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Research Article

A cytological study of the O_5 chromosomal inversion of *Drosophila subobscura* (Diptera, Drosophilidae)

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Abstract

The O_5 chromosomal inversion has been a cornerstone for understanding different aspects of the American colonization by *Drosophila subobscura*. To obtain more information of this evolutionary event it is important to know the pattern of bands of this inversion in detail. Comparing this pattern with that of *D. melanogaster* it is possible to predict which genes are located inside or close to the O_5 inversion and use them as genetic markers. In this study, the complete band pattern of the O_5 inversion is presented. Furthermore, the most important genes located inside it have been predicted. Finally, a constriction located close to the proximal breakage point of the O_5 inversion has been observed many times and its possible genetic significance is discussed.

Key words: Drosohila subobscura, chromosomal inversion, colonization, lethal genes.

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Introduction

Until 1978, *D. subobscura* was considered a species with a Palearctic area of distribution. In that year this species was first found in the Chilean locality of Puerto Montt (Brncic *et al.*, 1981), but in 1982 it was also detected in Port Townsend (Washington) (Beckenbach and Prevosti, 1986). The species is now found in Chile from La Serena to Punta Arenas and in many localities of Argentina in the Andes mountain range. In North America the species is spread along the West Coast (Prevosti *et al.*, 1987; 1989). This phenomenon of colonization has been studied at different genetic levels: the chromosomal polymorphism (Prevosti *et al.*, 1985; 1988; 1990), the allozyme polymorphism (Prevosti *et al.*, 1983; Balanyà *et al.*, 1994), the lethal genes (Mestres *et al.*, 1990; 1992; 1995; Solé *et al.*, 2000), the polygenes for quantitative traits (Pegueroles *et al.*, 1995; 1999; Huey *et al.*, 2000), the mtDNA (Latorre *et al.*, 1986; Rozas *et al*., 1990), the genomic DNA variation (Rozas and Aguadé, 1991) and the effect of inbreeding (Pegueroles *et al.*, 1996).

From the beginning our research group tried to search for the origin of the colonization. The chromosomal polymorphism obtained in the American populations of *D. subobscura* (Prevosti *et al.*, 1985; 1988; 1990) showed a high similarity with that from the Western Mediterranean area (Prevosti *et al.*, 1984) with the important exception of the O_5 inversion. This inversion is present in the American populations but it has never been found in the Western Mediterranean samples. The O_5 inversion from America has an interesting property, it is always completely associated with a lethal gene (Mestres *et al.*, 1990; 1992; 1995). In spite of its association with a lethal gene its frequency shows a clinal distribution with latitude in both colonized areas. However, in the Palearctic region the distribution of O_5 is rather erratic (Krimbas and Loukas, 1980; Krimbas, 1993; Mestres *et al.*, 1994; Zivanovic and Mestres, 2000) and its frequency is extremely low with the exception of South Scandinavia (Sperlich, 1964; Pinsker and Sperlich, 1981). Furthermore its combination with lethal genes is variable. Thus, an O_5 inversion from Gävle (Sweden) was lethal free, while another one from Lilla Edet (also in Sweden) was found to be semilethal (Mestres *et al.*, 1992). O_5 inversions from Taulé (France) and Zanjic (Yugoslavia), on the other hand, were associated with lethal genes. However, these lethals were neither allelic between each other nor with those from the American populations (Mestres *et al.*, 1992; Zivanovic and Mestres, 2000). The constant association of the lethal effect and the O_5 inversion in America can be used as a tracer to detect the local Palearctic population from which the colonization started. It is most probable that a single O_5 gene arrangement with the lethal gene was among the chromosome sample of the colonizing flies (Mestres and Serra, 1995). Thus, if an O_5 inversion carrying the lethal gene found in America is detected in a Palearctic population, this could be the one from which the

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colonization started. As the lethal genes from Taulé and Zanjic are different from those found in America, the origin of the colonization remains still unknown.

The breakage points of the O_5 inversions from Europe and America seem to be the same (Mestres *et al.*, 1992; Zivanovic and Mestres, 2000), but the behavior of the O_5 arrangement from America and from the Palearctic region is different. In America significant latitudinal clines can be found (Prevosti *et al.*, 1985; 1988; 1990); its frequency is higher than expected from inbreeding experiments (Pegueroles *et al.*, 1996) and a significantly higher segregation can be observed in recombination studies (Mestres *et al.*, 1998). All these results seem to support that the O_5 inversion is associated with an heterotic effect in America (Mestres *et al*., 2001). Although the breakage points of all O_5 inversions studied are identical, the possibility of recurrence in this inversion must be taken into account.

For all these reasons the O_5 inversion has a key function for understanding the American colonization by *D. subobscura*. The aim of the present study is to obtain more information about the cytology of this inversion. The detailed analysis of the banding pattern of the O_5 inversion will allow a comparison with the genetic and karyotypic information available in other species of *Drosophila* (mainly *D. melanogaster*), and it might be possible to recognize the genes that are located in the inversion or in its neighborhood.

Material and Methods

All O_5 chromosomal lines available carry a lethal gene and no homokariotypic strain O_5/O_5 could be established by inbreeding. However, the lethal gene of the O_5 chromosomal lines are not allelic in all cases. For instance, as previously mentioned, the lethal gene associated with the American O_5 inversion is not allelic with that from Taulé (France). Thus, it is possible to obtain O_5/O_5 individuals with O chromosomes of different origin. For this purpose the T15 (from Taulé) and the G7A (from Gilroy, California), both O_5 chromosomal lines with different lethal genes, were crossed. The strategy of these crosses is presented in Figure 1. One *Va*/O_5 male from Taulé was crossed with *Va*/O_5 virgin females from Gilroy. The parental individuals are heterokaryotypic balanced over a chromosome *Va* (*Varicose*) from the *Va*/*Ba* balanced lethal strain (Sperlich *et al*., 1977). The *Varicose* chromosome carries the X-ray induced overlapping inversions $O_{\underline{VIII + 210}}$ and the naturally occurring $O_{\underline{3+4}}$ region. Among the offspring the karyotypes: *Va*/O_5, O_5/O_5 and *Va*/*Va* are expected, but *Va*/*Va* genotypes die because the *Va* gene is a recessive lethal. The problem is the time of lethal effect of the *Va* gene. Since the *Va* gene of *D. subobscura* shows the same behavior as the *Delta* gene of *D. melanogaster*, it is generally assumed that they are homologous genes (Krimbas, 1993). In *D. melanogaster*, the *Delta* gene is embryonic lethal (Lindsley and Zimm, 1992). However, if the *Va*/*Va* genotypes can survive the larval stadium, then both homokaryotypes (O_5/O_5 and *Va*/*Va*) might be present in the F_1 larval offspring. Certainly, it would be possible to distinguish these genotypes by their pattern of bands, but this would slow down the analysis. To overcome this problem, one possibility is to perform a more complex strategy of crosses preventing the appearance of *Va*/*Va* individuals (Mestres *et al*., 1992). Another option is to determine the time of the lethal effect of *Va* lethal during development. This study can be done cytologically by examining the karyotype of a greater sample of larvae of the *Va*/*Ba* lethal balanced strain (*Va*/*Va* homozygotes are $O_{\underline{3+4}}/O_{\underline{3+4}}$ in the distal region of the chromosome O, whereas *Ba*/*Ba* homozygotes are O_{st}/O_{st}). When larvae of the *Va*/*Ba* strain were analyzed all lhomokaryotypic individuals were O_{st}/O_{st} (Figure 2). This result confirms that the *Va* gene (as expected by its equivalence with the *Delta* gene of *D. melanogaster*) is an embryonic lethal, and the *Ba* gene expresses lethal effects after pupation. Thus, in the

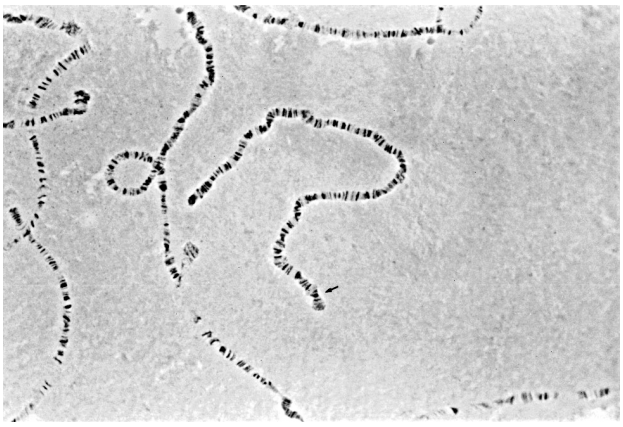

Figure 2 - Homokaryotypes O_{st}/O_{st} (*Ba*/*Ba*) obtained in the crosses among *Va*/*Ba* individuals. The arrow indicates the distal tip of the chromosome, where the pattern of bands allows clear differentiation of the O_{st} and the $O_{\underline{3+4}}$ chromosomal arrangements. 400X.

(P)	Genotype	*Va*/*O*$_5$ (Taulé)	x	*Va*/*O*$_5$ (Gilroy)		
	Phenotype	Va		Va		
		↓				
(F_1)	Genotype	*O*$_5$ (Taulé)/*O*$_5$(Gilroy) +	*Va*/*O*$_5$ (Taulé) +	*Va*/*O*$_5$ (Gilroy) +	Va/Va	
	Phenotype	+	Va	Va		lethal

Figure 1 - Crosses performed in order to obtain the homokaryotypes O_5/O_5.

crosses between the lethal chromosomal lines T15 and G7A all homokaryotypes must be O_5/O_5.

The polytene chromosomes were stained and squashed in aceto-orcein solution and observed using a light microscope.

Results and Discussion

The pattern of bands in the O_5/O_5 homokaryotypes is presented in Figure 3. The pairing is accurate indicating the full coincidence of the breakage points confirming previous observations (Mestres *et al.*, 1992; Zivanovic and Mestres, 2000). Although it is not possible to exclude a recurrent origin of new O_5 inversions by mutation, the probability of this phenomenon is expected to be very low. Using the method proposed by Sperlich and Pfriem (1986) the estimate of this recurrence would be between 1.368×10^{-7} and 9.488×10^{-8} (Mestres and Serra, 1995) assuming that the breakage points are randomly distributed along the O chromosome of *D. subobscura*. However, this is most unlikely, because many "hot spots" can be identified in this chromosome (Kunze-Mühl and Müller, 1958; Krimbas, 1993). The proximal breakage point is mapped in the 83 B-C section and very close to the "hot spot" located in the 83 C - 84 A section (where the breakage points of the O_{22}, O_{15} and O_9 inversions are also located). The distal breakage point is between the 87 D - 87 E, not far from the breakage points of the inversions O_{12}, O_{17} and O_{22}. Furthermore, in the chromosomes of the homokaryotypes O_5/O_5 a constriction was recognized in many cases close to the left breakage points of the O_5 inversion (Figs. 4a, 4b, 4c and 4d). This constriction could be related in some way to a "hot spot" of the O

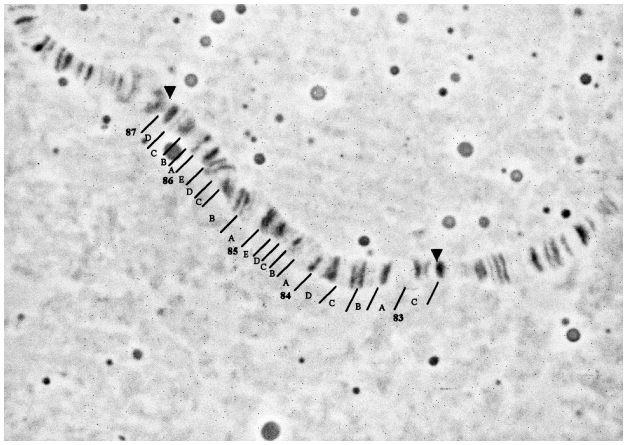

Figure 3 - Pattern of bands of the O_5 chromosomal inversion in the homokaryotypes O_5/O_5. 1000X. Arrows indicate the breakage points of the inversion.

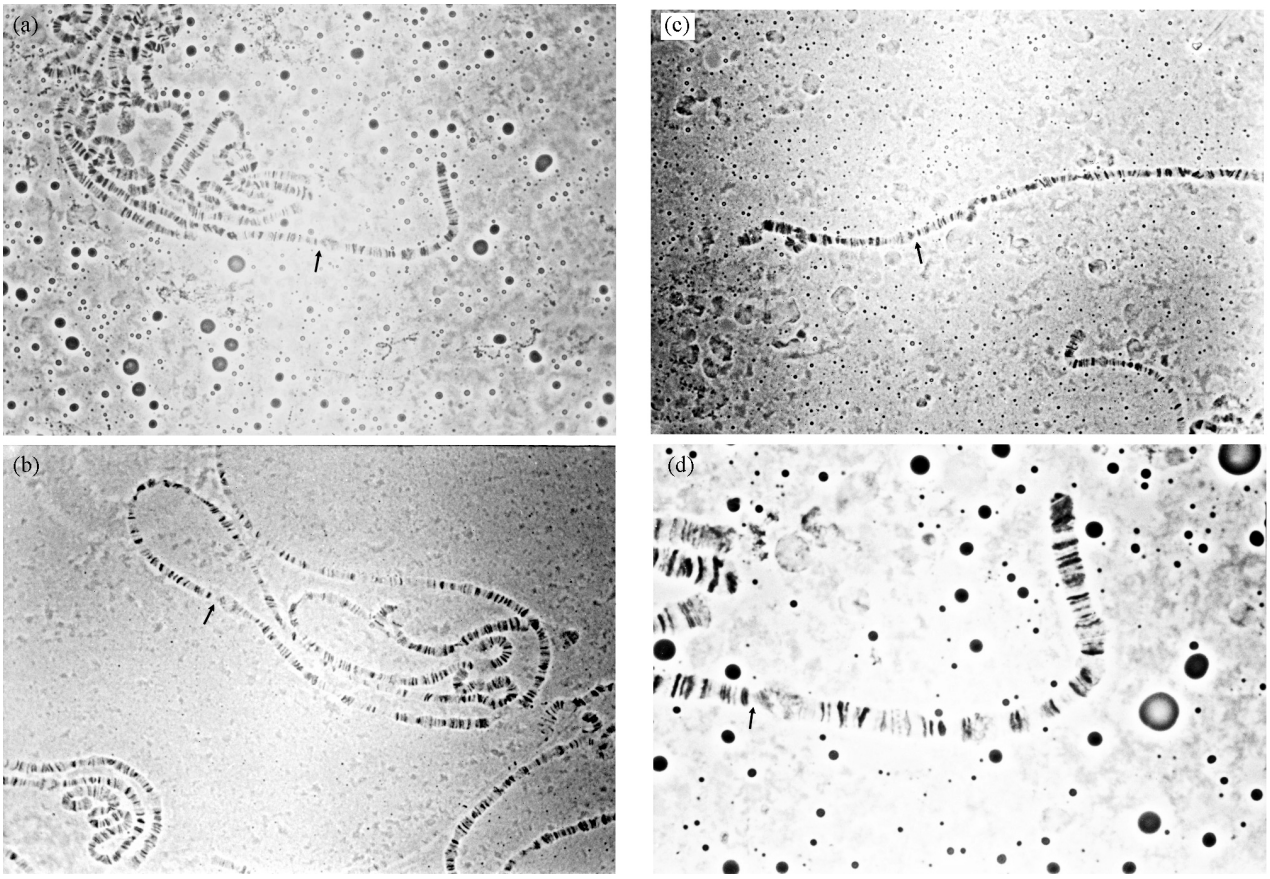

Figure 4 - Different pictures where the constriction located near the O_5 inversion proximal breakage point (indicated by an arrow) is presented. (a, b and c) 400X. (d) 1000X.

chromosomes of *D. subobscura* where breaks occur more frequently than in other regions and might be discussed in the context of a possible recurrence of the O_5 inversion.

As the pattern of bands obtained in the homokaryotype O_5/O_5 is very clear, it could be directly compared with that of the 3R - chromosome arm of *D. melanogaster* . The region covered by the O_5 inversion corresponds approximately to the section 86 - A to 89 - F of the 3R chromosome of *D. melanogaster*. According to Lindsley and Zimm (1992) the most important genes located in this segment are: *Odh* (*Octanol dehydrogenase*), *Sdh-2 (Sorbitol dehydrogenase*), *ants* (*antennas*), *cu* (*curled*), *man* (*mandarin*), *Hsp 70A* (*Heat - shock protein*), *ttr* (*tetrapter*), *ry* (*rosy*), *Ace* (*acetyl cholinesterase*), *aur* (*aurora*), *red* (*red Malpighian tubes*), *trx* (*trithorax*), *cv-c* (*crossveinless c*), *Tm1* (*Tropomyosin 1*), *jvl* (*javelinlike*), *Po* (*Pyridoxal oxidase*), *Sb* (*Stubble*), *ss* (*spineless*), *Ubx* (*Ultrabithorax*), *abd-A* (*abdominal A*), *Abd-B* (*Abdominal B*) and *cal* (*coal*). Thus, some of these genes could be used as molecular markers for the O_5 inversions from American and Palearctic populations. The DNA of the *Odh* (86 - D 1-4) and the *Sdh-2* (86 - D) genes has been sequenced in *D. melanogaster* (Luque *et al.*, 1994; Luque *et al.*, 1998). Both genes are located inside, but close to the proximal breakage point of the O_5 inversion in *D. subobscura*. The DNA sequence of a fragment of the *Odh* gene of *D. subobscura* has been recently used to characterize the O_5 and other inversions in American and Palearctic populations of *D. subobscura* (Abad, 2000). Homeotic genes such as *trx* (88 - B3), *Ubx* (89 - E1), *abd-A* (89 - E2) and *Adb-B* (89 - E2) have been also characterized at the molecular level in *D. melanogaster* (Martin *et al.*, 1995). These genes map inside and close to the distal breakage point of the O_5 inversion, thus it might become possible to cover the two ends of this inversion and use them for future investigations.

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