results that do not correspond to true choice. This factor is eliminated in male-choice as single type of males are present so this experimental design is preferred. Pattern of matings can be affected by mating propensity and factor of discrimination (Spieth and Ringo, 1983). Difference in mating propensities affects number of matings in a specified time. If mating propensity is more then it may give rise to erroneous interpretation. This factor is eliminated by using male-choice method.

With regard to choice-situations and marking procedures, patterns of matings are not affected. So, it may be concluded that marking procedures and choice-situations have no affect on pattern of matings in *D. ananassae*.

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A preliminary report on chromosomal polymorphism in Mexican populations of *Drosophila nebulosa.*

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Introduction

Drosophila nebulosa (Sturtevant) exhibits a moderate degree of chromosomal polymorphism with up to 17 different paracentric inversions in chromosome III plus two other inversions in the left arm of the sex chromosome (XL) and already described (Pavan, 1946). This neotropical species has broad geographical distribution extending from Buenos Aires, Argentina, in South America up to Central Mexico and Texas (Patterson and Wagner, 1943), and it is considered the most common member of the *willistoni* group found in Mexico and inhabiting eastern areas in this country (Patterson and Mainland, 1944). With respect to its inversion or chromosomal polymorphism in this species are few reports; examples of them are that of Pavan (1946), da Cunha *et al.* (1953), Bonorino

and Valente (1989), Bonorino *et al.* (1993), and in Mexico the only one report is that of Salceda (2005) reporting a long term study in a single population. Our aim in this occasion is to have a better knowledge of its spatial distribution and how the inversion polymorphism is represented by mean of a series of collections along an eastern transect of its distribution in Mexico.

Materials and Methods

We choose as areas of study nine locations across a north-south transect in the eastern of its distribution in Mexico and eventually two other sites, such localities are: Tuxpan, Arroyo Agrio, Las Tinajas and Cosamaloapan State of Veracruz; Lázaro Cárdenas, Macuspana and Kolemjaá State of Tabasco; La Malinche State of Tlaxcala in the highlands and Tepic State of Nayarit in the Pacific coast. We must point out that this is a preliminary report, since all the collections correspond to a prospective trip with the purpose to establish a more extensive study of this and other species in the country. So a trip was programmed in order to search for more appropriate places to conduct such a project and consequently it was a short trip with a one week duration, trying to perform collections in as many locations as possible, and they were those previously mentioned. We used 25-30 plastic buckets containing fermenting bananas to attract flies all of them distributed every 5-7 meters in the site as to cover a large area. Traps were visited every 10-15 minutes during the time collections were done. This depended on the amount of flies caught that according to our experience was appropriate for our purposes then moving to another place. For that reason the number of flies was small and different in each locality. Captured flies were sorted *in situ* by species group and kept in vials with fresh food until arrival to the laboratory in Mexico City. Once there, each *D. nebulosa* female was transferred into an individual half-pint bottle with fresh food, initiating an isofemale line, and incubated at 25±1ºC. A week later a new culture was done for each isofemale line, and to the original we added some drops of a heavy solution of live yeast and transferred to a cooler area at 15±1ºC temperature providing in such a way a maximum nourishment for the developing larvae and as a consequence large polytene chromosomes. When larvae started to crawl out the medium, from each isofemale line a single larva was taken, dissected and the salivary glands extracted; they were stained with a regular lacto-aceto-orcein solution and a smear prepared. Each smear was analyzed using a light microscope and its corresponding karyotype determined using as a guide the figures published by Pavan (1946) as reference, the heterozygous inversions found from each isofemale line were recorded, their relative frequencies calculated, and tables containing the information prepared.

Results

The number of flies captured in each locality was in most cases small for determining a proper genetic constitution based on a relative frequency of their inversions. Here we must remember that this is a prospective study to gather information for future projects. Nevertheless, the gathered data proved to be useful for our purposes providing information not only promising sites for further studies but also concerning chromosomal variation. Table 1 shows how many females were captured per site showing that all together we were able to karyotype in total 202 females and from them a total of 404 chromosome arms as represented in Table 2. Small discrepancies in number of chromosomes are due to an occasional occurrence of more than two inversions in a particular chromosome pair of some larvae. In our analysis we found eleven different inversions out of 17 already described for this species, which corresponds to 64.7% of the variability for this trait.

Table 1. Number of isofemale lines and analyzed number of chromosome arms in several populations of *Drosophila nebulosa* from Mexico.

Site	PR	AA	TI	CO	LC	MA	KO	TL	TE	Total
No. Chroms.	6	86	11	30	29	30	3	2	5	202
No. Chroms. Arms	12	172	22	60	58	60	6	4	10	404

Poza Rica (PR), Arroyo Agrio (AA), Tinajas (TI), Cosamaloapan (CO), Lázaro Cardenas (LC), Macuspana (MA), Kolemjaá (KO), Tlaxcala (TL), Tepic (TE).

Table 2. Relative frequency for each inversion found in chromosomes X and III of *Drosophila nebulosa*. Global frequencies.

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

Discussion

Since the sample size in each locality was small, we organized our analysis in a global form, adding every frequency as to have single one, in referring to relative frequencies of the different inversions found as if we were referring to a single population. Each inversion in this species is designated by a letter, and the normal sequence is referred as Standard. In this work we found eleven of them, 10 present in the third chromosome and one in the left arm of chromosome X referred as XL. Relative frequencies of each inversion varied as seen in Table 2. From it we observe that 37.9% of the chromosomes analyzed are free of inversions and the remaining have at least one inversion. The most frequent of them is "A" present in 15.6% of the cases, and it could be characterized as common; then follow those of intermediate frequency "D" and "G" in chromosome III and inversion XL with values of 9.6, 9.4 and 7.9%, respectively; those of lower frequency "E/G", "B", "E", and "C" with values between 5-7 percent; and rare inversions "H/h", "F", and "H" with frequencies close to one percent. Since out of 404 chromosome arms 37.9% are free of inversions, it means that we detected 251 inversions in the complete sample just in chromosome III; in the XL chromosome arm only 32 chromosomes carried inversion. From this information we could derive more information. It deals with the number of inversions carried by a single female; in our case it corresponds to 1.4. This measure has been considered important, since in the central area of distribution of the species it reaches values as high as nine inversions per female, it is due to the size of the involved inversions which are relatively small. This trait is also considered when dealing with the genetic variability. Finally as the general composition of our sample it could be considered in accordance with those two parameters analyzed as marginal populations. If a conclusion is pertinent we consider the necessity of more studies not only in a way to increase the sample size but also involving other areas and try to make observations referred to annual cycles, abundance and relationships with other species in or out of their group.

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New *piggyBac* insertional mutations on FRT chromosomes.

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Transposon insertional mutagenesis has been widely used in *Drosophila* forward genetic screens. We have recently described a new *piggyBac*-based insertional mutagenesis approach (Schuldiner *et al*., 2008) that allows us to perform mosaic screens that disrupt genes in specific tissues. We modified the *piggyBac* transposon to efficiently disrupt gene function even when inserted in introns and mobilized the transposon in a fly strain that contains FRT sites on all four autosomal arms. Each insertion was molecularly mapped by inverse PCR. Altogether, 1920 insertions targeting 1803 genes were reported (Schuldiner *et al*., 2008). These mutations can be readily used for FLP/FRT-based mosaic screens (Berdnik *et al*., 2008; Schuldiner *et al*., 2008).

We report here 410 additional insertional mutations we have generated since the original publication, which concludes our screen. Table 1 lists all new insertions with information including nucleotide position of insertions in the *Drosophila* genome as determined by inverse PCR, cytological location, ID number of the fly stock, gene symbol and CG number for target genes (and potential secondary target genes), insertion location with respect to gene structure, and Genbank accession number.

Among the 410 piggyBac alleles listed in Table 1, 329 are insertions into new genes. The rest are potentially stronger alleles of genes previously reported (Schuldiner *et al*., 2008; rightmost column), based on insertion location with respect to gene structure.

Table S1 combines all insertions we have generated, including those reported in Schuldiner *et al*. (2008) as well as new insertions reported here (in red italics).

As with all strains reported in Schuldiner *et al*. (2008), these new *piggyBac* alleles can be obtained from the Drosophila Genetic Resource Center in Kyoto, Japan.

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