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TEMPORALCHANGES IN RELATIVE FREQUENCIES OF THIRD CHROMOSOME INVERSIONS OF *DROSOPHILA PSEUDOOBSCURA* IN MEXICAN POPULATIONS

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Variations in relative frequencies of third chromosome inversions during 1974-2000 in three populations of *D. pseudoobscura* from Mexico were analyzed. The general constitution of these populations is formed from ten up to 15 different inversions, been prevalent, as data shows, the

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gene arrangements CU, TL, SC, OA, EP, and OL as main components of the studied populations. In Amecameca and Tulancingo we observed that they were constituted by a dominant pair in both cases represented by such partners as CU-TL inside each pair, in Amecameca the superior partner was CU and in Tulancingo TL. In Zirahuén two dominant pairs form the constitution they are SC-OA and CU-TL been the former superior. In all cases sporadic increases of some of inversions were observed. No directional changes were observed. The cyclic pattern is show within periods of 4-5 years.

Key words: frequency changes, inversion polymorphism, *Drosophila*

INTRODUCTION

Natural populations of many species of *Drosophila* are polymorphic for inversions in their chromosomes. In *Drosophila pseudoobscura* this polymorphism is found mainly in the third chromosome with at least 40 different gene arrangements up to now described by DOBZHANSKY and EPLING, (1944), OLVERA *et al.* (1979), POWELL, (1992). With respect to its chromosomal constitution populations could be uniform, present only one inversion type, but more common they present a constitution consisting in up to 6-7 different inversions, also the number and kind of gene arrangements vary from population to population. The relative frequencies of inversions in the populations show geographical and temporal variation, in these sense cyclic seasonal changes have been well documented in *D. pseudoobscura* by example DOBZHANSKY (1956) in a population from California, in there changes in frequency of inversions ST, AR and CH are well described; the same happens with some southwestern USA populations studied by DOBZHANSKY, (1958) in all these cases changes refer to seasonal changes. Furthermore DOBZHANSKY, (1963), DOBZHANSKY *et al.,* (1964 and 1966) and ANDERSON *et al.,* (1991), have reported long term changes occurred in several localities along western USA populations of *D. pseudoobscura.*

In Mexico there are some reports dealing with geographical changes in relative frequencies of the inversions present in several populations of this species, GUZMAN *et al* (1993), OLVERA *et al.,* (2005), GUZMÁN *et al.,* (2005) and SALCEDA *et al.,* (2007a and 2007b), all of them referring to a qualitative changes due to geographical location of the populations. In this occasion we are interested in describe changes observed in three populations of *D. pseudoobscura* belonging to two different geographic zones along a period of 25 years with respect to their relative frequencies of prevailing inversions present in each locality.

MATERIAL AND METHOD

We choose for our study three locations from two different geographical zones, they were described by GUZMÁN *et al.,* (1975) and data there reported correspond to our baseline. Name of populations and their approximate situation are: Amecameca, State of Mexico, (19º 03´ N, 98º 45´ W); Tulancingo, State of Hidalgo, (20º 06´N, 98º 21´W); and Zirahuén, State of Michoacán, (19º 30´N, 101º 46´ W). Our collections started in 1974 and during the period 1974-1977 they were done every three months in a way as to have a collection in every season; later collections were spaced even by years, averaging 3-4 five years between collections and the last collection was performed in February 2000, their corresponding collection dates are shown in tables I-III. In total we did 27 collections at Amecameca, 26 at Zirahuén and 25 at Tulancingo. Care was taken as perform the sampling in the same place.

Flies were attracted to plastic buckets containing fermenting bananas and collected by sweeping with an entomological net, this was done at regular intervals of 10-15 from sunrise to 9.00 h in the mornings and from 17.00 h until dark in the evenings during five days of each collecting trip. Trapped flies were sorted, and those belonging to the *obscura* group 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 collecting trip was finished. At arrival to the laboratory each female was placed into separate one-half-pint bottle with fresh food and enriched with a heavy solution of live yeast and allowed to lay eggs for a week; when the amount of females was small we also used males, in such cases males were crossed individually with 2-3 females of known karyotype strain in this case either Estes Park (EP/EP) or Tree Line (TL/TL) 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 in this way assure large salivary glands and as a consequence large polytene chromosomes.

When mature third instar larvae crawled out of the food, a single larva from each culture was dissected, its salivary glands extracted, and a smear prepared using the regular aceto orceine staining technique. Each slide was examined through a compound microscope to determine their corresponding karyotype, this was done, when necessary, with the help of a photographic atlas and figures reported by DOBZHANSKY and EPLING, (1944), KASTRIRSIS and CRUMPACKER, (1966 and 1967) and OLVERA *et al.,* (1979). The data obtained were used to calculate inversions frequencies and elaboration of Tables 1-3.

Regular corn meal-agar-sugar-yeast food normally used in the laboratory was employed to rear flies and larvae. All the cultures were incubated at 25 $\pm$ 1º C and 65 % of relative humidity. This study is based on the analysis of 15118 third chromosomes.

RESULTS

Considering the three populations we observed 24 different inversions out of40 already described gene sequences, they represent 60 per cent of the variability of the species for this trait their names and symbols are: Cuernavaca (CU), Tree Line (TL), Santa Cruz (SC), Olympic (OL), Oaxaca (OA), Pikes Peak (PP), Estes Park (EP), Chiricahua (CH), Hidalgo (HI), Michoacán (MI), Tarasco (TA), Pátzcuaro (PA), Zirahuén (ZI), Ozumba (OZ), San Antonio (SA), Amecameca (AM), Uruapan (UP), Iztacíuatl (IZ), Tzinzunzan (TZ), Tulancingo (TU), Paxtepec (PA), Miraflores (MF) and Popocatepetl (PO). Not all such inversions are present on each locality neither they were found in every collection. As for their abundance, inversions could be catalogued as very common with more than 30 per cent of relative frequency, moderately common between 5-15 per cent and rare less than 5 per cent of frequency. For easiness in describing the behavior of populations we will do it by separate.

In Amecameca, during the whole period we found 12 different inversions, among them CU showed an average relative frequency of 52.2 % and reached six maximums with over 60 % they were observed with regularity every 4-5 years, complementary with this inversion and forming a dominant pair is inversion TL with an average frequency of 37.5 % and presented two maximums with more than 50 %, the general constitution of that population is completed with the participation of inversions EP with an average frequency of 4.3 %, the remaining 5-6 % is represented by nine inversions in different relative frequencies and not always found in the sample they are: OL, HI, SC, CH, OA, OZ, SA, AM and PO, in Table 1 we present their frequencies and the behavior of CU and TL in figure 1.

Tulancingo, our second locality, this population behaves similar to Amecameca in having a dominant pair of inversions accounting for about 90 %, they are also CU and TL but both populations differ from each other in their relative frequencies, in Tulancingo as dominant partner we found TL with an average frequency of 51.8 % and showing four maximums over 60.5 per cent in 1981, 1984, 1994 and 2000, its partner CU was present with an average frequency of 39.5%; complete the constitution of this population those rare inversions: EP, OL, SC, HI, PO, CH, SA, OA, PP and TA, relative frequencies scored are shown in Table 2 and the behavior of TL and CU in figure 2.

In Zirahuén, its constitution is quite different, in it we found 15 different inversions the larger number ever found in a population of this species, as in previous localities most of them rare and in a variety of frequencies. In this population six inversions account up to 98.5 of the total and they form two main pairs of partners, let see how they operate. Inversions OL and EP were less abundant, both were found in all collections and showed variable frequencies averaging 4.7 and 8.4 % respectively. The remaining four CU, TL, SC and OA were common with average frequencies of 18.9, 20.8, 30.7 and 15.2 % respectively.

Table 1. Number of chromosomes (n) and relative frequencies of different third chromosome inversions of Drosophila pseudoobscura found in Amecameca.

DATE	CU	TL	EP	others	n
August,1974	42.4	51.2	6.4	0	125
Sept., 1974	51.8	44.8	2.2	1.2	272
Dec., 1974	50	45.9	2.7	1.4	218
Feb., 1975	53.6	41.8	2.2	2.4	356
May, 1975	62.1	30.2	2.6	5.1	232
July, 1975	52.1	31.6	11.6	4.7	190
Sept., 1975	52.1	42.5	5.3	0.1	188
Dec., 1975	63.6	33.9	1.2	1.3	330
March, 1976	65.6	28.3	2.8	3.3	396
June, 1976	56.3	35.4	3.1	5.2	229
Sept., 1976	59.2	38.9	0.4	1.5	270
Nov., 1976	66.1	30.7	2.3	0.9	619
Jan., 1977	58.7	31.4	7.5	2.4	973
August, 1977	54.2	37.5	8.3	0	24
Jan., 1980	53.1	32.8	7.8	6.3	64
Jan., 1981	51.9	37.9	3.8	6.4	79
May, 1981	50	31.8	4.5	13.7	22
Jan., 1982	65.4	30.2	2.8	1.6	179
May, 1982	37.5	50	0	12.5	16
Jan-March,83	47.7	39.1	4.7	8.5	128
May, 1983	49.5	45.6	2.2	2.7	184
Jan., 1984	63.6	29.2	3.9	3.3	335
Nov., 1994	53.7	39.5	2.9	3.9	134
Jan., 1995	61.8	38.2	0	0	136
May, 1995	57.5	33.7	5	3.8	80
Feb., 2000	49.2	38.4	7.1	5.3	185

They are accommodated forming two pairs of partners one represented by TL and CU whose added frequencies are lower than the other pair SC and OA which seems to be a dominant pair; in both cases when one member of a pair increases its partner diminish and the contrary when the first decreases the later increases. This is an easy way to look their behavior since other possibilities exist, for example when the added frequencies of TL, CU and OA are taken as a unite against SC we observed that when SC increases that unite decreases and the contrary, in general we could take any pair and see a similar pattern of ups and downs as shown in figure 3, their respective frequencies are shown in Table 3.

Table 2. Number of chromosomes (n) and relative frequencies of different third chromosome inversions of Drosophila pseudoobscura found in Tulancingo.

DATE	CU	TL	others	n
August,1975	51.8	48.1	0.1	54
Nov., 1975	52.9	42.8	4.3	287
Feb., 1976	39.2	54.1	6.7	74
May, 1976	35.7	51.3	13	193
August, 1976	50.8	43.8	5.4	297
Nov., 1976	46.1	48.3	5.6	178
Feb., 1977	47.1	47.5	5.4	1805
June, 1977	50.2	45.1	4.7	253
August, 1977	32.2	54.2	13.6	59
April, 1979	34	54	12	100
Feb., 1980	35.3	52.6	12	173
Feb., 1981	34.6	61.4	4	202
May, 1981	28.6	62.5	8.9	56
Jan., 1982	45.3	45.3	9.4	150
July, 1982	45.4	45.4	9.2	22
March, 1983	39.5	49.1	11.4	114
May, 1983	57.3	34.8	7.9	204
Jan., 1984	19.9	67.8	12.3	518
Nov., 1994	25.6	67.2	7.2	156
Jan., 1995	23	63	14	200
May, 1995	34.7	54.7	10.6	75
Feb., 2000	23.1	67.2	9.7	238

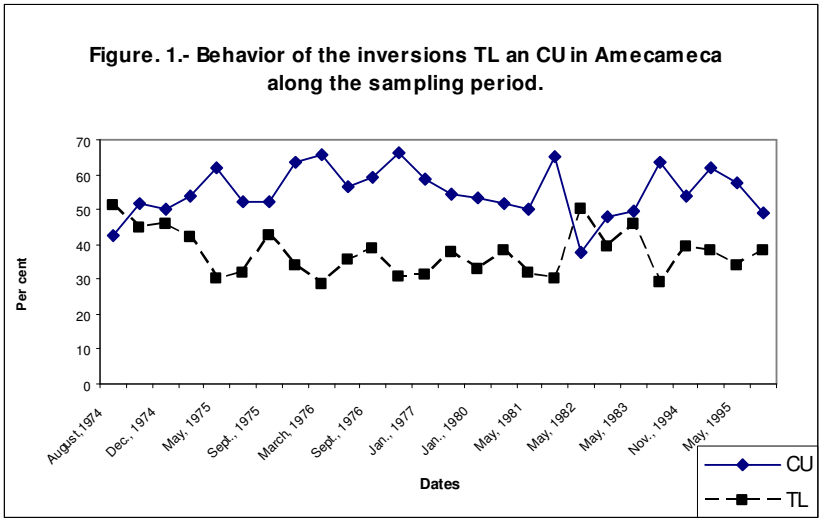
Table. 3- Number of chromosomes (n) and relative frequencies of different third chromosome inversions of Drosophila pseudoobscura found in Zirahuen.

DATE	CU	TL	SC	OA	OL	EP	n
Sept., 1974	12.5	0	31.2	43.7	6.2	6.2	16
Dec., 1974	26.3	13.1	11.1	23.2	4	18.2	99
Feb., 1975	24.9	23.8	26.9	7.6	5.6	8.6	197
April, 1975	21.1	20.5	29.2	11.8	3.7	10.5	161
June, 1975	17.2	22.5	17.9	24.5	6.6	5.9	151
Oct., 1975	26.9	25	25	13.7	1.9	6.9	160
Jan., 1976	24.7	19.9	41	7.2	2.9	2.6	307
April, 1976	21.2	25	37.4	6.5	4.5	4.1	444
Se-Nov., 1976	22.2	13.3	26.7	24.4	2.2	2.2	45
Jan., 1977	26.2	18.1	40.1	10.2	0.8	3.3	518
April, 1977	14.6	21	49.7	8.6	1.3	2.9	314
Sept., 1977	14.3	7.1	7.1	28.6	14.3	28.6	14
Jan., 1978	19.6	13	23.9	28.3	2.2	6.5	46
April, 1978	14.3	12.4	24.7	32.7	1.5	12.4	202
April, 1979	23.9	25	34.1	10.2	0	3.4	88
Jan., 1980	13.6	21.2	37.9	9.1	6.1	12.1	66
May, 1981	15.9	17	39.7	7.9	5.7	11.4	88
Feb., 1982	11.1	31.5	40.7	7.4	2.8	4.6	108
May, 1982	14.3	21.4	40.5	9.5	4.8	7.1	42
Jan., 1983	32.5	34.9	12.8	12.8	0	6.9	86
May, 1983	11.3	15.1	16.9	28.3	7.5	15.1	53
March, 1984	13.9	24.5	49.3	3.8	1.9	4.1	265
Nov., 1994	13.3	21.7	35	10	8.3	11.7	60
Jan., 1995	11.3	22.6	47.8	3.5	9.6	4.3	115
Feb., 2000	26.7	29.7	20.8	7.9	3.9	10.9	101

DISSCUSSION

Temporal studies of chromosomally polymorphic populations of *D. pseudoobscura* and other species seek to establish whether observed changes in frequency of the various inversions that form the genetic composition of such populations are changes of a long term directional nature or are transient variations in frequency caused by cycling factors or seasonal climatic ones. It is clear that polymorphisms of the three populations here described have been stable through a quarter century of our study, even do, some relevant changes deserve mention

In Amecameca, the dominant pair CU-TL remained almost constant been CU always superior to TL; also notorious is its increase in frequency of SC in four years, the same happened during two years with CH, in some of those cases, its cause could be due to a small sample size, also sporadic increases occurred with OA, PO and OL in one of those years with no further changes; the regular presence of EP with an average of 4.3 per cent complete its constitution.



Tulancingo population also presented a dominant pair formed by combination frequencies of CU and TL but in this case TL was the superior partner; of interest is the increase of OL starting in 1977showhing from then on an average frequency of 5.1 per cent and that of HI with an average frequency of 3.2 per cent as well sporadic rising of EP and SC, in general the frequency of this dominant pair remained constant.

In Zirahuén as was mention early, this population showed to be constituted by two dominant pairs, one of them represented by SC-OA that was always superior to the other one formed by combining inversions CU-TL, its constitution was completed with the regular presence of OL and EP that in some cases were relatively high. In general we could say that this population did not show long term variations of directional nature and that these observed changes were only ascribed to particular climatic changes occurring in those years.

Figure 2.- Behavior of inversions TL and CU in Tulancingo along the sampling period.

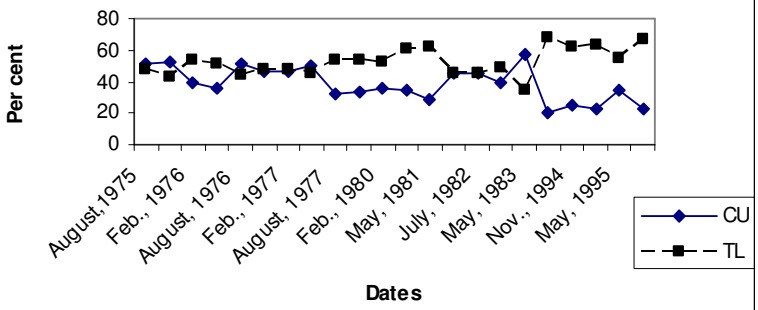
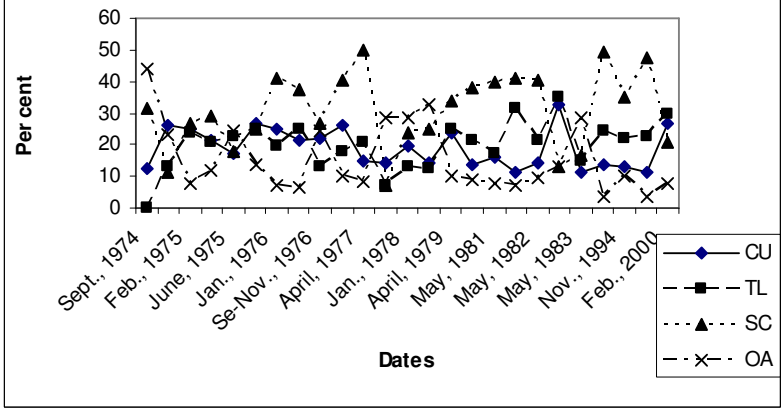


Figure 3.- Behavior of inversions TL, CU, SC and OA in Zirahuén along the sampling period.



According to the data obtained we could say that this populations did not show any trend of change as was observed for other authors for example those reported by DOBZHANSKY *et al* (1964 and 1966), and the one of ANDERSON *et al.* (1991) both in population of *D. pseudoobscura* from USA that assume changes due to pesticides; and neither to effects similar to those reported by SOLE *et al.* (2002) or BALANYA *et al.* (2004) in populations of the sibling species *D. subobscura* from Europe who ascribe changes in frequency of inversions in those populations to global warming and causing some clinal changes along a period of 20 years. Closer view of the data in tables gave information concerning cyclic changes along a single year mainly during years 1974-1977 in where samplings were done every season, there we could observe that the nature of a cyclic pattern is possible if we extrapolate them as seen in figures, remains constant and a regular periodicity of 4-5 years is noticed.

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PRIVREMENE PROMENE RELATIVNE UČESTALOSTI INVERZIJA TREĆEG HROMOZOMA KOD *DROSOPHILA PSEUDOOBSCURA* U POPULACIJAMA MEKSIKA

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I z v o d

U vremenu od 1974-2000. godine vršena su ispitivanja variranja relativne učestalosti inverzija trećeg hromozoma u tri populacije *D. pseudoobscura* u Meksiku. Dobijeni podaci pokazuju da je generalna konstitucija ovih populacija formirana od deset do petnaest različitih inverzija kao što su aranžiranje gena CU, TL, SC, OA, EP I OL kao glavnih komponenata ispitivanih populacija.

U populacijama Amecameca i TulacingoIn utvrdili smo da su konstituisane od dominantnog para u oba slučaja pretstavljena partnerima CU-TL unutar svakog para; kod Amecameca superiorni partner je bio CU a kod Tulancingo TL. Kod Zirahuen populacije dva dominantna para koji određuju konstituciju su SC-OA i prethodno su bila superiorna. U svim slučajevima su je utvrđeno sporadično povećanje broja nekih inverzija. Nisu nađene promene usmerenog tipa. Utvrđena je cikličnost pojava unutar perioda od 4 do pet godina.

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