La Presa, is 8 Km ahead of location Presidio and 15 Km clockwise to the south is Diego de Alcalá. Vegetation in this area is mostly arid shrubs and grass species, but forest type cover is present in La Presa with species of cedar and casuarinas at altitudes between 2800 and 2850 m. Around Santa María del Río, region GP, 35 Km to the north, the site Los Reyes which is 10 Km away from the site Tierra Nueva; vegetation in the area is mostly huizache shrubs at a mean altitude of 2200m. In the region CH the sites have an orientation east-west and are separated by 20 Km from each other; pine species dominant in the area and its elevation averaging 2250 m. Region SA has five locations along a semi circle with a radial distance less than 30 Km; site are as follows: 26 Km to the east of the city of Saltillo is found Los Lirios; heading clockwise to the Southeast, at 25 Km from Saltillo, is Jagüey de Ferniza, which is followed to the south 8 Km apart by Ejido Cuahutémoc, 18 Km away also to the south is El Pino, a location facing The Catana Sierra. Finally at 15 Km to the West of the city is found the location El Chiflón, the altitude of it is 1500 m and that of the other four sites is between 2100 and 2350 m. The vegetation cover is as follows: forest of pines and juniper in Los Lirios; thorny shrubs, pines and liquidambar in Jagüey de Ferniza and Cuahutémoc; subinerme shrubs in El Pino; and subinerme thorny shrubs in El Chiflón.

Fly trapping in each location of a particular region was done within a week in order to minimize errors. Once the site was selected, 25 to 30 traps were placed strategically in the area. The traps were plastic buckets containing fermenting bananas as bait. Collections were done every other day from sunrise to 8.30 in the mornings and from 17.30 to sunset in the afternoon and rounding every 15 minutes in each case.

Flies were caught using an entomological net and placed into glass vials with fresh food. At the end of the trapping week flies were carried to the laboratory of the Instituto Nacional de Investigaciones Nucleares based at Salazar, state of Mexico.

Once in the laboratory each female from nature was individually cultured in a 250 cc bottle with fresh food made of corn flour, yeast, agar, and sucrose plus some drops of a heavy solution of fresh yeast to nourish the larvae. A week after, a good number of third instar larvae were ready for dissection to obtain salivary glands tissue. The extracted glands were stained with an aceto-orcein solution and applying the squash technique in order to prepare slides for polytene chromosome observation. As was stated above, the third chromosome of *D. pseudoobscura* exhibits wide and diverse paracentric inversions, which in turn make distinctive arrangements easily identified. Polymorphism in this and other species are detected because of these specific banding patterns. The help of an atlas and the figures that appears in the publications of KASTRITSIS and CRUMPACKER (1966 and 1967) made identification of the different configurations possible.

RESULTS AND DISCUSSION

A total of 767thrid chromosomes obtained from 12 populations sampled were analyzed. Among this total, 11 different gene arrangements were identified as seen in Table 1. The different inversions are named as follows: Tree Line (TL), Cuernavaca (CU), Santa Cruz (SC), Estes Park (EP), Olympic (OL), Oaxaca (OA), Pikes Peak (PP), Arrowhead (AR), Chiricahua (CH), Standard (ST), all of them described previously by DOBZHANSKY and EPLING (1944), plus a new inversion not yet described (UN unknown). Data here analyzed showed evidences for differences in relative frequencies in the chromosomal inversion in some population samples

Using data in Table 1 a X^2 test was applied in order to establish the existence of statistical differences in inversion relative frequencies among population samples within a given region. The results are presented in Table 2. Clinal trends were also analyzed as an indicative of variation for each chromosomal arrangement within the corresponding geographical area. It is instructive to remember that the 12 locations sampled are grouped into four different areas in Mexico hundreds of kilometers apart one from the other. Meanwhile, locations or sites within a determined area are situated closely no more than 35 Km apart from each other; this condition allows comparisons among them due to their neighborhood. In the Durango area (DU) we found 10 different inversions; eight of them were detected in La Presa and Presidio sites, but nine in the Diego de Alcalá location (Tables 1 and 2). There were detected differences in relative frequencies only for inversions TL and SC ($p < 0.001$ and $p < 0.05$) in the Presidio population when compared to the relative frequencies in the other two locations (La Presa and Diego de Alcalá). Since Presidio is located geographically between La Presa and Diego de Alcalá it is relevant to notice that the frequencies of TL and CH decrease in both directions while SC shows the opposite condition as seen in figure 1a. these results suggest that there is a compensatory effect in getting an equilibrium, *i.e.* the increase in the relative frequency of SC is balanced with a decrease in TL or CH relative frequencies. The remaining inversions did not show significant changes or trends.

Table 1. - Relative frequencies of chromosome arrangements in 12 natural populations of Drosophila pseudoobscura from Mexico

Durango											
	TL	CU	SC	EP	OL	PP	AR	CH	ST	UN	n
LP	5.55	3.70	55.55	---	---	5.55	5.55	18.51	3.70	1.85	54
P	17.07	7.31	31.70	---	4.87	7.31	2.43	26.82	2.43	---	41
DA	8.51	---	48.93	6.38	2.12	2.12	2.12	19.14	2.12	8.51	47

Guanajuato – San Luis Potosí					
	TL	CU	SC	EP	OL	n
LR	51.61	3.22	16.12	6.45	22.58	31
TN	52.17	8.69	28.26	---	10.86	46

Chiapas						
	TL	CU	SC	EP	OA	n
O	45.52	1.62	41.46	9.75	1.62	123
SCC	33.33	1.28	61.15	3.54	---	78

Saltillo									
	TL	CU	SC	EP	OL	OA	PP	UN	n
CH	40.00	---	---	20.00	---	---	40.00	---	5
EP	71.43	4.76	---	4.76	---	---	19.05	---	21
CU	48.48	1.52	---	15.15	4.55	---	30.30	---	66
JF	35.66	23.08	10.49	5.59	23.78	1.40	---	---	143
LL	25.89	4.46	---	8.03	16.96	---	41.96	2.67	112

Note: first column the locations; La Presa (LP), Presidio (P), Diego de Alcalá (DA), Los Reyes (LR), Tierra Nueva (TN), Ocosingo (O), San Cristóbal las Casas (SCC), Chiflón (CH), El Pino (EP), Cuahutémoc (CU), Jagüey de Ferniza (JF) and Los Lirios (LL). First row the corresponding name of the inversions as described in the text: Tree Line (TL), Cuernavaca (CU), Santa Cruz (SC), Estes Park (EP), Olympic (OL), Oaxaca (OA), Pikes Peak (PP), Arrowhead (AR), Chiricahua (CH), Standard (ST), Undescribed (UN)

Table 2. - Relative frequencies of chromosome inversions and X^2 test for the different comparisons among 12 Mexican natural populations of Drosophila pseudoobscura

	TL	CU	SC	EP	OL	PP	CH
LP	5.55	3.70	55.55	---	---	5.55	18.51
P	17.07***	7.31	31.70*	---	4.87	7.31	26.82
DA	8.51	---	48.93	6.38	2.12	2.12	19.14
LR	51.61	3.22**	16.12*	6.45	22.58*	---	---
TN	52.17	8.69	28.26	---	10.86	---	---
O	45.52	1.62	41.46**	9.75	---	---	---
SCC	33.33	1.28	61.15	3.84	---	---	---
CH	40.00	---	---	20	---	40	---
EP	71.43	4.76	---	4.76	---	19.05	---
CU	48.48**	1.52	---	15.15***	4.55	30.30**	---
JF	35.66***	23.08***	10.49	5.59	23.78***	---	---
LL	25.89***	4.46	---	8.03	16.96***	41.96**	---

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

The first column indicates the names of the localities: La Presa (LP), Presidio (P), Diego de Alcalá (DA), Los Reyes (LR), Tierra Nueva (TN), Ocosingo (O), San Cristóbal las Casas (SCC), Chiflón (CH), El Pino (EP), Cuahutémoc (CU), Jagüey de Ferniza (JF) and Los Lirios (LL). The first row corresponds to the names of the different inversions as denoted in the text: Tree Line (TL), Cuernavaca (CU), Santa Cruz (SC), Estes Park (EP), Olympic (OL), Pikes Peak (PP), and Chiricahua (CH).

Data for the GP region, localities Los Reyes and Tierra Nueva, indicates the presence of five inversions, of them only four are common in both localities. No statistical differences were found. However, the relative frequencies for the arrangements SC and OL were found in opposite amounts in the locations, the one that is high in one site is low in the other (figure 1b). The remaining inversions are in low frequency and there are no relevant changes.

Five inversions were also detected in the Chiapas region (CH) but only four are shared by the two localities: San Cristóbal las Casas and Road to Ocosingo. True difference ($p < 0.01$) was obtained only for inversion SC when locations were compared. However, as can be seen in figure 1c, there is an inverse relationship between arrangements TL and SC, while TL decreases from Ocosingo to San Cristóbal las Casas, SC shows the opposite condition. This a situation similar to that found in the GP region. In both cases, it seems that the inversions alternate.

In regard to the five locations of the Saltillo area (SA), we detected eight different chromosome arrangements. From them, three were present in location El Chiflón, four in El Pino, five in Cuahutémoc, and six in both Jagüey de Ferniza and Los Lirios. Due to the small sample size, data from El Chiflón was not used in the comparisons

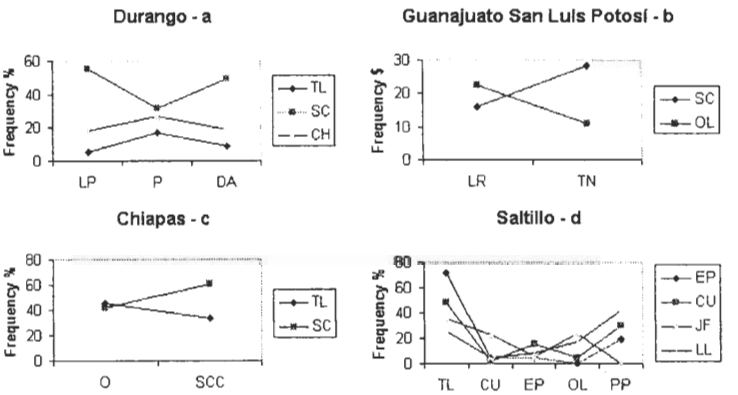

Figure 1.- Relative frequencies of the different chromosomal arrangements in natural populations of *Drosophila pseudoobscura* from four different regions in Mexico.

Note: first column the locations; La Presa (LP), Presidio (P), Diego de Alcalá (DA), Los Reyes (LR), Tierra Nueva (TN), Ocosingo (O), San Cristóbal las Casas (SCC), Chiflón (CH), El Pino (EP), Cuahutémoc (CU), Jagüey de Ferniza (JF) and Los Lirios (LL). First row the corresponding name of the inversions as described in the text: Tree Line (TL), Cuernavaca (CU), Santa Cruz (SC), Estes Park (EP), Olympic (OL), Oaxaca (OA), Pikes Peak (PP), Arrowhead (AR), Chiricahua (CH), Standard (ST), Undescribed (UN). The places at the left correspond to the West

The four SA locations available for comparison had in common five out of eight inversions found in the region. Their relative frequencies and corresponding X^2 test are shown in Tables 1. and 2. Information in these tables shows clearly that inversion TL has a gradual reduction of its frequency when we move from west to east, according to the situation of the sites. On the other hand, the inversion CU in Jagüey de Ferniza is present in a very high frequency ($p < 0.001$) with respect to the other locations where its frequency is less than 5 %.

Another important features of this region concerns the frequencies of inversion EP. It is present in high amount in the locality Cuahutémoc but decreases toward the other two locations having a small recuperation in Los Lirios (the eastern most location). The inversion OL increases from Cuahutémoc to Jagüey de Ferniza but decreases in Los Lirios. All these changes are significant (Table 2).

Finally, also in the SA region, inversion PP shows a clear gradient as we move West to East increasing gradually its relative frequencies despite the fact that it was not detected in Jagüey de Ferniza, a location situated in the Southeast position. The absence of this arrangement in Jagüey de Ferniza might be compensated with the presence of the inversion SC, which was found solely in this location. Trends of the different inversions showed geographical gradients in both up and down directions (figure 1 d) in three out of five inversions in common in the four localities under comparison. The other two inversions differ only in their relative frequency.

The results of this study as those reported by DOBZHANSKY (1939b) suggest the existence of genetic differences among populations of *D. pseudoobscura* that inhabit sites geographically close to one another. It also detects the presence of certain trends that might be indicative of gradients similar to those pointed out by GUZMAN *et al.* (1993), GUZMAN *et al.* (2005) in populations from Central Mexico, OLVERA *et al.* (2005) in southern Mexico, and those reported by DOBZHANSKY and EPLING (1944) in their study of certain American populations of the same species.

The results of this report also are illustrative of the kind of variation documented, and it seems to be worthwhile to investigate further this subject to determine if trends found here really correspond to true gradients in frequency changes. It is desirable to not only study more regions in Mexico but also to increase considerably sample sizes and sampling all the year round in order to have an insight to seasonal trends and to discover any other types of alteration in the inversion frequencies and the dynamics of the populations.

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REFERENCES

DOBZHANSKY, T. H. (1939 a): Genetics of natural populations. IV. Mexican and Guatemalan populations of *Drosophila pseudoobscura*. Genetics 24: 391-412.

DOBZHANSKY, T. H. (1939 b): Microgeographic variation in *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. USA. 25: 311-314.

DOBZHANSKY, T. H. and C. EPLING. (1944): Contributions to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Institute of Washington Publication 554: 183p.

DOBZHANSKY, T. H., A. S. HUNTER, O. PAVLOVASKY, B. SPASSKY and B. WALLACE (1963): Genetics of natural populations. XXXI. Genetics of an isolated marginal population of *Drosophila pseudoobscura*. Genetics 48: 91-103.

GUZMAN, J., L. LEVINE, O. OLVERA, J. R. POWELL, M. E. DE LA ROSA, V. M. SALCEDA, T. H. DOBZHANSKY and R. FELIX. (1975): Genética de poblaciones de Drosophilas de México. III. Estudio preliminar sobre la variación cromosómica de *Drosophila pseudoobscura* en dos zonas geográficas del Centro de México. An. Ins. Biol., Universidad Nacional Autónoma de México, 46 Seric Zoología 1: 75-86.

GUZMAN, J., O. OLVERA, M. E. DE LA ROSA and V. M. SALCEDA. (1993): East-West distribution of inversion polymorphism in *Drosophila pseudoobscura*. Southwestern Nat. 38: 52-57.

GUZMAN, J., V. M. SALCEDA, O. OLVERA and L. LEVINE. (2005): Geographical gradient of chromosomal polymorphism in Mexican populations of *Drosophila pseudoobscura*. Rev. Int. Contam. Ambient. 21 (Supl. 1): 21-25.

KASTRITSIS, C. D. and D. W. CRUMPACKER. (1966): Gene arrangements in the third chromosome of *Drosophila pseudoobscura* . I. Configurations with tester chromosomes. J. Heredity 57: 150-158.

KASTRITSIS, C. D. and D. W. CRUMPACKER. (1967): Gene arrangements in the third chromosome of *Drosophila pseudoobscura*. II. All possible configurations. J. Heredity 58: 112-129.

OLVERA , O., J. R. POWELL, M.E. DE LA ROSA, V.M. SALCEDA, M.I. GASO, J. GUZMAN, W.W. ANDERSON and L. LEVINE. (1979): Population genetics of Mexican Drosophila. VI. Cytogenetics aspects of the inversion polymorphism in *Drosophila pseudoobscura*. Evolution 33: 381-393.

OLVERA , O., M. E. DE LA ROSA, V. M. SALCEDA, M.I. GASO and J. GUZMAN. (1982): Population genetics of Mexican Drosophila. VII. Inversion polymorphism in populations of *Drosophila pseudoobscura* from Southern Mexico. Southwestern Nat. 27: 361-36.

OLVERA , O., V. M. SALCEDA, L. LEVINE and J. GUZMAN. (2005). Chromosomal polymorphism of *Drosophila pseudoobscura* from southern Mexico. Rev. Int. Contam. Ambient. 21 (Supl. 1): 27-30.

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MICRO-GEOGRAFSKO VARIRANJE INVERZIJA U PRIRODNOJ POPULACIJI *DROSOPHILA PSEUDOOBSCURA*

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Izvod

Polimorfizam trećeg hromozoma *Drosophila pseudoobscura* je ispitivan u velikom obimu u cilju utvrđivanja genetičke varijabilnosti u različitim uslovima sredine ili na različitim lokacijama. Mali broj informacije je dostupan kada su u pitanju geografska mesta koja su veoma blizu. Moguća geografska varijabilnost je ispitivana kod 12 meksičkih populacija grupisnih u četiri regiona: Durango (DU), granična oblst Guanajuato-San Luis Potosí države (GP), Chiapas (CH) i Saltillo (SA). Procesi fermentacije banane su bili kao mamac u njihovoj prirodnoj sredini. Individue su gajene zajedno u laboratorijskim slovima u cilju proizvodnje larvi. Tkivo za ispitivanje polimorfizma politenih hromozoma su uzimani samo od jedne larve u kulturi svake populacije. Identifikovane su inverzije i izvršena njihova kalkulacija učestalosti. Od ukupnog broja ispitivanog trećeg hromozoma, 767, detektovano je 11 različitih inverzija. Učestalost inverzija je varirala između populacija. Od 11 utvrđenih inverzija 10 istih je nađeno u Durangu, pet u Guanajuato-San Luis Potosí i Chiapas-u a osam u Saltillo. Utvrđeno je postojanje geografskog gradijenta između regiona: četiri vrste inverzija u Saltillo and Durango regionima, tri u Guanajuato-San Luis Potosí i jedna regionu Chiapas. U svim ispitivanim regionima je utvrđena mikrogeografska varijabilnost. Potrebna su obimnija istraživanja da bi bila utvrđena značajnost učestalosti inverzija u susednim populacijama kao i postojanje učestalosti variranja u toku sezone ili godine.

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