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*Original scientific paper*

# RELATIVE FREQUENCIES OF THE "SEX-RATIO" INVERSION IN NATURAL POPULATIONS OF *Drosophila pseudoobscura* FROM MEXICO

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Sex-ratio (proportion of males) in a species is related to a not entirely explained mechanism of sex determination. In *Drosophila*, sex is determined by the proportion of X chromosomes vs. autosomes. A decrease in the proportion of males vs. females, known as sex-ratio, is characteristic to several *Drosophila* species, and is related to an inversion in the sexual chromosome the so called "sex-ratio" (SR) condition. In this occasion we study the presence of that inversion in several populations of *Drosophila pseudoobscura* from Mexico. With this purpose we did collections of this species on Nevado de Colima,Col., Valparaíso, Zac, Zirahuén, Mich., Tulancingo, Hgo. and Amecameca, Mex. Flies were captured in nature and carried to the laboratory were individual cultures of each female were established, when the offsprig emerged salivary glands of

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a single larva from each culture were extracted and stained with an aceto-orcein solution and the corresponding genotype for the third and sex chromosome determined and their relative frequencies calculated. The corresponding frequencies of the "sex-ratio" inversion in the localities analyzed were: Nevado de Colima 21.8 %, Valparaiso 18.8 %, Zirahuén 11.4 %, Tulancingo 6.8 % and Amecameca 6.8 %. Apparently an East-West cline distribution is present in these populations. Relative frequencies for inversions in the third chomosome were recorded and relationships between third and X chromosomes inversions performed. Comparisons with similar studies on this and other species are pointed out.

*Key words*: *D.pseudoobscura*, natural population, sex ratio

## INTRODUCTION

DARWIN (1859) mentions that selection tends to equalize the numerical proportion of sexes, and then this tendency produces an equal number of individuals of each sex, which is advantageous for the species, this condition leads to the concept of balance of FISHER (1930) which brings into play the adaptive value afforded by this parameter. Later on, DARWIN (1871) in his book "The descent of man ..." devotes a good part of a chapter to the problem of the numerical proportion between the sexes, which indicates the importance that represents the preponderance of either sex, among other observations mentioned that the dominance of one sex favors polygamy in the opposite sex and particularly notes the importance of this ratio in relation to natural selection in terms of sex and of course, the advantage to the species.

The sex ratio in the species is related to a mechanism not yet fully explained of sex determination. Most of the species, regardless of sex determination system they present, show an equal proportion of individuals for both sexes. Thus, when sex is determined by the sexual chromosome, then the proportion of males is controlled by chromosome segregation by the meiosis mechanism in the heterogametic sex, usually males. In diploid species the sex ratio is mainly determined by the gene balance in various combinations of sex chromosomes.

In *Drosophila,* sex is determined by the ratio of sex chromosomes versus the autosomes (STURTEVANT, 1949), however are known mechanisms by which the numerical proportions between males and females are affected.

Thus, a mechanism that causes distortions in the sex ratio in *Drosophila* is the so called meiotic drift first reported by NOVITSKI (1947) in *D. affinis*, and this phenomenon has continued to be studied in other species, in a recent report appointment of CAVASINI *et al*. (2008) that includes a short list of authors.

A second type of studies related to sex ratio distortion in *Drosophila* and in particular *D. melanogaster* is that initiated by KERR and KERR (1952) and continued by DRESCHER (1964) and called by them sex limited effect.

The sex ratio is also related to the presence of a complex of three non-overlapping paracentric inversions in the right arm of the sexual chromosome (X)

which causes a decrease and even absence of males in the offspring of female carriers of this inversion, it was originally detected by MORGAN *et al*. (1925).

These three types of mechanisms that alter the sex ratio are important genetic issues, but on this occasion we will not discuss all of them with the intensity of other studies instead we will only talk about another case of deviations that happen in several species of this genus, as is the case of *Drosophila pseudoobscura*, *D. persimilis* and *D. subobscura* and others that are affected by the presence to a related inversion the so called "sex-ratio" or simply "SR", present in the right arm of chromosome X. This is an issue extensively studied in natural populations of those species mentioned above, so for example we have the works of STURTEVANT and DOBZHANSKY (1936), DOBZHANSKY and EPLING (1944), BRYANT *et al.* (1982), MILOSEVIC-BROCKETT and ANDERSON (2009) in D. *pseudoobscura* and D. *persimilis* in populations inhabiting U.S. and Mexico, similar studies have been reported for *D. subobscura* by KRIMBAS (1992), TERZIC *et al*. (1997), PASCUAL *et al*. (2004) among others in Europe. In Mexico, is only known the data of DOBZHANSKY and EPLING (1944) for some localities at the north of the country and with the present study we hope to contribute to a better understanding of the phenomenon and chromosome structure of the populations that live there.

Furthermore, studies on relationships between inversions in the third chromosome with the "SR" of the right arm of the sex chromosome were performed in *D. pseudoobscura* by STURTEVANT and DOBZHANSKY (1936), DOBZHANSKY and EPLING (1944) and ANDERSON, KASTRITSIS and DOBZHANSKY (1967), with this information we decided to conduct a similar analysis with those populations that we studied, that is the reason to include the relationship between the chromosomal arrangements of the third chromosome and simultanelly the "SR" inversion.

## MATERIALS AND METHODS

We selected five sites located along the Volcanic Axis where individuals of the species *D. pseudoobscura* were collected, they were caught using plastic traps containing 20-25 fruit in fermentation, the traps were placed every 5-6 meters so as to cover as much area as possible; once the flies began to visit the traps we started collecting them in rounds every 15-20 minutes during the time of collection, usually 6 to 10 pm and from 17 am to dusk, and with the help of an entomological net flies were caught and sexed on the site, females were placed in groups of 20 - 30 individuals into homeopatic tubes with fresh food to be transported to the laboratory in Mexico City.

Once in the laboratory, each female was placed individually in a one quater milk flask with fresh food and in this way allow them to lay eggs, and then incubated for 8 days, after this time from each culture we extracted a single larva that was dissected for the extraction of its salivary glands and stained with a 2% lacto-aceto-orcein solution, commonly used in similar studies, to observe the polytene chromosomes and thus determine its genotype; once known the genotypes from the larval offspring of the females captured in nature, this information allowed us to determine the frequencies of the inversion of which we are now concerned;

simultaneously we also determined the relative frequencies of the inversions of the third chromosome present in the populations here analyzed. The food used was that of common use in the laboratory made of agar, cornmeal, sugar, yeast with the addition of propionic acid and tegosept as a preservative and fungicide treatments, all the cultures were maintained at a temperature of 25 $\pm$ 1º C and 65 % of relative humidity. Once all the cytological analysis were performed, the data were put together and then frequencies calculated and the respective tables prepared.

## RESULTS AND DISCUSSION

A total of 397 females were analyzed as above and the corresponding frequencies calculated from the corresponding sample size, which varied in each locality sampled, including genotypic associations between two chromosomes, these data are presented in Tables 1, 2 and 3.

As noted in the previous section, we also determined the relative frequencies for the third chromosome to be used to see if there is a relationship between the inversions in this chromosome with those of the inversion "SR."

With regard to this second set of frequencies, the same females were analyzed and thus enable us to find an association that may exist between the two pairs of chromosomes. To count the frequencies are considered two chromosomes III per female and only one for the inversion “SR”.

With regard to inversions of third chromosome Valparaiso population is the most polymorphic with 10 different inversions, followed by the localities Nevado de Colima and Zirahuén which had eight different inversions each and finally Tulancingo and Amecameca sites with only five different inversions each one. Their relative frequencies are presented in Table 2, which do not display all frequencies since those inversions with values lower than 3% were added and named as "OTHERS". With the exception of Tulancingo and Amecameca populations in the remaining three, the most frequent chromosomal arrangements were SC (Santa Cruz) and TL (Tree Line), in the case of Tulancingo and Amecameca SC was replaced by CU (Cuernavaca), the designations of other inversions in Table II are: OL (Olympic), OA (Oaxaca), AR (Arrow Head), CH (Chiricahua) and EP (Estes Park), conventionally used in similar studies.

*Table 1. - Frequencies of the inversion "sex-ratio" (SR) in five natural populations of Drosophila pseudoobscura from Mexico*

| | SR Freq. (%) | Num. females |
|---|---|---|
| Nevado de Colima | 21.8 | 101 |
| Valparaíso, Zac. | 18.8 | 149 |
| Zirahuén, Mich. | 11.4 | 44 |
| Tulancingo, Hgo. | 6.9 | 29 |
| Amecameca, Mex. | 6.8 | 103 |

In relation to inversion "SR", we must note first that it is only detected in the female in heterozygous condition, for this reason to analyze it is necessary to take it into account only once for female, for that it produces a decrease in the sample size to a half when compared with the chromosome III that is represented two times in a single female as shown in Table I; the highest frequency of this inversion occurred in Nevado de Colima population with 21.8%, followed by Valparaíso with 18.8%, with 11.4% Zirahuén, finally Tulancingo and Amecameca with a 6.8% each. Also in Amecameca was possible to analyze 33 males that were crossed with females from the laboratory strain TL / TL, this led us in the offspring to sample two individuals of genotype TL / CU and SR and the remaining 31 as TL/TL and SR. In the other locations was not possible to analyze males. Table 3 shows different chromosome III genotypes carrying the SR inversion and their respective frequencies.

*Table 2- Relative frequencies of the inversions in chromosome III present in five Mexican populations of Drosophila pseudoobscura.*

| | SC | TL | CU | OL | OA | AR | CH | EP | others |
|---|---|---|---|---|---|---|---|---|---|
| N. C | 65.3 | 20.3 | 4.9 | 5.9 | 1.5 | --- | --- | --- | 1.5 |
| Val. | 43.3 | 33.9 | --- | --- | 2.0 | 7.7 | 5.4 | 2.7 | 5.0 |
| Zir. | 34.1 | 25.0 | 22.7 | --- | 11.4 | --- | --- | --- | 6.4 |
| Tul. | --- | 62.1 | 29.3 | 5.2 | --- | --- | --- | --- | 3.4 |
| Ame. | --- | 31.1 | 66.0 | --- | --- | --- | --- | --- | 3.0 |

Sample size is the same as in Table 1.

*Table 3. Frequencies of different genotypes for the thrird chromosome carriers of inversion "SR" in five Mexican natural populations of Drosophila pseudoobscura.*

| Nev. Colima | Valparaíso | Zirahuén | Tulancingo | Amecameca |
|---|---|---|---|---|
| SC/SC = 10 | SC/SC = 8 | SC/TL = 3 | TL/CU = 2 | TL/CU = 5 |
| SC/TL = 4 | SC/AR = 6 | SC/CU = 1 | | CU/CU = 2 |
| SC/CU = 2 | SC/TL = 3 | CU/OA = 1 | | |
| SC/OL = 2 | SC/CU = 1 | | | |
| SC/HI = 1 | TL/TL = 3 | | | |
| TL/TL =1 | TL/EP = 3 | | | |
| OL/ OA = 1 | TL/OA = 2 | | | |
| OL/HI = 1 | TL/CH = 1 | | | |
| | CH/CH = 1 | | | |
| n = 22 | n = 28 | n = 5 | n = 2 | n = 7 |

## DISCUSSION

The characteristic "sex ratio", which manifests itself as a distortion of sex ratio, and in *Drosophila* occurs in three forms, the first due to the action of a single gene as in *D. melanogaster*, the second caused by a microbial infection as manifested in *D. willistoni* and the third due to the presence of an inversion on the X chromosome which has been shown to exist in several species of the *obscura* group as are the species *D. azteca, D. affinis, D. obscura, D. persimilis* and *D. pseudoobscura* as have been reported by STURTEVANT and DOBZHANSKY (1936), DOBZHANSKY and EPLING (1944), KRIMBAS (1992) and POWELL (1997) among others. Few studies are known about the geographical distribution of inversion SR in the chromosome X of *D. psudoobscura* and other species of the group, the most completely is that done by DOBZHANSKY and EPLING (1944).

On this occasion we refer to the presence of this inversion in five natural populations of *D. pseudoobscura* from Mexico. This condition has been found in different parts of their range, and their frequencies in them vary according to the aforementioned authors, and has not been reported in Canada and Northwestern United States or Colombia, but starting from its presence in California it increases going south, reaching at the U.S. border with Mexico values around 20%, and in Mexican territory has values reaching up to 30% in the states of Nuevo León and Sonora, but nevertheless in some populations of Chihuahua is rare with a frequency as low as 6%, further on as it moves south presents intermediate values in the central regions of Mexico with values ranging between 7 and 19% and reaches its lowest level in Guatemala with 1.2%, this data clearly shows a gradient North-South starting at a low frequency in California from where it increases up to the middle part of their distribution in the United States until the border with Mexico reaching in Sonoran populations its highest values around 30 percent and begin to decrease reaching finally its minimum in Oaxaca, in southern Mexico, and Guatemala with values of 1.5 %.

In this study, the localities are situated in the Central part of Mexico within the area corresponding to the neovolcanic axis and covers a distance between the end points of about 400 km, along this transect and from the western extreme we have the Nevado Colima that shows a frequency of 21.8% and gradually decreases to in the highlands to Amecameca where the frequency is 6.8%, showing clearly the presence of an East-West gradient, so this feature has two types of gradients: North-South and East-West. This situation does not occur in the polymorphism for inversions in the third chromosome, which has shown only an East-West gradient. This random distribution may be due to the great diversity of habitats that cause a selective pressure on the carrier populations of such inversions.

Simultaneous analysis was performed for the inversions in the third chromosome using the same females, and their frequencies, which are shown in Table II, reflect the genetic structure for this feature in the populations under study. Previous studies on the possible relationship between chromosome polymorphism in the third chromosome and inversion "SR" in *D. pseudoobscura* (STURTEVANT and DOBZHANSKY1 (1936), DOBZHANSKY and EPLING (1944), and ANDERSON, KASTRITSIS

and DOBZHANSKY, (1967) indicate that there is no evidence to link the two polymorphisms.

In our case, possible associations were detected as well, and considering that the different genotypes for the third chromosome, with some exceptions, were heterozygous, genotype combinations for the third chromosome and the inversion "SR" that we found are mainly with the following genotypes: SC / TL, SC / CU, and TL / CU, other possible combinations with homokariotypes such SC / SC and TL / TL also occur and the reader can infer them by looking at Table II, and we consider unnecessary to mention them in this moment.

Thus, in Nevado de Colima population, 101 females were analyzed cytologically for inversion frequency in the third chromosome, and the corresponding frequencies found were: SC (Santa Cruz) of 65.3%, TL / TL (Tree Line) 20.3% and the remaining percentage splited between six other inversions which frequencies were lower than six per cent, with respect to inversion "SR", 22 individuals were carriers of it representing to a 21.8% of the sample, of these 22 individuals with "SR", 20 of them, which corresponds to 90.1% are carriers also of the "SR" inversion and are too associated to inversion SC.

In the population of Valparaiso, we found that inversion SC is present with a frequency of 43.3% and 33.9% with TL, the sample represented by 149 females, 28 of them, equivalent to 18.8% are inversion "SR", of these 28 individuals 19 of them are associated with inversion SC equivalent to 67.8% that carried also the "SR" inversion.

In Zirahuén the situation is that the most frequent arrangements are SC 34.1%, TL 25.0% and 22.7% CU, our population size was only 44 females, out of them, five equivalent or 11.4% carried the "SR" inversion, and from them four equivalent to 9.1% of the total, were associated with the chromosomal gene arrangement sequence SC.

In Tulancingo, the situation is different due to the absence of the SC inversion, here the relative frequencies of chromosome III gene arrangements were TL 62.1%, 29.3% CU, OL 5.2% and HI 3.4% from a sample of 29 females , only two individuals were carriers of the inversion "SR" that is equivalent to 6.8 %.

In Amecameca the situation is similar to Tulancingo since inversion SC is practically absent SC represented only in 0.5% of the sample, showing relative frequencies for CU and TL of 66.0 and 31.1 percent respectively, in this case the sample size was 103 females, here only eight corresponding to a 7.8 percent of the sample, carried the inversion "SR" and all of them associated with the inversion CU of the third chromosome.

Also in this population (Amecameca) it was possible to analyze the genotype of 33 males from nature captured together with the females, when the were crossed with females of the laboratory strain TL / TL in their offspring larvae, we recovered two carriers of the SR inversion equivalent to 6.1% and in both cases they were associated with the inversion CU of the third chromosome.

All these relationships between heterozygotes for the third chromosome and their association with "SR" are shown in Table III which is presented to illustrate these relationships.

Finally, as it is shown, inversion "SR" exhibits an East-West gradient, but if we consider the populations from northern United States, Sonora and Nuevo Leon in there also exists a North-South gradient, so we can assume that this inversion shows simultaneously both kinds of gradient, which is quite remarkable.

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## RELATIVNA UČESTALOST INVERZIJE " ODNOS POLOVA" U PRIRODNOJ POPULACIJI *Drosophila pseudoobscura* IZ MEKSIKA

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I z v o d

Odnos polova (proporcija mužjaka) u biološkoj vrsti zavisi od nedovoljno objašnjenih mehanizama determinacije pola. Kod vrste *Drosophila* pol je determinisan proporcijom X hromozoma prema autosomima. Smanjenje odnosa mužjaka prema ženkama, poznato kao odnos polova, je karakteristika nekoliko *Drosophila* vrsta i u odnosu je sa inverzijom u polnom hromozomu takozvanog "sex-ratio " (SR) uslova. U radu su prikazani rezultaati ispitivanja prisustvA ovih inverzija kod nekoliko populacija *Drosophila pseudoobscura* u Meksiku. Formirane su kolekcije vrsta Nevado de Colima, Col.,Valparaíso, Zac., Zirahuén, Mich., Tulancingo, Hgo i Amecameca. Meksičke populacije su sakupljane u prirodnim uslovima i u laboratoriji su utvrđene individualne kulture svake ženke. U potomstvu su iz larvi koje su se pojavile izolovane pljuvačne žlezde iz svake pojedinačne larve svake kulture, bojene rastvorom aceto-orseina. Određen je genotip koji je odgovarao trećem i polnom hromozomu i izračunata njihova relativna učestalost. Odgovarajuća učestalost "sex -ratio" inverzija u analiziranim lokalitetima su: Nevado de Colima 21.8 %, Valparaiso 18.8 %, Zirahuén 11.4 %, Tulancingo 6.8 % i Amecameca 6.8 %. Očigledno prisutna je distribucija u pravcu istok – zapad u ispitivanim populacijama. Relativne učestalosti u trećem hromozomu su evidentirane i odnos između trećeg i inverzija u X - hromozomu je ispitivan.
U radu su diskutovana slična ispitivanja kod drugih *Drosophila* vrsta.

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