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GEOGRAPHICAL CHANGES IN RELATIVE FREQUENCY OF INVERSIONS IN CHROMOSOME III OF *Drosophila pseudoobscura* AMONG NATURAL POPULATIONS FROM MEXICO

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Chromosomal polymorphism in natural populations of *Drosophila pseudoobscura* have been broadly studied in the USA but scarcely in Mexico where only about 60 localities have been analyzed. Differences among both regions are notorious with respect to their chromosomal constitution. Northern populations, those of USA, have as representative inversions the sequences ST, AR and CH contrasting with those in Southern populations (Mexico) in which prevail the gene arrangements TL, CU and SC. Assuming as a probable mechanism that has allowed these substitutions the flow generated by the presence of a North - South clines, we took as a goal find out if such clines really exist. With that objective in mind we studied 29 populations of this species distributed along four North - South transects. Specimens of *D. pseudoobscura* caught by attracting them with fermenting bananas were carried to the laboratory where from each female an isofemale line was established. When their offspring appeared a single larva from each

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isofemale was taken, its salivary glands extracted and stained with a solution of lacto- aceto- orcein, by these means the polytene chromosomes were obtained. On these chromosomes we identified, for each larva, the inversion (s) carried in the third chromosome, in such a way 3439 third chromosomes were analyzed. Among the 29 localities we identified 17 different inversions but the number of them varied from population to population from three to eleven. Relative frequencies of each inversion at every location were calculated and with them for each transect the presence or absence of clines was determined. Among each transect the existence of clines was observed only between two or three near by populations, but we were not able to find a clear manifestation of the presence of clines along a complete transect. Our results at this respect are similar to those previously reported for USA populations. A mechanism that explains North – South substitutions of predominant inversions remains as open question.

Key word: chromosomal polymorphism, *Drosophila pseudoobscura,* geographical gradients

INTRODUCTION

Naturally occurring inversions are widespread in the genus *Drosophila* and were indirectly detected as early as 1917 by Sturtevant, more than half of the *Drosophila* species up to now examined is naturally polymorphic for inversions in one or more chromosome arms. These polymorphisms display an interesting geographical differentiation. The first species to be studied at this respect and probably the best studied is *Drosophila pseudoobscura,* for a detailed review see DOBZHANSKY and POWELL (1975) and POWELL (1992).

Drosophila pseudoobscura is polymorphic for its third chromosome and has a geographical distribution which goes from British Columbia in Canada, Western USA, Mexico and Guatemala and a small colony near Bogotá in Colombia (DOBZHANSKY *et al.* 1963), inhabiting mainly coniferous forests and mixtures of other temperate trees as well as areas covered with chaparral; depending on the locality in altitudes from sea level up to 3000 meters above sea level.

The inversion polymorphism of this species is primary in the third chromosome as seen in the polytene chromosomes and with up to 40 different gene arrangements, all of them paracentric inversions.

The degree of chromosomal polymorphism in natural populations of the species along the Mexican territory has been reported by several authors, among them we have those realized by DOBZHANSKY (1939, 1948), DOBZHANSKY *et al.* (1975), GUZMÁN *et al.* (2005), LEVINE *et al.* (1995), OLVERA *et al.* (1979, 2005) and SALCEDA *et al.* (2007a,b).

Other aspects dealing with the distribution of inversions is that concerning the presence of clines or gradients, the gradual directional changes in frequency on adjacent localities.

The presence of clines is evident in several species of *Drosophila,* for instance the studies in European populations of *D. subobscura* reviewed by KRIMBAS and LOUKAS (1980) and KRIMBAS (1992, 1993) and those done by DOBZHANSKY and EPLING (1944) in American and Mexican populations of *D. pseudoobscura.* Other known studies in Mexican populations of *D. pseudoobscura* are: GUZMÁN *et al.* (1993, 2005), OLVERA *et al.* (2005) and SALCEDA and ESPINOZA-VELÁZQUEZ (2006a, b), the three former reports correspond to East-West clines at long distances and the latter to clines in near by populations.

Now**,** considering that in Northern populations the prevailing inversions are ST, AR and CH and in Southern ones prevail TL, CU and SC, we assume that the mechanism with conferred such substitution is the presence of North-South clines, so we decided to analyze such possibility in several populations of this species along the Mexican territory.

MATERIAL AND METHODS

During 2000-2004 populations samples of *D. pseudoobscura* were taken along the major part of distribution of the species in Mexico. Specimens were collected in 29 locations and grouped into four regions according to a corresponding four North-South transects as follow: The 29 localities were distributed into four groups or transects arranged according to representative meridians as follow: transect "A" includes longitudes 92º - 96º 59´W, transect "B" from 97º to 98º 59´ W, transect "C" 99º - 101º 59´ W and transect "D" 102º - 106º W. Tables 1 to 4 show this grouping and includes: locality, relative frequencies of each of those more abundant inversions and sample size; all these in their corresponding North-South transect. All this was done for easiness for analyzing the data in such a way as to include in few groups as much as possible localities that could share close or related environments hopping that this clustering could be the best option to analyze the information.

Each collecting trip lasted for a week to assure a good sample size, even do they were of variable sample size. Flies were captured using as traps 25-30 plastic buckets containing fermenting banana as bait and scattered in the locality to cover a large area. When flies started to visit them, we did collecting rounds at regular intervals, 15-20 minutes, from sunrise to 9.00 h and from 17.00 h until darkness during the five days of collection. Flies were caught using an entomological net, sorted to separate those *D. pseudoobscura* from other species, then placed into glass vials with fresh food in groups of 20-30 individuals per vial, females and males in separate groups to prevent double insemination, and kept there until the arrival to the laboratory in Mexico City.

Once in the laboratory, each female was put in an individual flask with fresh food and left them to incubate for a week; when the number of captured females was small, also the males were used, in these cases each male was crossed individually with 2-3 females from the laboratory stocks EP/EP (Estes Park / Estes Park) or TL/TL (Tree Line / Tree Line) chromosomal constitution. A week latter, when larvae started to crawl out the culture bottle, adults were transferred to a new bottle with fresh medium to serve as a reserve and to the original culture we added drops of a heavy solution of live yeast to assure ample nourishment for the larvae and consequently large polytene chromosomes. At the appearance of third instance larvae, a single larva was taken from each culture and it was dissected with the aid of a stereoscopic microscope; salivary glands extracted and stained for 3-4 minutes in a 2 % solution of lacto-aceto-orceine and the smears prepared for each isofemale culture. Smears were analyzed using a light microscope and the karyotype of each larva determined with the help of an atlas and figures provided in DOBZHANSKY and EPLING (1944), KASTRITSIS and CRUMPACKER (1966, 1967) and OLVERA *et al.* (1979). After all determinations were done for each collection, we calculated the relative frequency of each inversion and with them the corresponding tables and figures were built.

The medium employed was prepared with a mixture of agar-sugar-corn flour-yeast of regular use in our laboratory and all cultures kept at 25$\pm$1º C and 65 % of relative humidity.

RESULTS AND DISCUSSION

Considering the 29 localities sampled, a grand total of 3439 third chromosomes were analyzed, this is the data we will describe. Throughout the study we found 16 different gene arrangements including a new one not yet described, these inversions were previously described by DOBZHANSKY and EPLING (1944) and OLVERA *et al.* (1979), their corresponding identification are given by their names as follow: Tree Line (TL), Cuernavaca (CU), Santa Cruz (SC), Estes Park (EP), Olympic (OL), Oaxaca (OA), Hidalgo (HI), Pikes Peak (PP), Chiricahua (CH), Standard (ST), Arrow Head (AR), Tarasco (TA), Ozumba (OZ), Iztaccíhuatl (IZ) and Pátzcuaro (PA), here listed in decreasing order of their global abundance and presented in Tables 1 – 4. The data here analyzed shows evidence for differences in relative frequency in chromosomal inversions in some of the populations sampled. Also is important to mention that not all the inversions are present in all populations since the number of them varied from three to eleven per locality. Taken this in account in tables we present only relative frequencies of the six more representative inversions and include the remaining as “OTHERS”.

Table 1. Relative frequencies of inversions of Drosophila pseudoobscura found in transect "A".

	TL	CU	SC	EP	OA	n
Oaxaca	35.7	54.1	7.1	2.0	1.0	98
Ocosingo	45.5	1.6	41.5	9.8	1.6	123
S.C.Casas	33.3	1.3	61.5	3.9	---	78

(Tree Line = TL; Cuernavaca = CU; Santa Cruz = SC; Estes Park = EP; Olympic = OL; Oaxaca =OA; n = sample size).

Table 2. Relative frequencies of inversions of Drosophila pseudoobscura found in transect "B". (Symbols as in Table 1).

	TL	CU	SC	EP	OL	OA	others	n
Lobo	54.6	14.6	9.1	1.8	12.7	1.8	5.4	55
Pinal	56.9	6.3	4.9	---	25.0	---	7.0	144
Tulancingo	67.6	22.9	---	6.3	2.5	0.4	0.4	240
C. Nuclear	66.2	21.1	---	12.7	---	---	---	71
Amecameca	39.0	48.0	2.5	8.0	1.0	1.5	---	200
Malinche	66.3	20.2	---	9.0	1.1	2.3	1.1	89
Seco	47.4	39.6	5.8	5.2	1.4	0.4	0.2	515
Perla	59.7	32.2	2.7	4.7	0.7	---	---	149
F. de Caballo	32.7	53.3	7.5	0.9	1.9	2.8	0.9	107

Table 3. Relative frequencies of inversions of Drosophila pseudoobscura found in transect "C". (Symbols as in Table 1).

	TL	CU	SC	EP	OL	OA	PP	others	n
C. de Caballo	9.7	6.4	32.3	22.6	---	9.7	19.4	---	31
Lirios	35.7	23.1	10.5	5.6	23.8	1.4	---	---	143
Matehuala	34.3	14.3	5.7	14.3	20.0	---	8.6	2.9	35
Jerez	30.9	7.1	33.3	11.9	2.4	4.8	---	9.6	42
Congoja	36.3	2.9	42.6	2.1	4.6	2.6	---	9.1	240
Río Verde	59.3	5.4	2.8	7.6	22.4	1.0	---	1.6	317
Reyes	51.6	3.2	16.1	6.5	22.6	---	---	---	31
T. Nueva	57.2	8.7	28.3	---	10.9	---	---	---	46
Huimilpan	45.2	26.2	4.8	4.8	14.3	2.4	---	2.4	42
Victoria	30.9	16.2	29.4	1.5	2.9	7.4	---	11.8	68
Zirahuén	30.5	25.7	20.9	10.5	2.9	9.5	---	---	105

Table 4. Relative frequencies of inversions of Drosophila pseudoobscura found in transect "D". (Symbols as in Table 1).

	TL	CU	SC	EP	OL	OA	CH	others	n
Torreón	36.6	11.6	16.1	9.8	16-1	---	---	9.8	112
Presidio	17.1	7.3	31.7	---	4.9	---	26.8	12.1	41
Diego de A.	8.5	---	48.9	6.4	2.1	---	19.2	14.8	47
Presa	5.6	3.7	55.6	---	---	---	18.5	16.8	54
Valparaiso	34.9	6.8	6.8	2.7	3.4	5.5	---	8.3	146
Cd. Guzmán	21.4	8.6	8.6	5.7	1.4	2.9	---	1.4	70

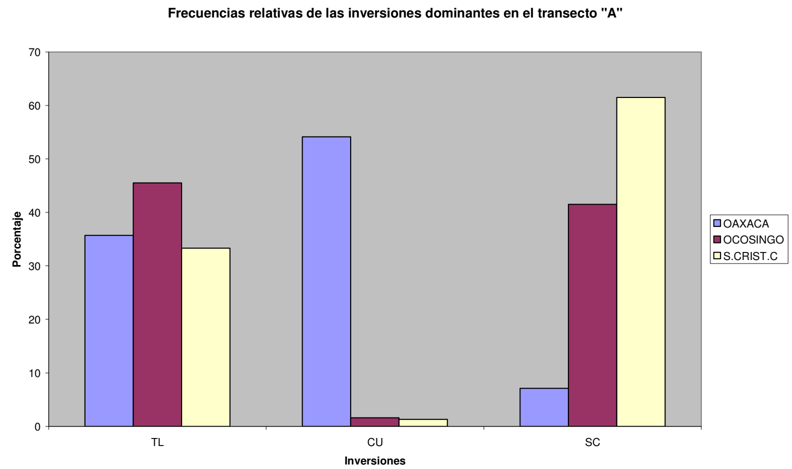

Figure 1.- Graphic representation of changes in relative frequency of dominant inversions of *Drosophila pseudoobscura* in transect "A".

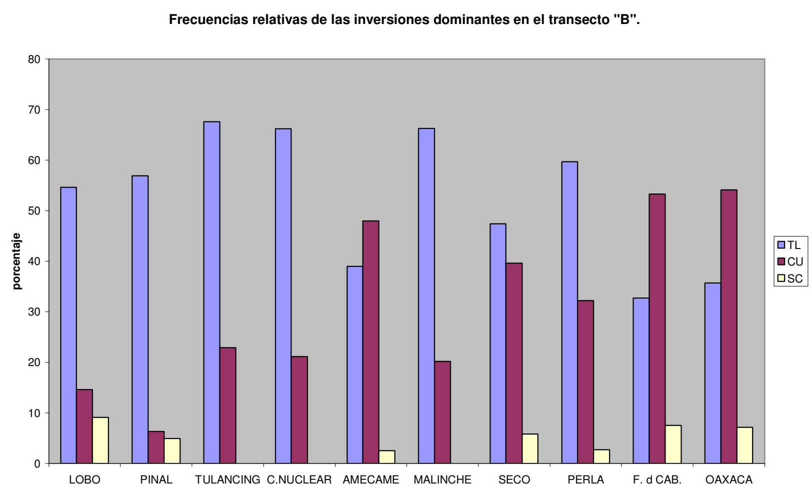

Figure 2.- Graphic representation of changes in relative frequency of dominant inversions of *Drosophila pseudobscura* in transects "B".

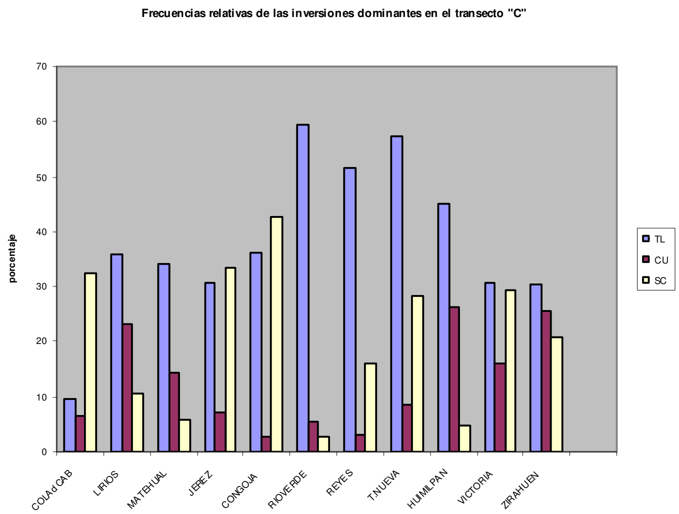

Figure 3.- Graphic representation of changes in relative frequency of dominant inversions of *Drosophila pseudoobscura* in transects "C".

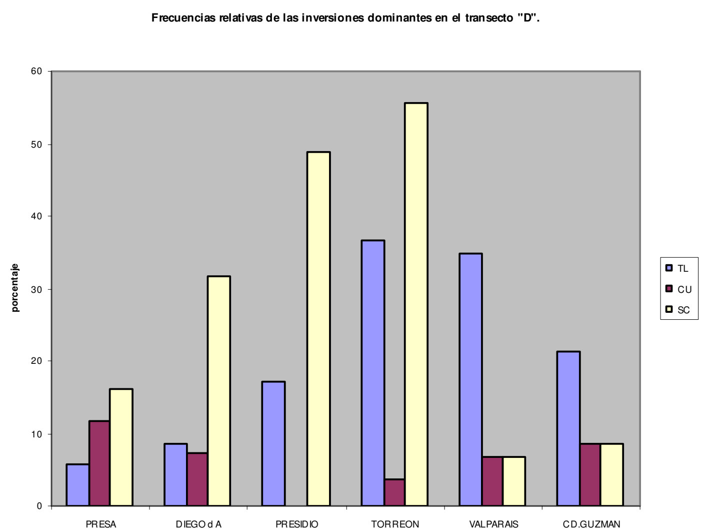

Figure 4.- Graphic representation of changes in relative frequency of dominant inversions of *Drosophila pseudoobscura* in transects "D".

DISCUSSION

First we must point out that most of the populations of *D. pseudoobscura* up to now studied for chromosomal polymorphism exhibit a pattern in which a pair of inversions cover up to 90% and represent the dominant pair of the sample and the remaining ten percent is divided in three, four or more inversions present in that particular population. Those populations now analyzed present in general the same pattern with some peculiarities inherent to each population.

Let now show how the relative frequencies behave on each group. In transect “A”, (see Table 1 and Figure 1), we found 4-5 different inversions in them inversion TL is the dominant one and form a pair with CU in Oaxaca the northern population but as we moved to the South it was replaced by SC in Ocosingo and reached a maximum in San Cristobal las Casas; gene arrangements EP and OA complete the constitution.

Transect “B”, (see Table 2 and Figure 2), in this area we observed an average of six different inversions, of them, only TL and CU that complement each other as a dominant pair, showed some changes in frequency as we go from North to South, these changes are not consistent as to define a cline and they could be due to different times of collection, the remaining inversions did not show considerable changes.

In transect “C”, (see Table 3 and Figure 3), we found between six and eleven different inversions depending on the locality. This transect presented notorious changes as we moved North- South, so in the northern most population inversions SC, EP and PP showed to be dominant but in moving South such condition changed and they were substituted by TL, CU and OL, further South a new substitution occurred been now dominant inversions TL, CU and SC, then a new change OL instead SC and finally TL, CU and SC as dominant representatives. In all cases minor contributions of 3-4 inversions are present.

In transect “D”, (see Table 4 and Figure 4), we found as main components, in the Northern populations, inversions TL, SC and OL they were substituted in dominance by inversions SC and CH, later a change occurred with a rise of TL and very similar values for the remaining five inversions.

This short account on differential changes in relative frequency of the inversions involved in each transect, easy seen in tables, shows what complicated is the response of each inversion in different localities and consequently clear presence of cline in a North-South direction is hard to demonstrate actually we did not find it.

It is well known that observed clines for relative frequency of different inversions, as another feature of this and other species of *Drosophila* could be considered as a clear effect of selection as response to differential changes of environment, POWELL (1990), but among the populations here analyzed we did not find a clear evidence of clines in North-South direction, at least when we took a whole transect since occasionally in some sections of a transect, going South from one locality to the next, some changes were observed that could show a possible directional trend, for example in transect “A” it clearly showed a cline in which inversion CU is substituted by SC; transect “B” did not show any evidence of trend and always presented as main components inversions TL and CU; in transect “C” we did not observe what could be a trend but instead some substitutions of inversions in they relative frequency for instance inversions PP and EP were substituted as they frequencies concern by rises in inversions TL, SC or OL.

In transect ”D” it seems that the main components in the North are TL, SC and CU but TL and CU were substituted by CH in the middle localities and in the Southern populations again TL, CU and SC remain as main components.

Finally we must mention that East-West clines have been reported by LEVITAN (1990) in *D. robusta,* and in *D. pseudoobscura* in several transects in populations of this species in USA by DOBSHANZKY and EPLING (1944), and in Mexico by GUZMÁN *et al.* (1993 and 2005) and OLVERA *et al.* (2005). Of interest is the case reported by KRIMBAS (1990) that in *D. subobscura* three different kinds of clines have been observed they are North-South, East-West and Central-Marginal, and also is the information of SOULÉ (1973) who found that among 16 species analyzed for clines 15 showed central-marginal clines. Studies of DOBSHANZKY and EPLING (1944) and of POWELL (1990) in *D. persimilis,* who shares most of its distribution with *D. psudoobscura,* demonstrated that this species shows North-South clines but not East-West. In the 29 different population here studied in search of North-South clines, no clear evidence was found, however, this same 29 populations in most of the cases showed clines in an East-West direction. We assume that this absence could be ascribed in the first place by the random way in which collections were done and suggest a possibility to find it if we do new collections, taking care at choosing the localities that share the very same meridian and located at considerable distances from each other orientated North-South. Anyway we showed that these Mexican populations behave similarly to those from USA for this trait not showing clines in this direction. Other mechanisms that explain how the different inversions are substituted must be investigated.

We also suggest that new studies are necessary to really find mechanism that allowed the gradual substitution of one inversion for other as the species moved in North-South direction and complete its actual distribution.

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GEOGRAFSKE PROMENE U RELATIVNOJ FREKVENCIJI INVERZIJA NA HROMOZOMU III *DROSOPHILA PSEUDOOBSCURA* IZMEĐU PRIRODNIH POPULACIJA U MEKSIKU

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I z v o d

Hromozomski polimorfizam u prirodnim populacijama *Drosophila pseudoobscura* je široko ispitivan u SAD ali u Meksiku je analizirano samo 60 lokaliteta. Razlike između oba regiona su opštepoznate sa obzirom na njihovu hromozomsku konstituciju. Severne populacije, u SAD, imaju kao reprezentativnu inverziju sekvenca ST, AR i CH nasuprot onim u Južnim regionima (Meksiko) u kojima preovladavaju genski arnžmani TL, CU i SC. Ispitano je 29 populacija ove vrste raspoređenih duž Sever-Jug sekcije. Vrste *D. pseudoobscura* uhvaćene sa fermetisanom bananom kao mamcem su prenete u laboratoriju gde je od svake ženke uspostavljena izolinija. Na hromozomima izolovanim iz pljuvačnih žlezda pojedinačnih larvi, potomstva izoženki, identifikovane su za svaku larvu, inverzije na trećem hromozomu, 3439 hromozoma je analizirano. Između 29 lokaliteta identifikovano je 17 različitih inverzija ali je broj varirao od 3 do 11. Relativna frekvencija svake inverzije na svakoj lokaciji je izračunata. Dobijeni rezultati su u saglasnosti sa rezultatima objavljenim za populacije iz SAD. Mehanizam koji objašnjava Sever-Jug substituciju predominatnih inverzija ostaje otvoreno pitanje.

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