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INVERSION POLYMORPHISM IN SOME NATURAL POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA* FROM CENTRAL MEXICO

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Samples of *D. pseudoobscura* were taken in seventeen localities in Central Mexico inside the parallels 18^o – 20^o N, with the purpose of determine the chromosomal polymorphism in the third of the different populations of this species. From each captured female a single larva of its offspring was taken, its salivary glands extracted and stained with a solution of aceto orcein to observe the polytene chromosomes. From these smears the corresponding karyotype of each larva was determined, keeping a record of them. With the information gathered the relative frequency of each one of the fourteen different inversions found was calculated. A grand total of 1894 third chromosomes were analyzed. The

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fourteen different inversions found are equivalent to a 34.1 % of the total chromosomal variation of the species. The most abundant inversions found were: TL 50.6 %, CU 27.2 5, SC 9.1 % and EP 5.5 %; the remaining ten inversions detected are in general grounds rare ones with variable relative frequencies depending on the locality. Analysis of the predominant inversions for each population was done. The presence of West-East gradients is reported, even if in cases not so well defined, since as one moves in a particular direction the ups and downs in relative frequency for the alternating pairs TL-CU; TL-SC in the western populations and TL-CU in the eastern ones were observed. The assignment of each population to a particular race was also done, and such a way we were able to recognize three different races coexisting in the area of study.

Key word: *Drosophila pseudoobscura*, natural populations, polymorphism

INTRODUCTION

Natural populations of *Drosophila pseudoobscura* show a remarkable variability in the gene arrangements of their third chromosome with up to 40 different inversions. Normally two or as many seven gene arrangements of this chromosome occur in the majority of the populations of this species in most of the localities in which it has been studied. In there, the relative frequencies of each gene arrangement normally vary from region to region. A large amount of populations have been analyzed for that purpose only in the U.S.A. see for example DOBZHANSKY and EPLING (1944), ANDERSON *et al.* (1991) and POWELL (1992).

In Mexico the number of populations studied to know the amount of variability in the third chromosome of *D. pseudoobscura* is much inferior than those analyzed in the USA, among them we have: GUZMAN *et al. (*1975), OLVERA *et al.* (1979), GUZMAN *et al.* (1993), LEVINE *et al.* (1995), OLVERA *et al.* (2005) and GUZMAN *et al.* (2005).

In this occasion our interest is focused in some populations located along the parallels 18^{o} and 20^{o} North in Central Mexico in order to find out their chromosomal composition represented by the relative frequencies of the different gene arrangements or inversions present in these localities and tray to understand the dynamics on the spatial distribution of those inversions along the selected area of study.

Up to date 27 out of 40 different known inversions have been detected in Mexico, OLVERA *et al.* (1979) and POWELL (1992). Even that some localities in this area have been already reported by us, OLVERA *et al.* (1979), for their chromosomal composition and for the presence of geographical gradients, GUZMAN *et al.* (1993 and 2005), we are now extending the number of populations as well some previously analyzed but including in them more recent data.

MATERIAL AND METHODS

Along the parallels 18^{o} – 20^{o} N in Central Mexico, seventeen localities were sampled in order to collect specimens of *D. pseudoobscura*, these localities are, in a West - East direction: Ciudad Guzmán, Mazamitla in Jalisco; Jerez in Zacatecas; Zirahuén in Michoacán; Victoria de Cortazar and Tierra Nueva in Guanajuato; Huimilpan, Juriquilla, Pinal de Amoles and El Lobo in Querétaro; Tulancingo in Hidalgo; Centro Nuclear and Amecameca in the State of Mexico, Tlaxcala and El Seco in Tlaxcala; La Perla and Laguna Verde in Veracruz. The collections were done during the years 2000-2004, the approximate position of each locality is shown in figure 1.

Figure 1. Approximate position of the different localities in which collections of *Drosophila pseudoobscura* were done.

1.- Ciudad Guzmán, Jal.
2.- Mazamitla, Jal.
3.- Jerez, Zac.
4.- Zirahuén, Mich.
5.- Victoria de Cortazar, Gto.
6.- Tierra Nueva, Gto.
7.- Huimilpan, Qro.
8.- Juriquilla, Qro.
9.- Pinal de Amoles, Qro.
10.- Centro Nuclear, Edo. de México.
11.- El Lobo, Qro.
12.- Amecameca, Edo de México.
13.- Tulancingo, Hidalgo.
14.- Tlaxcala, Tlax.
15.- El Seco, Pue.
16.- La Perla, Ver.
17.- Laguna Verde, Ver.

In order to capture the flies we used as baits 25-30 plastic buckets containing fermenting banana scattered in the locality as to cover a large area. Once the baits were settled and the flies started to visit them, we did collecting rounds every 15-20 minutes and with the help of an entomological net we swept each trap and caught the flies. This operation was done from sunrise to 9.00 h and from 17.30 h to sunset during the five days of each collecting trip.

The flies captured were selected and those belonging to the obscura group were placed in vials with fresh food in groups of 20-30 individuals, males and females in separate vials to prevent double insemination, and ready to carrier them to the laboratory in Mexico City once the collection was finished.

Once in the laboratory, every female was put in an individual quarter of a liter bottle with fresh food and remained there for a week; when the amount of females was small we used the males, in such case they were also put individually in a bottle and crossed with 2-3 females of a known karyotype strain, in this case Estes Park (EP) in order to increase the sample size.

A week later, flies were transferred to a new bottle with fresh food to serve as a reserve, and to the original culture we added some drops of a heavy solution of live yeast to allow a better nourishment of the developing larvae and so assure large salivary glands and as a consequence large polytene chromosomes.

When larvae started to crawl out, from each culture a single larva was taken, a dissection performed and its salivary glands extracted making a smear, staining it with a 2% aceto-orcein solution for 3-5 minutes. After preparing the smears they were analyzed under the light microscope to determine the corresponding karyotype, this was done with the help of a photographic atlas and the figures reported by KASTRITSIS and CRUMPACKER (1966, 1967) and OLVERA *et al.* (1979).

When all the cultures of each collection were analyzed the relative frequencies for each gene arrangement were calculated and with them we elaborated Tables 1 and 2. The food employed was the regular corn meal-sugar-yeast-agar normally used in the laboratory, all the cultures were kept at 25$\pm$ 1^{o}C and 65 % of relative humidity.

RESULTS AND DISCUSSION

A grand total of 1894 third chromosomes were analyzed from the seventeen localities sampled. In there we found fourteen different gene arrangements, including a new one not yet described, they are: Tree Line (TL), Cuernavaca (CU), Santa Cruz (SC), Estes Park (EP), Olympic (OL), Oaxaca (OA), Hidalgo (HI), Tarasco (TA), Ozumba (OZ), Iztaccihuatl (IZ), Chiricahua (CH), Pátzcuaro (PA), Pikes Peak (PP) and the new one (UN, for unknown), the relative frequency of each of them in the different localities are shown in Table 1 and 2. Assuming a total of 40 known inversions our data represents a 32.5 % of total chromosomal variation for this species, in this calculation we did not include the newly found, but if we do so the corresponding value raises to 34.4 % of the known chromosomal variability which is a quite representative value of the hidden variability for this species.

Table 1.- Relative frequencies in percentage of inversions in the third chromosome of Drosophila psudoobscura in some natural populations from Central Mexico.

	TL	CU	SC	EP	OL	Others	n
Ciudad Guzmán	21.4	8.6	58.6	5.7	1.4	4.3	70
Mazamitla	25.0	25.0	25.0		25.0		4
Jerez	31.0	7.1	33.3	12.0	2.4	14.2	42
Zirahuén	30.5	25.7	21.0	10.5	2.9	9.5	105
VictoriadeCortazar	31.0	16.2	29.4	1.5	2.9	19.1	68
Tierra Nueva	52.2	8.7	28.3		10.8		46
Huimilpan	45.2	26.2	4.8	4.8	14.3	4.8	42
Juriquilla	78.8			15.4	1.9	3.8	52
Pinal de Amoles	57.0	6.2	4.9		25.0	7.0	144
Centro Nuclear	66.2	21.1	12.7				71
Lobo	54.5	14.5	9.1	1.8	12.7	7.3	55
Amecameca	39.0	48.0	2.5	8.0	1.0	1.5	200
Tulancingo	67.5	22.9		6.2	2.5	0.8	240
Tlaxcala	66.3	20.2		9.0	1.1	3.4	89
El Seco	47.4	39.6	5.8	5.2	1.4	0.6	515
La Perla	59.7	32.2	2.7	4.7	0.8		149
Laguna Verde	100.0						2
TOTAL	50.6	27.2	9.1	5.5	4.2	3.2	1894

The first column gives the name of the population. The first line corresponds to the gene arrangement as in text.

In general grounds, it is, from the total 1894 chromosomes analyzed, the inversions TL and CU represent together the 77.8 % of the total sample and we could considered to both as of high frequency, more than 30 % each; followed by those that we catalogue as of moderate frequency fluctuating between 10 – 20 percent in our case SC and EP which in average gave values of 9.4 % and 5.5 % respectively; the remaining ten inversions had values less than 2 percent and we considered them as rare. Even do, not in all the localities this status is the same as we will see.

At Ciudad Guzmán, SC represent 58.6 % of the sample meanwhile TL represents the 21.4 % and they together are the main components of the population, then CU and EP have a low representation with 8.6 % and 5.7 % respectively and the remaining three OL, OA and TA with values around 2 % .

In Jerez, also SC and TL are the main components with values around 30 %, CU and EP are moderate and the remaining five inversions OL, OA, HI, CH and PP as rare with values less than 5 % each.

In Zirahuén, three main arrangements are present: TL, CU and SC with values of 30.1 %, 25.7 % and 21.0 % respectively, followed by EP with 10.5 %, OA with 9.5 % and OL with 2.9 %; notice here the presence of only six inversions,

considering that it is the most polymorphic population up to now reported with sixteen different inversions according to OLVERA *et al.* (1979).

Table 2. Relative frequency in percentage of inversions in the third chromosome of Drosophila pseudoobscura from some localities of Central Mexico. The frequencies correspond to those inversions included as others in Table 1.

	OA	HI	TA	OZ	IZ	CH	PA	PP	UN
GUZMAN	2.8		1.4						
JEREZ	4.7	4.7				2.3	2.3		
ZIRAHUÉN	9.5								
VICTORIA	7.3		8.8	1.4	1.4				
HUIMILPAN	2.3	2.3							
JURIQUILLA		3.8							
PINAL		4.2				0.7	1.4		0.7
LOBO	1.8	1.8							3.6
AMECAMECA	1.5								
TULANCINGO	0.4	0.4							
TLAXCALA	2.2	1.1							
SECO	0.4	0.2							

First line corresponds to the name of inversion as in the text. First column to the locality.

In Victoria de Cortazar the situation is quite similar with three main components as follows TL 30.1 %, CU 16.2 % and SC 29.4 %; two inversions with values between 5 and 10 percent, OA and TA; and four with values less than 2 %, EP, OL, OZ and IZ; this locality has among the ones sampled by us a vary good variability with up to nine different inversions and considering not a very large sample only 68 chromosomes.

Contrasting in number of inversions is Tierra Nueva with only four different inversions, two as a main components accounting for 80 % of the total TL and SC, and two with values around 10 %, CU and OL; this population behaves quite similar to most of the USA sampled populations, DOBZHANSKY and EPLING (1944).

In Huimilpan the prevailing arrangements are TL with 45.2 %, CU with 26.2 % and OL with 14.3 % being the remaining SC, EP, OA and HI rare with values less than 5 percent each.

In Juriquilla out of four inversions two represent the main components with combined relative frequency of almost 95 % of the total represented by TL and EP, the two other inversions are OL and HI which account for the remaining 5 %.

In Pinal de Amoles even of having eight different inversions only two of them TL and OL are representative of the 80 percent of the chromosomes, the remaining six are rare with frequencies close to 5 % or less.

In Centro Nuclear, this is the population with less number of inversions, only three, TL, CU and EP, seems to be the one that follows the most common pattern of inversion polymorphism in the USA in which two inversions are

responsible for up to 80 % of the total and the other one or two inversions for the remaining 20 percent. The same situation occurs only with rare variation concerning the inversions involved in the populations: El Lobo, Amecameca, Tulancingo, El Seco and La Perla in which two inversions are the main components and have up to five inversions present in low frequency.
For populations Mazamitla and Laguna Verde and due to the small sample size we could not make any consideration.

All the previous results here in show could be seen in numerical terms in Table 1 and 2 and graphically in figures 1- 3.

With all this information an ANOVA test was performed to detect the significance of the possible differences present in the populations, the result of it is:

Source of Variation	Sum of Squares	d.f.	Mean Squares	F
Between	1184	14	84.55	0.1829
Error	2.9594 E+04	64	462.4	
Total	3. 0778 E+04	68		

The probability of this result, assuming the null hypothesis, is 0.999.

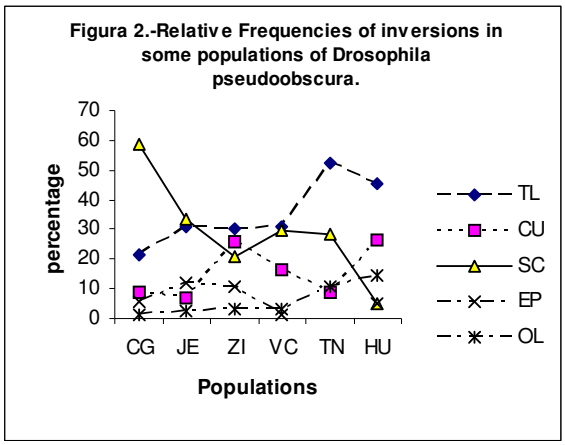
DISCUSSION

The presence in this region of fourteen different gene arrangements indicates that this area of good amount of chromosomal polymorphism. Thirteen out of seventeen populations here reported showed the most common pattern of distribution of its alleles found in the majority of the populations studied up to now, consisting in the presence of two or more, occasionally seven different gene arrangements, we found up to nine, and characterized by the prevalence of two or even three of them with relative frequencies that together represent about an 80-90 percent of the total, being the remaining gene arrangements rare ones and with relative frequencies according to the number of them, but normally the highest with no more than 5 %, to sum up the 10-20 percent of the total.

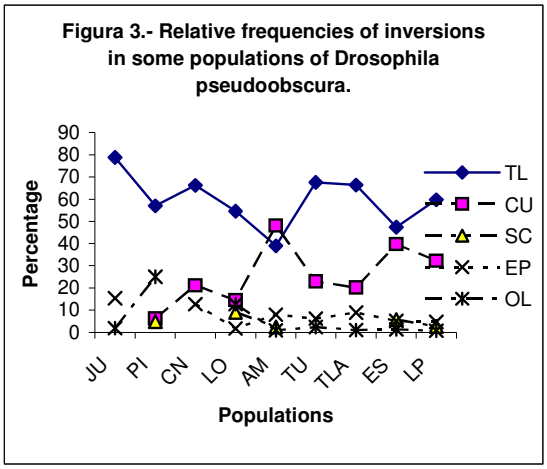
Thus, three genes arrangements TL, CU and SC or EP were found in each of the seventeen populations, the relative frequencies of these arrangements varied widely from place to place as is expected between populations separated by long distances. Notorious is in the population of Zirahuén the close similarity in relative frequency of the four main inversions in contrast with the prevailing situation of only two in the other populations.

Due to the general similitude found in the different populations it is difficult to point out the existence of a well defined gradient as was found by GUZMAN *et al.* (1993, 2005) for some of the populations here also reported. Even do, the presence of gradients was detected two of them were observed in a West-East direction.

The first gradient involves populations: Ciudad Guzmán, Jerez, Zirahuén, Victoria de Cortazar, Tierra Nueva and Huimilpan; in this gradient three inversions are involved: TL, CU and SC, and their behavior is as follows: at the starting point in Ciudad Guzmán, TL has a frequency of 21.4 %, it increases slowly up to Victoria de Cortazar with 31 % going up to 52.2 % in Tierra Nueva and decreasing to 45.2 % in Huimilpan. As for CU it begins with 8.4 % in Ciudad Guzmán increases up to 25.7 in Zirahuén goes down to 8.7 % in Tierra Nueva and climbs again up to 26.2 in Huimilpan. The third inversion SC starts with 58.6 % in Ciudad Guzmán decreases to Zirahuén with 21 % increases slowly to 29.4 % in Victoria de Cortazar and again decreases to 4.8 % in Huimilpan. It seems that both inversions TL and CU together are the counterpart of SC. Graphically this behavior is shown in figure 2.



The other gradient is more evident and involves inversions TL and CU in this case as TL decreases CU increases and vice versa; here the populations involved are again located in a West-East direction: Juriquilla, Pinal de Amoles, Centro Nuclear, El Lobo, Amecameca, Tulancingo, Tlaxcala, El Seco and La Perla. Coincidentally at the beginning, in Juriquilla, TL is at its maximum with a frequency of 78.8 % and CU is absent; now the former starts to decrease and the later to increase; in a mid-point; Amecameca, the situation seems to be at the inflection point since CU attained its maximum and TL its minimum, consequently here come the changes in direction, so TL goes up until the last locality, La Perla in where it presents a 59.7 % frequency and CU reaches 32.2 %; no further localities exists as to find again a maximum or a minimum, graphically it is shown in figure 3.



Considered our results we could assign the position of each population as to belong to the following races of the species as described by DOBZHANSKY (1948); so populations Ciudad Guzmán, Jerez, Zirahuén, Victoria de Cortazar, Tierra Nueva and probably Mazamitla as representatives of the Michoacán-Guatemala race, characterized by the presence of TL and SC as the commonest gene arrangements, with CU being fairly common and the remaining inversions in varied amounts constituting a mixture of four or more gene arrangements at low frequency.

Populations Huimilpan, Juriquilla, Pinal de Amoles and El Lobo seem to belong to the Northeast-Mexican race in which OL and TL are the prevalent gene arrangements, with mixtures of other rare elements. The remaining seven populations are included into the Central Mexico race that is represented by TL, CU and EP as principal components of the populations and with mixtures at different frequencies of the more rare inversions SC, OL, OA and HI. These results are in accordance to the description given by DOBZHANSKY (1948).

It seems to us that the gradients are related to the main components of each race since in the populations representatives of the Michoacán-Guatemala race the dominant inversions TL, SC and CU are the ones involved in the gradient. The same happens with the Central Mexico race in where the involved inversions in the gradient are TL and CU and they are the representatives of the race.

Also is important to mention that the population of Zirahuén was previously, OLVERA *et al.* (1979), considered as the most polymorphic for inversions of all the population of this species studied with up to sixteen different gene arrangements, but in contrast in this occasion we were able to detect only six of them, differences of the weather conditions prevailing at the moment we did the collection could be the responsible of such low variability. Also is necessary to mention that the presence of *D. pseudoobscura* in Laguna Verde, placed in the

coast of the Gulf of Mexico is quite rare, say an accident, since we have monitored the site for more than ten years for other purposes and only in this occasion we found the species and only a single female, most probably it was carried by the wind, as a consequence it does not represent a the population.

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INVERZIONI POLIMORFIZAM U NEKIM PRIRODNIM POPULACIJAMA *Drosophila pseudoobscura* IZ CENTRALNOG MEKSIKA

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Izvod

Uzorci *D. pseudoobscura* su sakupljeni sa sedamnaest lokacija u centralnom Meksiku unutar paralela 18^o – 20^o N, sa ciljem da se odredi hromozomski polimorfizam u različitim populacijama ove vrste. Od svake uhvaćene ženke po jedna larva od potomstva je uzeta, njihove pljuvačne žlezde su uklonjene i obojene sa rastvorom aceto orceina da bi se posmatrali politeni hromozomi. Odgovarajući kariotipovi svake larve su određeni. Ukupno 1894 trećih hromozoma je analizirano. Nađenih 14 različitih inverzija su ekvivalentne 34.1 % ukupne hromozomske varijabilnosti vrste. Najčešće inverzije su: TL 50.6 %, CU 27.2 5, SC 9.1 % i EP 5.5 %; ostalih 10 inverzija su retke i njihova relativna frekvencija zavisi od lokaliteta. Analiza dominantnih inverzija za svaku populaciju je urađena. Prisustvo zapad-istok gradijenta je utvrđeno za alternativni par TL-CU; TL-SC u zapadnoj populaciji i TL-CU u istočnoj. Svrstavanje svake populacije određenoj rasi je urađeno, i na taj način smo mogli da prepoznamo koegzistenciju tri različite rase u oblasti koja je ispitana.

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