Table 2. Differences between migration patterns of developing worms

Worm groups	p*
20 & 21 days old	0.8205
21 & 23 days old	0.0005
23 & 28 days old	0.0204
20 & 28 days old	0.0001
20 & 23 days old	0.0001
21 & 28 days old	0.0002

* Kruskal-Wallis one-way ANOVA.

worms, the distance between worms equaled or exceeded the initial distance at all time periods. In one experiment worms migrated to one-half their initial distance during the first three minutes, but then moved apart. Attraction was observed in two of three experiments with 21-day-old worms, and one worm pair made contact. In the third experiment, the 21-day-old worms moved away from each other. Attraction was pronounced in all of six experiments with 23- and 28-day-old worms, and five of the six worm pairs made contact.

Differences between the migrations of 20- and 21-day-old worms were not significant (table 2). However, differences between the migrations of 20-day-old worms and 23- or 28-day-old worms were highly significant, as were those between 21 and 23, and 21 and 28 days. Also, the migrations of 23-day-old worms differed significantly from worms 28 days old.

Discussion. These data clearly demonstrate that in vitro attraction begins when *Schistosoma mansoni* males and females are approximately 23 days old, which is in general agreement with reports of pairing in vivo. Standen [9] reported worm-pairing on day 30 when mice were infected with both male and female cercariae simultaneously and day 23 when mice previously infected with female cercariae were superinfected with male cercariae. Wilson [10] reported that pairing in vivo takes place between days 28 and 35 post-infection.

The initial distances between worms varied between experiments because when the small worms were pipetted into the bioassay channel there was an initial drifting related to early fluid disturbance. This also provides another insight into worm behavior because it demonstrates that worm responses can occur over varying starting distances. This variable has not been tested before. It is also of interest that in experiments with 20- and 21-day-old worms the initial starting distances were less than for 23-day-old worms, and attraction only occurred in the latter. To assess worm responses, the formula used [11,12] expressed a mean migration index (MMI), which reflected both positive and negative responses. Inasmuch as a positive MMI reflects attraction, we propose that a negative MMI reflects repulsion. Such a negative interaction has been noted before [7].

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Differences in chromosome A arrangement between *Drosophila madeirensis* and *Drosophila subobscura*

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Summary. The proximal half of the A (= X) chromosome of *D. madeirensis* has a gene arrangement very similar to the A1 or A6 inversions found in *D. subobscura*. Polytene chromosome analysis of hybrids between *D. madeirensis* and strains of *D. subobscura* homozygous for such inversions shows, however, that *D. madeirensis* has a gene arrangement different from any known for *D. subobscura*. These results provide evidence for a greater differentiation of the X chromosome in these species than has previously been described; it seems that the X chromosome is the only one that has undergone structural variation during the speciation process.
Key words. Drosophila-related species; chromosome arrangements; hybrids; speciation.

D. madeirensis [1], an endemic species of the island of Madeira, belongs to the obscura group, being closely related to *D. subobscura*. Krimbas and Loukas [2], studying chromosomal homologies between these species, based on analysis of F1 female hybrid larvae and using as male parents individuals from a strain of *D. subobscura* with the A (= X) standard gene arrangement, observed that the proximal half arrangement of the *D. madeirensis* A (= X) chromosome (segment I) is identical to the A1 gene arrangement of *D. subobscura*. *D. subobscura* has another inversion very similar to A1 in this chromosomal segment, the A6 inversion which is always found in association with A2 inversion located in segment II.

In order to ascertain unambiguously whether the proximal end of chromosome A of *D. madeirensis* has an arrangement which is identical to any one of those found in *D. subobscura*, crosses between *D. madeirensis* and two homozygous strains of *D. subobscura*, A1 and A2 + 6 respectively, have been carried out and polytene chromosomes of female hybrid larvae have been analyzed.

Female hybrid larvae were obtained by crossing *D. madeirensis* females with *D. subobscura* males. Polytene chromosome preparations were obtained from salivary glands of 3rd late instar larvae and stained with lacto-aceto-orcein (acetic orcein 70%, lactic acid 30%). The sections and sub-

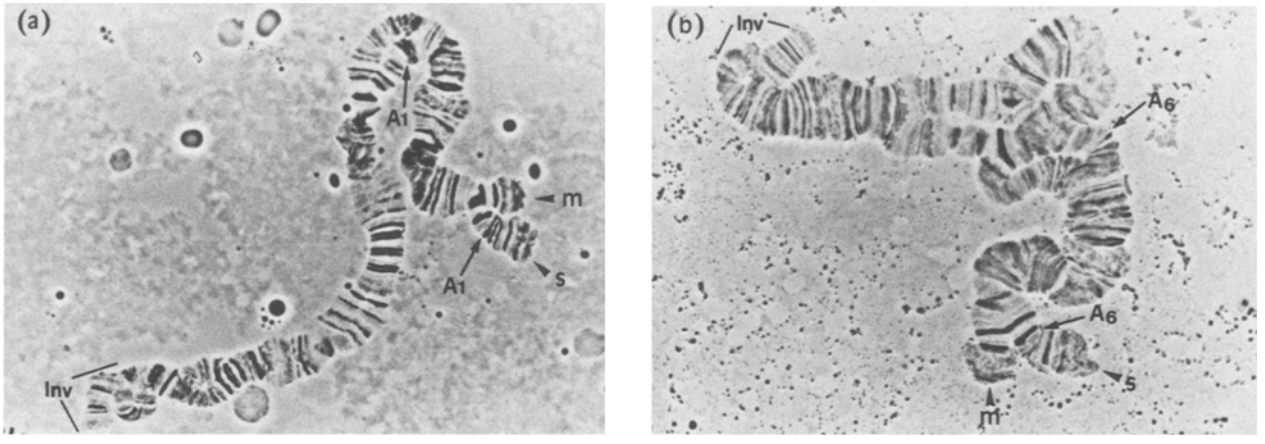

Figure 1. A (= X) chromosomes of F1 hybrids between *D. madeirensis* and strains A1 (*a*) and A2 + 6 (*b*) of *D. subobscura*. (s = *D. subobscura* chromosome, m = *D. madeirensis* chromosome, A1 = proximal and distal ends of the A1 gene arrangement, A6 = proximal and distal ends of the A6 gene arrangement, Inv = inversion of the terminal section 16 which is specific for *D. madeirensis*).

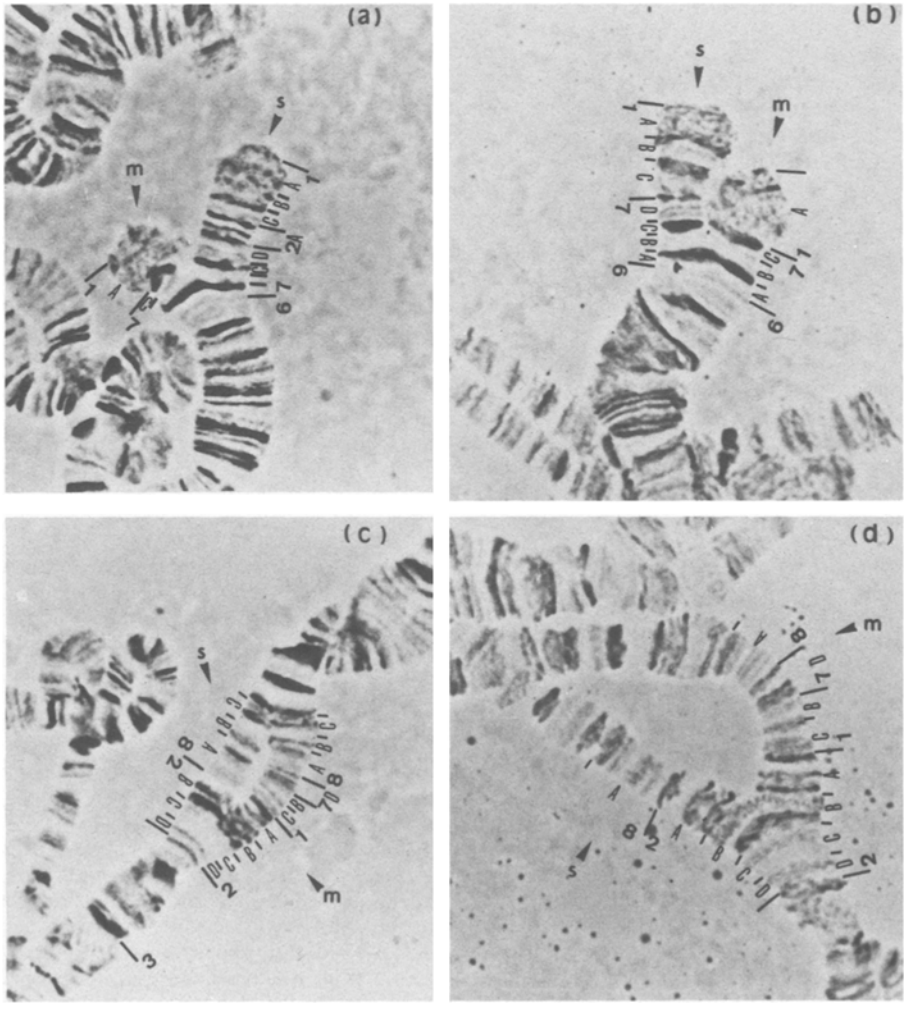

Figure 2. *D. madeirensis* gene arrangement and breaking points of A1 and A6 inversions. Proximal ends in hybrids with A1 (*a*) and A6 (*b*) gene arrangements of *D. subobscura*. Distal ends in hybrids with A1 (*c*) and A6 (*d*) gene arrangements of *D. subobscura* (s = *D. subobscura* chromosome, m = *D. madeirensis* chromosome).

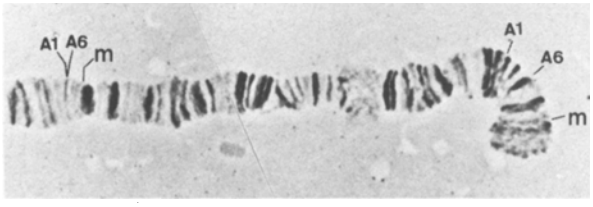

Figure 3. Proximal half (segment I) of the *D. subobscura* A (= X) chromosome with standard gene arrangement, where the *D. subobscura* A1 and A6 inversion break points and the *D. madeirensis* gene arrangement (m) are indicated.

sections shown in the figures and mentioned in the text are based on the standard salivary gland chromosome map of *D. subobscura*[3].

Figure 1 shows the A chromosome of F1 hybrids between *D. madeirensis* and strains A1 and A2 + 6 of *D. subobscura*. This figure shows unambiguously that the *D. madeirensis* gene arrangement is different from any of the *D. subobscura* arrangements. A1 and A6 inversions have the same distal break point, located at the 7D/8A subsections (figs 2, 3)[4], but they differ in the proximal one. This break point is located between subsections 2A and 2B in the A1 inversion and between 1C and 2A in the A6 inversion (figs 2, 3)[4]. The gene arrangement found in *D. madeirensis* differs from A1 and A6 inversions in both break points. The distal one is located between subsections 7C and 7D; in figure 2 it can be seen that the chromosomes of *D. madeirensis* show clearly the three bands of subsection 7D in the distal position, whereas in A1 and A6 *D. subobscura* chromosomes at least the first two bands of subsection 7D can be seen in the proximal position. The proximal break point is located possibly between subsections 1A and 1B, although in *D. subobscura* chromosomes the 1B/1C zone appears as a thick dark band, more apparent than in the *D. madeirensis* chromosomes. Figure 3 shows the proximal half of the *D. subobscura* standard X chromosome with the break points of A1 and A6 and *D. madeirensis* gene arrangements.

Thus, the gene arrangement of the X chromosome of *D. madeirensis* is different from any known arrangement of *D. subobscura,* both for segment I, that we have just analyzed, and for segment II, in which a small inversion is found in the distal end (fig. 1)[2]. Since all the other chromosomes of *D. madeirensis* are homosequential with the corresponding chromosomes of *D. subobscura* (Jst, U1 + 2, Est, O3)[2], the X chromosome is the only one that has undergone structural variation during the speciation process. In *D. guanche,* a closely related species, a greater structural variation in the X chromosome in comparison with the autosomes is also observed[5]. These results are in agreement with the expected higher incidence of rearrangements involving the X chromosome when related species are compared, as was proposed by Charlesworth et al.[6] in their model for the relative rates of evolution of sex chromosomes and autosomes.

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Single amino acid substitutions in *sn*-glycerol-3-phosphate dehydrogenase allozymes from *Drosophila virilis*

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Summary. The amino acid sequence was compared among the three allelic variants (allozymes) of *sn*-glycerol-3-phosphate dehydrogenase in *D. virilis,* which are detected by one-dimensional electrophoresis. The αGPDH^f variant was different from the αGPDH^m by only one substitution of 68-lysine for asparagine; αGPDH^s differed from αGPDH^m by substitution of 127-glycine for arginine. No electrophoretically 'silent' substitutions were found in a total of 352 amino acid residues.
Key words. Drosophila virilis; *sn*-glycerol-3-phosphate dehydrogenase; allozymes; single amino acid substitution.

Glycerol-3-phosphate dehydrogenase (*sn*-glycerol-3-phosphate: NAD^+ 2-oxidoreductase, EC 1.1.1.8) in adult *Drosophila* functions in flight muscle metabolism[1]. *Drosophila virilis* in Japanese populations has three alleles at the αGPDH structural locus, one of which (*αGPDH^m*) comprises more than 95% of the genes[2]. Biochemical studies of the allozymes (αGPDH^s, αGPDH^m, and αGPDH^f) produced by homozygotes for the three alleles revealed that although no significant differences in Michaelis constants for NADH, G-3-P and NAD^+ were detectable, subtle but significant differences were observed in thermostability at 35 °C and inhibition by excess dihydroxyacetone phosphate, one of the substrates. The αGPDH^f allozyme was most thermolabile, and the αGPDH^s form was most susceptible to the inhibition[3]. We take an interest in the relation between functional differences between allozymes produced by the *αGpdh* locus and their structural differences. We have already determined the primary structure of αGPDH^m[4]. Therefore, we have undertaken complete sequence analysis of the αGPDH^s and αGPDH^f forms. The present paper demonstrates that the three allozymes differ from each other by a single amino acid substitution only.

The two *D. virilis* allozyme strains, αGPDH^s and αGPDH^f, were used. These strains are homozygous for each of these alleles at the NAD^+-dependent glycerol-3-phosphate dehydrogenase locus. Each allozyme was purified from frozen 1-2-day-old flies according to the methods described before[4]. Since the αGPDH^f enzyme was partly leaked from a hydroxyapatite gel column at pH 7.4, this pH being the same as that for preparation of αGPDH^s, the pH was lowered to 6.8 for αGPDH^f. Final preparations gave a single band on polyacrylamide gel electrophoresis.