

**A PROSPECTIVE STUDY OF INVERSION POLYMORPHISM IN  
NATURAL POPULATIONS OF TWO *DROSOPHILA* SPECIES FROM  
EASTERN MEXICO**

Victor M. SALCEDA

Departamento de Biología, Instituto Nacional de Investigaciones Nucleares,  
México

Salceda M.V. (2010): *A prospective study of inversion polymorphism in natural populations of two Drosophila species from Eastern Mexico*-Genetika, Vol 42, No. 3, 407 - 414.

Relative frequencies for heterozygous inversions in nine populations of *D. nebulosa* and six of *D. willistoni* were analyzed. The analysis corresponds to a grand total of 1828 arm chromosomes in which their genotype were determined, of them 404 correspond for each one of the two polymorphic chromosomes, X and III, of *D. nebulosa* and 204 per chromosome arm in *D. willistoni*. The more abundant inversions, according to their relative frequencies in *D. nebulosa* were the XL inversion with 7.9

---

*Corresponding author:* Victor M. Salceda, Departamento de Biología, Instituto Nacional de Investigaciones Nucleares. Carretera México/Toluca S/N, La Marquesa, Ocoyoacac. México, C.P. 52750. Mexico. [victor.salceda@inin.gob.mx](mailto:victor.salceda@inin.gob.mx).

% and inversion "A" in the third chromosome with 15.6 %, the remaining inversion found did not reach the ten percent. In the case of *D. willistoni* the more frequent inversions were for the XL arm inversions "A" and "D" with 13.7 and 12.2 percent respectively the remaining five did not reach a ten percent; in the XR arm the higher frequencies were for inversions "D" and "E" with 9.3 % each been the other four inversions with values less than five percent; in the IIL arm none of the seven inversions registered had values higher than six percent; inversion "E" in the IIR arm showed a frequency of 24.5 % and the five remaining barely reached a frequency of 5 %; among the 12 different inversions recorded in the third chromosome, only inversion "B" surpassed the ten percent in this case with 16.1 %. We must mention that the Standar sequence in all cases, for both species, always had high frequencies as shown in Tables II and III. The corresponding variability for this characteristic is for *D. nebulosa* 61.4 % and 72.2% for *D. willistoni*. Concerning other parameter observed we have the average number of inversions per female in our case for *D. nebulosa* was 1.4 and for *D. willistoni* 3.6 which indicate the both species are marginal with respect to the distribution of this character in both species since in the central areas of distribution reach values up to nine inversions per female.

*Key words:* *Drosophila*, inversion polymorphism, natural populations

#### INTRODUCTION

Natural occurring chromosomal inversions are widespread in *Drosophila* and more than half of those species examined in any detail are naturally polymorphic for them in one or more chromosome arms. In the Americas the species *D. pseudoobscura*, *D. persimilis*, *D. robusta*, *D. willistoni*, *D. nebulosa* and *D. paulistorum*, among others have been intensively studied at this respect, all of them have been found to carry a large amount of inversion polymorphism in their genome (KRIMBAS and POWELL, 2000). Our interest in this occasion is centered in the chromosomal polymorphism of *D. willistoni* and *D. nebulosa* in some natural populations from Eastern Mexico. Both species are originous from South America and those populations from Mexico are considered as marginal. The first species, *D. willistoni* is a tropical one, quite common in South America, its geographical distribution extends from Buenos Aires, Argentina up to Central Mexico and Florida (TOWNSEND, 1958). According to VALENTE and MORALES (1985) this species is considered the most polymorphic for inversions among all the drosophilids studied with up to 70 different inversions described all of them along the three main chromosomes. Its fundamental chromosome map was described by DOBZHANSKY (1950); later DA CUNHA *et al.* (1950), DA CUNHA and DOBZHANSKY (1954), DA CUNHA *et al.* (1959), DOBZHANSKY (1957) and AYALA *et al.* (1971) reported relative frequencies of its inversions in numerous populations from South America, in the same way TOWNSEND (1952, 1958) reported the inversion polymorphism in

populations from Cuba, Florida, Hispanola, Puert Rico and other Caribbean Islands. More recently VALENTE *et al.* (2001, 2003) did the same in some populations from Uruguay, and in Mexico, SALCEDA (2006) studied a single locality from Veracruz along eight years analysis. Meanwhile, *D. nebulosa* has also a broad geographical distribution extending from Buenos Aires, Argentina in the South up to Central Mexico and Texas PATTERSON and WAGNER, (1943). According to PATTERSON and MAINLAND (1944) this species is the most abundant member of the *willistoni* group found in Mexico, occupying the Eastern part of the country. This species has been well analyzed with respect to its inversion polymorphism by PAVAN (1946), DA CUNHA *et al.* (1953), BURLA *et al.* (1949), BONORINO *et al.* (1989 and 1993) and the only information available from Mexico is the one of SALCEDA (2005). Its chromosome map was described by PAVAN (1946) reporting the presence of polymorphism in two of the chromosome arms of this species, namely the left arm of the sex chromosome (XL) and the third chromosome (III) reaching a total of 18 different inversions.

#### MATERIALS AND METHODS

In order to carry a long term project on the inversion polymorphism for the sibling species *D. willistoni* and *D. nebulosa* a prospective collecting trip was realized in the eastern part of Mexico covering the states of Veracruz and Tabasco and including two more localities, one in Central Mexico in the state of Tlaxcala and other in the western state of Nayarit, in such a way the following localities were sampled: Poza Rica, Arroyo Agrio, Tinajas and Cosamaloapan in Veracruz, Teapa, Lázaro Cárdenas, Macuspana and Kolemjaá in Tabasco; La Malinche in Tlaxcala and Tepic in Nayarit. The amount of flies of each species as well the number of chromosomes analyzed is shown in Table I.

Table 1. Number of females and number of chromosome arms analyzed in several populations of *Drosophila willistoni* and *D. nebulosa*.

Locality	<i>D. nebulosa</i>		<i>D. willistoni</i>	
	# females	# chrom	# females	# chrom.
Poza Rica	6	12	5	10
Arroyo Agrio	86	172	57	114
Tinajas	11	22	---	---
Cosamaloapan	30	60	---	---
Teapa	---	---	16	32
Comalcalco	---	---	19	38
L.ázaro Cárdenas	29	58	---	---
Macuspana	30	60	---	---
Kolemjaá	3	6	4	8
Tlaxcala	2	4	---	---
Tepic	5	10	1	2
Total	202	808*	102	1020*

\*these totals correspond to number of chromosome per female multiplied by number of chromosome arms.

Once in the corresponding locality the flies were attracted to 15-20 plastic buckets containing fermenting bananas and collected by sweeping the traps with an entomological net at regular intervals of 10-15 minutes. The trapped flies were sorted and those females of the species of interest, in groups of 15-20 individuals, were put into vials containing fresh medium and kept there until the arrival at the laboratory in Mexico City. Once there, each female was placed individually into a separate one-half-pint bottle, with fresh food enriched with drops of a heavy solution of live yeast, and allowed to lay eggs and incubated for a week and then transferred to a new bottle as reserve. When mature, third instar larvae, in the first culture, crawled out of the food, a single larva from each culture were dissected, its salivary glands extracted and a smear prepared using the regular lacto-aceto-orcein staining technique. Each slide was examined through a compound microscope to determine the chromosomal arrangements carried by it. The data such way obtained were used to calculate inversion frequencies and the average number of inversion per female. Since in most of the cases the amount of flies, as a consequence the number of chromosomes analyzed were small, we decided to plot all the data globally and in such way have a single set of information. All the cultures were incubated at  $25 \pm 1^\circ \text{C}$  and 65 % relative humidity; the medium used was the regular corn meal-sugar-yeast employed at the laboratory.

## RESULTS

Tables 1-3 summarize the number of females analyzed as well the relative frequencies, in percentage of the different heterozygous inversions corresponding to each of the 5 chromosome arms of *D. willistoni*, XL, XR, IIL, IIR and III, and the two chromosome arms of *D. nebulosa*, XL and III. From the different localities sampled. In this survey we were able to analyze the chromosomes of 102 *D. willistoni* females and 202 of *D. nebulosa* accounting for a total of 204 and 404 chromosomes respectively, equivalent to a total of 608 chromosomes.

The analysis allowed us to determine the presence of 43 different inversions in *D. willistoni* that represent a 61.4 % of the variability for this characteristic in the species. In the case of *D. nebulosa* 13 different inversions were found and its corresponding percentage of variability is 72.2 %; in both cases the number of inversion includes the standard arrangement.

Other parameter observed was the average number of inversions carried by a single female, in our observations, for *D. willistoni* we found 3.6 inversions per female and the value for *D. nebulosa* was 1.4, in both cases the value is quite inferior to those found in central populations as reported by different authors.

Table 2. Relative frequencies for each one of the chromosomes (XR, XL, IIR, II L and III) of *D. willistoni*. Global data for six localities.

XL												
A	C	D	E	F	G	H	ST*					
13.7	0.9	12.2	0.9	10.3	8.3	0.4	53.3					
XR												
A	B	C	D	E	F	ST*						
0.9	0.4	2.9	9.3	9.3	0.4	76.8						
IIR												
B	C	D	E	G	H	ST*						
3.9	6.8	6.3	24.5	0.4	0.4	57.7						
II L												
A	B	C	D	E	F	G	ST*					
2.9	1.4	3.9	0.4	1.4	4.4	5.4	80.2					
III												
A	B	C	D	E	F	H	I	J	K	L	M	ST*
4.9	16.1	2.4	1.4	0.4	4.9	0.9	1.9	5.3	2.4	3.4	2.9	53.1

\*ST, means Standard sequence, and all other inversions are combined with it.

Table 3. Relative frequencies for each one of the chromosomes (X and III) of *D. nebulosa*. Global data for nine localities.

X		XL										
92.1	7.9											
III												
A	B	C	D	E	F	G	H	H/h	E/G	ST*		
15.6	6.2	5.2	9.6	5.9	1.2	9.4	0.7	1.9	6.4	37.9		

\*ST, means Standard sequence, and all other inversions are combined with it.

## DISCUSSION

The populations of the two species we are now analyzing are characterized by present inversion polymorphism that could be denominated as high since in *D. nebulosa* we found 13, including the Standard arrangement different inversions out of 18 reported showing a 72.2 % of that for the species *D. nebulosa* and for *D. willistoni* 43 out of 70 inversions, also including the Standard gene sequence, in this case its value is 61.4 %. In both species the inversions are paracentric and in general small and independent, these characteristics allow us that occasionally in each chromosome some times we could observe two or more inversions simultaneously, situation that during the determination of the genotype it becomes heterozygous and hard to determine each inversion and obtain homozygous stocks, for instance in the central area of distribution it is possible to find up to nine different inversions per individual female and also make difficult to build phylogenetic trees as it was done in *D. pseudoobscura* (OLVERA *et al.*, 1979). Even both species in Mexico have a high variability for their polymorphism in all the populations studied and due to the small population size the relative frequencies found are low. Taken it in account we

arranged our data as shown in Tables II and III in a global form but not showing in tables we could mention the following information. Among all populations sampled, only three localities of each species contributed with a sample size appropriate to give reliable relative frequencies, they are for *D. willistoni* Arroyo Agrio, Comalcalco and Teapa and for *D. nebulosa* those of Comalcalco, Lázaro Cárdenas and Macuspana that yield for analysis respectively 57, 19, 16, 30, 29 and 30 females, enough to provide some interpopulation comparisons.

In them the more frequent inversions, based on their relative frequencies and considering only those that exceeded a ten percent frequency are: for *D. willistoni*, in chromosome arm XL, inversion "F" Arroyo Agrio with 14 %, in Comalcalco inversion "A" with 16 % and in Teapa also inversion "A" with 12.5% frequency. For XR arm chromosome, inversion "E" in Arroyo Agrio with 14% and inversion "D" in Teapa with 22 % were the more abundant. In IIL chromosome arm none of the populations reached values higher the ten percent. In chromosome arm IIR inversion "E" was the most frequent with values of 20, 29 and 28 percent respectively for locations Arroyo Agrio, Comalcalco and Teapa. Finally chromosome III showed that inversion "B" as the more frequent in localities Arroyo Agrio and Comalcalco with values of 21 % in both places.

In the case of *D. nebulosa* inversion XL had considerable contribution only in Cosamaloapan with 13.3 % frequency and in Macuspana with 10%. For chromosome III those inversions with higher values were in Cosamaloapan inversions "A", "C", "D" and "G" with values of 20, 18.3, 11.7 and 15 percent respectively. In Lázaro Cárdenas inversions "A", "B", "D" and "G" with percentages of 27.6, 13.8, 10.3 and 13.8 respectively were the more abundant. In Macuspana the values found were inversion "D" with 16.7 %, inversion "C" with 10%, inversion "D" with 18.3%, inversion "E" with 11.7 % and inversion "G" with 13.3% as the more abundant.

As noted above, even with a lack of a good sample size, we considered that the present information would serve as a base line for further studies in Mexico. Also with this information we suggest that studies of inversion polymorphism are quite promisorious for a better understanding of those populations of both species in the northern hemisphere and complete our recommendation to sample as many as possible populations.

#### ACKNOWLEDGMENT

The authors gratefully acknowledge the continuous support of the authorities of ININ during the course of this study. Particular thanks are given to CONACyT for partial support for this study by Contract 31736-N to VMS. Thanks are also given to my colleagues Citlali Guerrero, Carolina Arceo and Josefina González for their encouragement and friendship.

Received March 2<sup>nd</sup>, 2010

Accepted December 6<sup>th</sup>, 2010

## REFERENCES

- AYALA, J.F., J.R. POWELL and TH. DOBZHANSKY.(1971): Polymorphism in continental and island populations of *Drosophila willistoni*. Proc. Nat. Acad. Sci. USA. 68, 2480-2383.
- BONORINO, C.B.C. and V.L.S. VALENTE (1989): Studies on wild and urban populations and breeding sites of *Drosophila nebulosa*. Rev. Bras. Bio.49, 771-776.
- BONORINO, C.B.C., V.L.S. VALENTE and S.M. CALLEGARY-JAQUES (1993): Urbanization and chromosomal polymorphism of *Drosophila nebulosa*. Rev. Bras. Genet. 16, 59-70.
- BURLA, H., A.B. DA CUNHA, A.R. CORDEIRO, TH. DOBZHANSKY, C. MALOGLOWKIN and C. PAVAN (1949): The *willistoni* group of sibling species of *Drosophila*. Evolution 3, 300-314.
- DA CUNHA, A.B., D. BRNCIC and F.M. SALZANO (1953): A comparative study of chromosomal polymorphism in certain South American species of *Drosophila*. Heredity 7, 193-202
- DA CUNHA, A.B., H. BURLA and TH. DOBZHANSKY (1950): Adaptive chromosomal polymorphism in *Drosophila willistoni*. Evolution 4, 212-235.
- DA CUNHA, A.B. and TH. DOBZHANSKY. (1954): A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. Evolution 8, 119-134.
- DA CUNHA, A.B., TH. DOBZHANSKY, O. PAVLOVSKY and B. SPASSKY (1959): Genetics of natural populations. XXVIII. Supplementary data on the chromosomal polymorphism in *Drosophila willistoni* in its relation to the environmen. Evolution 13, 389-404.
- DOBZHANSKY, TH. (1950): The chromosomes of *Drosophila willistoni*. J. Heredity 41, 156-158.
- DOBZHANSKY, TH. (1957): Genetics of natural populations. XXVI. Chromosomal variability in island and continental populations of *Drosophila willistoni* from Central America and the West Indies. Evolution 11, 279-283.
- KRIMBAS, C.B. and J.R. POWELL (2000): Inversion polymorphism in *Drosophila*. In R.S. SINGH and C.B. KRIMBAS (Eds.) Evolutionary genetics: from molecules to morphology. Vol. 1, 284-299. Cambridge University Press. Cambridge, United Kingdom.
- OLVERA, O., J.R. POWELL, M.E. DE LA ROSA, V.M. SALCEDA, M.I. GASO, J. GUZMÁN, W.W. ANDERSON and L. LEVINE (1979): Population genetics of Mexican *Drosophila*. VI. Cytogenetic aspects of the inversion polymorphism in *Drosophila pseudoobscura*. Evolution 33 (1), 381-395.
- PATTERSON, J.T. and R.P. WAGNER (1943): Geographical distribution of species of the genus *Drosophila* in the United States and Mexico. Univ. Texas Publ. 4313, 217-282.
- PATTERSON, J.T. and G.B. MAINLAND (1944): The *Drosophilidae* of Mexico. Univ. Texas Publ. 4445.
- PAVAN, C. (1946): Chromosomal variation in *Drosophila nebulosa*. Genetics 31, 546-557.
- SALCEDA, V.M. (2005): Inversion polymorphism in a natural population of *Drosophila nebulosa*. A ten years record. Rev. Int. Contam. Ambient. 21 (Supl. 1), 31-34.
- SALCEDA, V.M. (2006): Chromosomal polymorphism in a natural population of *Drosophila willistoni* (Diptera: Drosophilidae) from Laguna Verde, Veracruz, Mexico. An eight year record. Southwester Naturalist 51(3), 320-325.
- TOWNSEND, J.I. (1952): Genetics of marginal populations of *Drosophila willistoni*. Evolution 6, 428-442
- TOWNSEND, J.I. (1958): Chromosomal polymorphism in Caribbean Island populations of *Drosophila willistoni*. Proc. Nat. Acad. Sci. USA. 44, 38-42.
- VALENTE, V.L.S. and N.B. MORALES (1985): New inversions and qualitative description of inversion heterozygotes in natural populations of *Drosophila willistoni* inhabiting two different regions in the State of Rio Grande do Sul, Brasil. Rev. Bras. Genet. 8(1), 167-173.

VALENTE, V.L.S., C. ROHDE, V.H. VALIATI, N.B. MORALES and B. GOŃI (2001): Chromosome inversions occurring in Uruguayan populations of *Drosophila willistoni*. Dros. Inf. Serv. 84, 55-59.

VALENTE V.L.S., B. GOŃI, V.H. VALIATI, C. ROHDE and N.B. MORALES (2003): Chromosomal polymorphism in *Drosophila willistoni* populations from Uruguay. Genet. Mol. Biol. 26, 163-173.

### ISPITIVANJA POLIMORFIZMA INVERZIJA U PRIRODNIM POPULACIJAMA DVE VRSTE VINSKE MUŠICE (*Drosophyla*) IZ ISTOČNOG MEKSIKA

Victor M. SALCEDA

Odeljenje za Biologiju, Nacionalni istraživački Institut, Meksiko

#### I z v o d

Vršena su ispitivanja relativne učestalosti heterozigotnih inverzija u devet populacija *D. nebulosa* i šest populacija *D. willistoni*. Analiza odgovara ukupnom broju 1828 krakova hromozoma čiji genotip je određivan, od kojih 404 odgovara svakom od jednog ili dva polimorfna hromozoma., X i III kod *D. nebulosa* odnosno 204 po kraku hromozoma kod *D. willistoni*. Najbrojnije inverzije, prema njihovoj učestalosti kod *D. nebulosa* su tipa XL sa 7,9 procenata i inverzije tipa "A" na trećem hromozomu sa 15,6procenata. Preostale nađene inverzije nisu dostizale deset procenata. U slučaju *D. willistoni* najučestalije utvrđene inverzije su za krak XL i inverzije "A" i "D" sa 13, 7 odnosno 12, 2 procenta. Preostalih pet nije dostiglo deset procenata; na XR kraku najveća učestalost je utvrđena za inverzije tipa "D" i "E" sa 9,3 procenta. Preostale četiri inverzije su sa učestalošću nižom od pet procenata. Na kraku IIL ni jedan tip nađenih inverzija nije imao učestalost višu od šest procenata, na kraku IIL su imale učestalost od 24 procenta a preostalih pet su sporadično dostizale učestalost od 5 procenata inverzije tipa "E"; među 12 različitih utvrđenih tipova inverzija na trećem hromozomu samo je tip "B" imao učestalost od 16,1 procenat. Potrebno je naglasiti da Standard sekvenca u svim slučajevima, u obe ispitivane vrste uvek ima visoku učestalost kako je prikazano u tabelama II i III. Odgovarajuća učestalost ovih karakteristika je 61, 4 procenta kod *D. nebulosa* i 72,2 procenta za *D. willistoni*. Kada se radi o drugim utvrđenim parametrima prosečan broj inverzija za ženke *D. nebulosa* je 1,4 a kod *D. willistoni* 3,6 što ukazuje da su ispitivane populacije marginalne u odnosu na distribuciju tih osobina ispitivanih populacija dok u centralnim oblastima distribucije dostižu vrednosti do devet inverzija po jedinki ženke.

Primljeno 2. III. 2010.

Odobreno 06. XII. 2010.