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Studies of the species barrier between *Drosophila subobscura* and *D. madeirensis* IV. A genetic dissection of the X chromosome for speciation genes

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Abstract

The genetics of four traits contributing to the isolation mechanism between the two closely related species of *Drosophila* belonging to the *obscura* group, *D. subobscura* and *D. madeirensis*, have been investigated, especially regarding the influence exerted by the X chromosome. This chromosome has been roughly dissected genetically by the use of four markers. It was found that factors affecting viability of backcross males are spread from the centromeric end of the chromosome up the region marked by Bx. Three sections were responsible for male sterility/fertility. The abnormal head shape of the backcross males was affected by factor(s) on the *madeirensis* and the *subobscura* sex chromosome located at the region of MAD1 inversion. Finally, an abnormal trait in these males (presence of extra sex combs) was found to be controlled by four sections, two on the *madeirensis* X chromosome and two on the *subobscura* one.

Introduction

Several alternative views have been advanced concerning the genetic mechanisms of speciation, a crucial part of the evolutionary process. Are sterility and lethality in interspecific hybrids due to the presence of a large number of loci or to a small number of genes with major effect? White (1954) opted for the first possibility; Gould (1980), Zouros (1981) Templeton (1981), Wu and Beckenbach (1983) Coyne and Charlesworth (1986) and Orr (1992) for its alternative. More recently the presence of multiple genetic factors of weak effect which interact strongly with

heterospecific but also with conspecific genes is accepted (Cabot et al., 1994; Davis et al., 1994; Wu and Palopoli, 1994). This is in accordance with the views of the founding fathers of neodarwinism (Mayr, 1963; Dobzhansky, 1970; Wright, 1977, 1982).

This is perhaps the reason why the search for so-called speciation genes has met with success in only a few cases, and a partial one at that. Using the technique invented by Dobzhansky (1936), several scientists succeeded in identifying such genes. Wu and Beckenbach (1983) studied the differences between X chromosomes of *D. pseudoobscura* (sex ratio and standard gene arrangements) and of *D. persimilis*. They argued for the existence of at least four factors controlling interspecific sterility. Historically, the more precise mapping of a gene distant at about 1 cM from the mutant *forked* in *Drosophila simulans*, a gene responsible for sterility of hybrids between this species and the close relatives *D. mauritiana* or *D. sechellia* came first (Coyne and Charlesworth, 1986). Later, these authors proved the presence of at least three such genes with strong effects (Coyne and Charlesworth, 1989). In the hybrids between *D. melanogaster* and *D. simulans* the only viable F_1 progeny is of the sex of the *melanogaster* parent; Watanabe (1979) was the first to discover in *D. simulans* a gene rescuing the lethal males, the gene *lethal hybrid rescue*, *Lhr*. Hutter and Ashburner (1987) described in *D. melanogaster* a gene rescuing the males from the same cross, the *hybrid male rescue*, *Hmr*. That single genes can rescue lethal hybrids from this cross has been confirmed by Sawamura et al. (1993a), Sawamura et al. (1993b) and Sawamura and Yamamoto (1993). An autosomal speciation gene restoring fertility in hybrids between *D. mojavensis* and *D. arizonae* was identified by Pantazidis and Zouros (1988) and Pantazidis et al. (1993). Finally Perez and Wu (1995) have identified and characterized a gene, *Odysseus*, *Ods*, responsible for the sterility between *D. simulans* and *D. mauritiana*. It is a sex linked gene positioned between the markers *forked* and *Beadex*. However, this gene is apparently only one member of a group of genes affecting the fertility of these interspecific hybrids.

In the present study we have investigated the genes or chromosomal segments of the X chromosome responsible for viability reduction, sterility, and two morphological abnormalities of male hybrids between two closely related species of the *obscura* group of *Drosophila*, namely *D. subobscura* and *D. madeirensis*. This study continues previous ones on the same species cross (Khadem and Krimbas, 1991a, 1991b, 1993). The first species mentioned is highly polymorphic for the gene arrangements in all its five acrocentric chromosomes (one pair of dot chromosomes is also present). In contrast, *D. madeirensis* is monomorphic, but its gene arrangements resemble those of *D. subobscura*: in some chromosomes they are identical with one of the gene arrangements encountered in this latter species, while in other chromosomes the respective gene arrangements differ by a simple inversion. The two species can be crossed in both directions, but one of the crosses is more productive, *madeirensis* females crossed to *subobscura* males. The cross in the other direction usually (but not with all strains) produces a sex ratio distortion, i.e. more males than females (the ratio varies from 1:30 to nearly 1:1.2). All F_1 males are sterile, having either small testes empty of sperm or normal size testes with

immotile sperm. Most of the F_1 females are fertile and thus a backcrosses progeny can be produced. This progeny can be used to study the genetics of sterility, viability and morphological abnormalities with the help of appropriate markers. It should be noted that there is a genetic heterogeneity within marker classes of these progenies due to autosomal segregation. It was found that a combination of X and Y chromosomes from different specific origins leads to male sterility. In homospecific combinations of the sex chromosomes sterility depends on autosomal factors, positioned in four different autosomes. Female fertility is influenced by the origin of the cytoplasm combined with a heterospecific X chromosome and heterospecific autosomal factors. In two morphological abnormalities of the backcross males, i.e., the presence of extra sex combs and the abnormal head shape of the adult fly, the X chromosome plays an important role. It induces the traits when combined with a heterospecific Y and with a majority of heterospecific autosomes, two of which seem to have a more important role than the others. We decided, therefore, to analyse further the influence exerted by the X chromosome by dissecting it with the help of four markers.

Material and methods

Strains and markers

A single strain *D. madeirensis* was used. The X chromosome of this strain bears the allele for light antennae colour (ac^L), characteristic of this species; also the wild type allele of vermilion ($+^{ve}$), the wild type allele of *Beadex* (+recessive to Bx) and the allele controlling the electrophoretic allozyme of Diaphorase specific to this species (Dia^m). The *madeirensis* X chromosome in comparison to that of *subobscura* is fixed for two inversions, MAD1, a large one near the centromeric end (the chromosome is acrocentric) and 16BCD, a very small inversion at the tip of the chromosome (its symbol designates the inverted sections). Genes $+^{ve}$ and ac^L are located within inversion MAD1, while Dia^m is probably within 16BCD. Thus the order of the genes in the *madeirensis* X chromosome is as follows: centromere --- proximal break point inv MAD1 --- [ac^L; $+^{ve}$]---- distal break point of MAD1 ---- Bx^+ ------ Dia^m --- tip. Brackets indicate that we are not yet sure of the relative positions of the markers ac and ve.

A single strain of *D. subobscura* was used. Its X chromosome was marked by the alleles ve, ac^D (dark antennae colour, characteristic of this species), Bx and Dia^s. The gene arrangement of the X chromosome of this strain being A_{ST}, the corresponding order of genes is: centromere ------- [ve; ac^D] ----- Bx -------Dia^s --- tip. From interspecific backcrosses the distance between Bx and the distal break point of inversion MAD1 was found to be equal to 21.1 cM and that between Bx and Dia to 38 cM. These distances apparently are grossly underestimated, since double crossing overs were not detected between the positions of these markers.

The crosses

The progeny from two backcrosses were examined: F_1 females, originating from a cross of females of the strain of *madeirensis* to males from the marked strain of *subobscura*, were backcrossed to males either of the strains of their *madeirensis* parent or of their *subobscura* one. Male progeny from these two crosses were separately classified according to the phenotypic markers of the four characters examined. For every class recorded the genetic background is heterogeneous, since female F_1 were used for the cross. This contributes to the within class variance. Fertility was diagnosed by the presence of motile spermatozoa, established by microscopic examination.

Results

Viability

Several phenotypic classes were produced from both crosses: parentals; those derived from one crossover between Dia and Bx; those from one crossover between Bx and [ve – ac]; those from two crossovers, one between [ve – ac] and Bx, and another between Bx and Dia; finally those from two crossovers within the inversion in heterozygote state, MAD1/ST, which brings $+^{ve}$ out of MAD1 in the otherwise *subobscura* chromosome A_{ST} and vice versa. Since each of these classes appears with a different frequency depending on genetic distances between loci, we can only make two types of assessments: one is to compare the two complementary types of the same class. These should be of equal number if they have the same viability. We cannot assume that the numerically larger class has a normal viability (a 100% one) since all genotypes from an interspecific backcross are generally affected, each to a different extent. We may, however, estimate the reduction of viability compared to the corresponding numerically larger class. The relevant information is shown in Table 1. When the male parent is of *madeirensis* origin (*madeirensis* background) the only significant difference is found between males differing by an entire X chromosome. The viability of individuals bearing a *subobscura* X chromosome is diminished, being only 62% of the viability of those having a *madeirensis* X chromosome.

In contrast, all parts of the *madeirensis* X chromosome, except for the region marked by Dia, on a foreign background decrease viability when the male parent is of a *subobscura* origin. The viability decreases, being 46% or less of that of the complementary class. The region of the markers ve – ac exerts the greatest influence. This is the part covered by inversion MAD1; contiguously to it, but with a milder effect on viability reduction, comes the region marked by Bx.

Sterility

An inspection of Table 2 reveals the presence of three factors affecting sperm motility. First, in the case where the male parent is of *madeirensis* origin (this means

Table 1. Dissection of chromosome X, segments affecting male viability in the backcross progeny. The same four markers were used on the *subobscura* X chromosome; s indicates that the region of the marker is of *subobscura* origin and m of *madeirensis* one. Two different backcrosses were used, A and B, listed in separate columns. The chi-square tests whether every two complementary classes are of equal numbers, as expected (1 d.f.). At the second part of the Table chi-squares test the effect of each marker on viability (1 d.f.).

Backcross males from: A/♀ F_1 [*madeirensis* X *subobscura*] X ♂ *D. madeirensis* B/♀ F_1 [*madeirensis* X *subobscura*] X ♂ *subobscura* X chromosome constitution				A	χ^2	B	χ^2
ve	ac	Bx	Dia				
s	s	s	s	41		103	
m	m	m	m	66	5.53	48	19.18
s	s	s	m	35		68	
m	m	m	s	41	0.47	31	13.09
s	s	m	s	5		12	
m	m	s	m	9	1.14	4	4.00
s	s	m	m	12		34	
m	m	s	s	11	0.08	16	6.48
s	m	m	m	1		0	
m	s	s	s	0		0	

Marker	A χ^2	B χ^2
ve	0.50	22.03***
ac	0.50	22.03***
Bx	3.56	13.78**
Dia	2.62	0.01

that the males of the backcross possess a *madeirensis* Y chromosome and majority of autosomes), an entire *subobscura* X chromosome leads to sterility. Fertility is only restored when the segment marked by the gene ac is of *madeirensis* origin, not when Dia or Bx segments are *madeirensis* (males with an X chromosome s --- m --- m --- m had fertility restored, but not those with a *subobscura* simple replacement of the region marked by Dia or Bx or a replacement of both of them; by *s* and by *m* we denote the origin of the region or segment, of *subobscura* or of *madeirensis* respectively). A *madeirensis* X chromosome in a background mostly of *madeirensis* origin is somewhat fertile. The percentage of males with motile sperm is increased when this chromosome has exchanged its Dia segment with a *subobscura* one (see chromosome m -- m -- m -- s in Tab. 2). This *subobscura* factor, increasing sperm motility, can also function in a *subobscura* genetic background. It is attested by the examination of the last four X chromosome genotypes from the second backcross reported in Table 3. The presence of a third factor is revealed by the inspection of the first four X chromosome genotypes in Table 3, referring to the second backcross reported. It is evident that males with an m -- m -- m -- m X chromosome in a *subobscura* background (Y and majority of autosomes) are sterile, as expected, but when the Bx region is replaced by a *subobscura* marker the fertility, is, at least partly restored.

Table 2. Factors affecting male sterility on the X chromosome. Four markers were used on the *subobscura* X chromosome, from centromere to the tip in the following order ve ac Bx Dia; s indicates that the region of the marker is of *subobscura* origin and m of *madeirensis*. Two different backcrosses were used. F means percentage of fertile males, N sample size.

Backcross males from:
♀ F_1 [*madeirensis* X *subobscura*] X ♂ *D. madeirensis*
X chromosome constitution (Y and autosome's majority are m)

	ve	ac	Bx	Dia	F	N
1)	s	s	s	s	0	41
2)	s	s	s	m	0	35
3)	s	s	m	s	0	5
4)	s	s	m	m	0	12
5)	s	m	m	m	100	1
6)	m	m	m	m	35	65
7)	m	m	m	s	61	41
8)	m	m	s	m	33	9
9)	m	m	s	s	18	11

Comparisons:	chi-square (1 d.f.)
6 to 7 (effect of Dia)	5.77*
6 to 8 (effect of Bx)	n.s.
6 to 9 (effect of Bx + Dia)	1 85

Backcross males from:
♀ F_1 [*D. madeirensis* X *D. subobscura*] X ♂ *D. subobscura*
X chromosome constitution (Y and autosome's majority are s)

	ve	ac	Bx	Dia	F	N
1)	m	m	m	m	0	48
2)	m	m	m	s	0	31
3)	m	m	s	m	50	4
4)	m	m	s	s	13	11
5)	s	s	s	s	69	103
6)	s	s	s	m	26	68
7)	s	s	m	s	42	12
8)	s	s	m	m	9	34

Comparisons:	chi-square (1 d.f.)
5 to 6 (effect of Dia)	31.395***
5 to 7 (effect of Bx)	3.551
5 to 8 (effect of Bx + Dia)	41.521***
4 to 5 (effect of ve + ac)	15.637***
3 to 5 (effect of ve + ac + Dia)	n.s.

Abnormal head shape in males

Table 3 contains the data relevant to the head shape in males. The first part of the Table presents the different X chromosomes in a *madeirensis* genetic background (Y chromosome and majority of autosomes). In this background, an abnormal shape of the head is induced by a *subobscura* X chromosome especially

Table 3. Factors affecting abnormal head shape in the males located on the X chromosome. Data from two different backcrosses. ABN: percentage of males with abnormal head, other symbols as in Table 1.

Backcross males from:
♀ F_1 [*madeirensis* X *subobscura*] X ♂ *madeirensis*
X chromosome constitution (Y and autosome's majority are m)

ve	ac	Bx	Dia	ABN	N
s	s	s	s	50	50
s	s	s	m	53	30
s	s	m	s	38	8
s	s	m	m	58	12
s	m	m	m	100	2
m	m	m	m	23	71
m	m	m	s	19	42
m	m	s	m	43	7
m	m	s	s	13	15

Chi-squares (1 d.f.) testing homogeneity between s and m segments of the markers concerning the production of abnormal head, thus testing the influence of the segments in the production of the pathological character:

ve	ac	Bx	Dia
22.01	19.61	1.50	0.30

Backcross males from:
♀ F_1 [*madeirensis* X *subobscura*] X ♂ *subobscura*
X chromosome constitution (Y and autosome's majority are m)

ve	ac	Bx	Dia	ABN	N
s	s	s	s	11	102
s	s	s	m	22	68
s	s	m	s	10	10
s	s	m	m	15	34
s	m	m	m	100	1
m	m	m	m	93	44
m	m	m	s	83	29
m	m	s	m	71	17
m	m	s	s	93	14

Chi-square tests as above in the same Table.

ve	ac	Bx	Dia
150.62	162.65	41.46	0.30

by the part within the sections delimited by the inversion MAD1 in the other species, *D. madeirensis*. These sections include the loci ve and ac. The two loci seems to be nearly completely linked, since the F_1 females are heterozygous for this inversion. The remaining part of the chromosome up to its tip, that is the segments marked by Bx and Dia, do not play any important role. Thus, the presence of at least one gene in the [ve – ac] region is assumed in the *subobscura* X chromosome. In the second backcross, the Y chromosome and the majority of the autosomes are of *subobscura* origin. The abnormal head shape is influenced by that part of the *madeirensis* X chromosome which includes inversion MAD1, that is genes ve and

Table 4. Factors located on the X chromosome affecting the presence of extra sex combs in males. Data from two different backcrosses, A and B. ESC: number of individuals with extra sex combs, NESC number of males without extra sex combs, other symbols as in previous Tables. The second part of the Table presents chi-square testing the influence exerted by the different segments.

Backcross males from:
A/♀ F_1 [*madeirensis* X *subobscura*] X ♂ *madeirensis*
B/♀ F_1 [*madeirensis* X *subobscura*] X ♂ *subobscura*

X chromosome constitution				A		B	
ve	ac	Bx	Dia	ESC	NESC	ESC	NESC
s	s	s	s	2	46	0	100
m	m	m	m	16	53	35	12
s	s	s	m	2	31	1	67
m	m	m	s	4	42	24	3
s	s	m	s	0	3	0	11
m	m	s	m	1	8	5	1
s	s	m	m	0	12	0	35
m	m	s	s	1	13	7	8
s	m	m	m	0	2	1	0
m	s	s	s	0	0	0	0

Marker	A χ^2	B χ^2
ve	8.68*	204.67***
ac	8.68*	205.14***
Bx	3.51	77.45***
Dia	4.34*	1.79

ac, and also by a region outside MAD1 marked by Bx. No influence is detected by the segment marked by Dia. Thus, we should postulate the presence of at least two genes on the *subobscura* X chromosome, on in the MAD1 region and another at the Bx segment.

Extra sex combs in males

Table 4 displays all relevant information concerning the presence of an abnormal phenotypic character, the presence of extra sex combs in the male (Khadem and Krimbas, 1991b, 1993; Papaceit et al., 1991). These appear when the X and Y chromosomes are heterospecific (incompatible) and in combination with autosomes of mixed origin. When the X chromosome is of *madeirensis* origin and the Y of *subobscura* along with a majority of *subobscura* autosomes, then the segments that influence the appearance of this trait are those of [ve – ac], and of Dia. Thus, the existence of at least two different factors should be assumed on the X chromosome of *madeirensis*, one at the [ve – ac] region and another near Dia. When the X chromosome is of *subobscura* origin together with a *madeirensis* Y and majority of autosomes, the segments that influence the appearance of extra sex combs are those marked by [ve – ac] and Bx. Thus, the presence of at least two loci should be postulated on the *subobscura* X chromosome, one in the [ve – ac] region and another in the Bx one.

Discussion

In previous publications we already presented data on the genetics of these four traits (Khadem and Krimbas, 1991a, 1991b, 1993). We found an important reduction of viability in the males from the backcross which carry a *madeirensis* X chromosome together with a Y and a majority of autosomes from *subobscura* origin. In the present publication we further analyse this result and attribute it to several (at least two) factors on the *madeirensis* X chromosome located in the region comprising MAD1 inversion and the contiguous to it segment marked by Bx.

The sterility of F_1 males was attributed to the different origin of the two sex chromosomes: males with such an incompatibility either had small testes or if they were of normal size either they did not contain sperm or they were fillled with immotile sperm. Of course, as with viability, several autosomes were also implicated. In the present study we have identified three segments responsible for male sterility: in incompatible combinations of the sex chromosomes, a *madeirensis* segment marked by antennae colour, ac, in an otherwise *subobscura* X chromosome, may restore its fertility when combined with a *madeirensis* Y. Another *subobscura* segment, marked by Bx, bears a factor or factors responsible for restoring male fertility—at least partially—in the cases where the remaining X chromosome is of *madeirensis* origin in the presence of a Y *madeirensis* chromosome and a *madeirensis* majority of autosomes. These autosomes apparently contain some factors controlling fertility, since only 4 out of 20 such individuals are fertile (the last two lines, 8 and 9, from the first cross reported in Tab. 2). The third factor was unexpected: it is located near the tip region of the *subobscura* X chromosome and seems to increase sperm motility in both species!

Males normally have sex combs in the first and second segments of the tarsi of the first pair of legs. The presence of extra sex combs in males (in the second and sometimes in the third pair of legs) was already found to be highly dependent on the X chromosome when Y and autosomes were of a different specific origin. Cytoplasm was also implicated as well as the E autosome (this last chromosome also seems to control the number of teeth in the sex combs of normal males, together with chromosomes J and O; Khadem, unpubl. data). In a parallel study, Papaceit et al. (1991), using another *madeirensis* strain, have detected an influence of the X

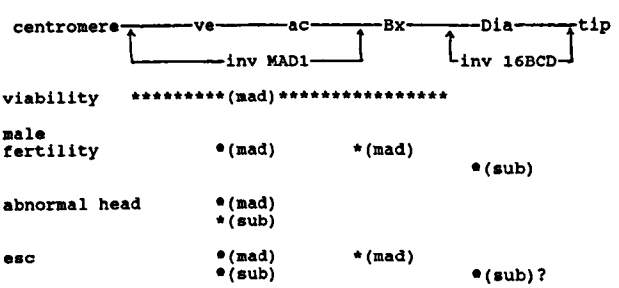

Fig. 1. Map of the X chromosome.

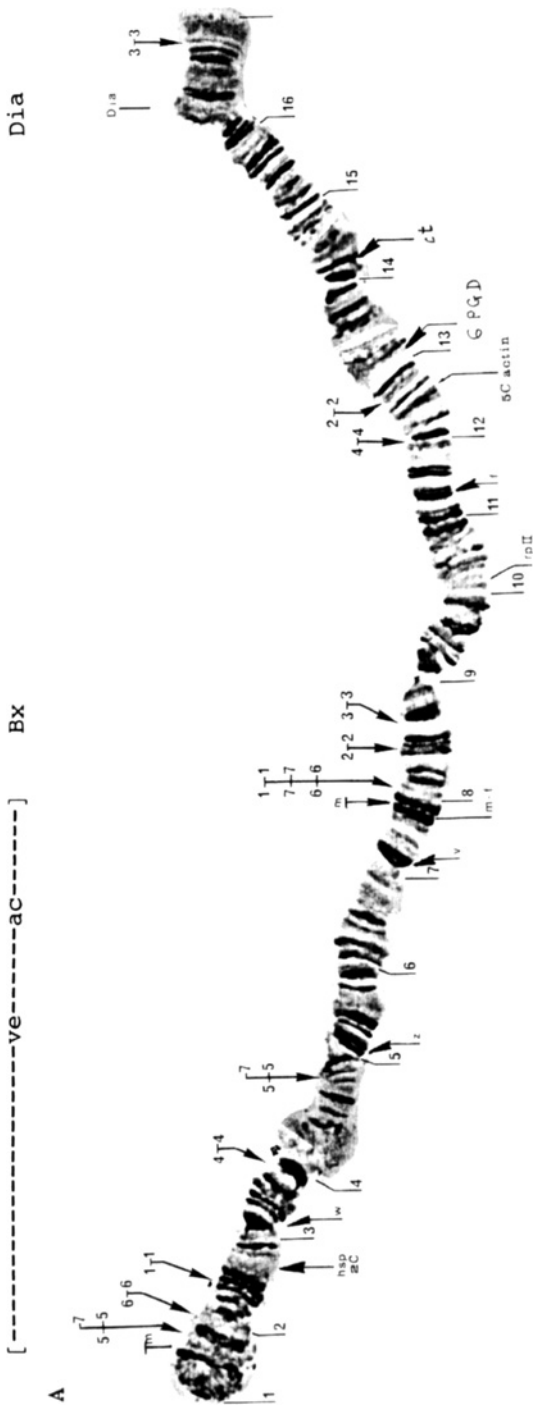

Fig. 2. The X chromosome of *Drosophila subobscura*. The 16 sections are indicated along with the breakpoints of inversions and some cytologically positioned genes. Also are shown the segments discussed in this study together with the approximate locations of the markers used.

chromosome and/or cytoplasm as well as that of the four autosomes mentioned here according to the decreasing order of their effect: U, E, O and J. In the present study, we have detected two regions on the X chromosome of *madeirensis* controlling the presence of extra sex combs, one at the part contained by MAD1 and another one near Dia (however, the data show a statistical significance only at the 0.05 probability level). On the other hand, the *subobscura* X chromosome also carries such genetic factors, one in the region corresponding to the MAD1 inversion and another in the region marked by Bx.

In the previous studies, the abnormal shape of the head was found to be influenced by an X chromosome combined with a heterospecific Y and a heterospecific majority of autosomes. A *madeirensis* X chromosome displays a greater effect than otherwise. Two autosomes, E and O, also contribute to this effect. The dissection of the X chromosome, in the context of the present study, permitted the identification of two regions on the *madeirensis* and one on the *subobscura* X chromosomes responsible for these effects. Thus, at least two *madeirensis* and one *subobscura* gene are responsible for the development of this abnormal character. The relative positions of the factors postulated are shown in Figure 1, while Figure 2 is a photograph of the *subobscura* X chromosome divided into 16 sections and the positions of some landmarks. Our estimates of the number of genes contributing to the isolation mechanisms are probably too low: first, segments identified may contain more than one unit. Second, the paucity of markers used to use in the two species examined may have left undetected the presence of other factors on the X chromosome. The available data does not allow a meaningful answer to the question whether the factors described here are similar to those which other authors have identified in the *melanogaster* group of species; e.g., *Ods* is located midway between *f* and Bx in *D. mauritiana*, that is between sections 15F and 17C of the giant chromosome. However, the X chromosome has been much rearranged: in *D. subobscura*, one of the most complete genetic maps of X chromosome indicates that the locus *f* is at 82.4 (the marker most near to the tip of the chromosome is *m*, being at position 0) and *Bx* is at position 171.03 (the entire chromosome is 258 units long) (Krimbas, 1993). Thus, the two markers are at a distance of about half the chromosome length, that is, they are not so closely linked as in *D. melanogaster* or its sibling species. Thus *Ods* cannot be compared to the factors near Bx we have detected here. A further study for a more precise localization of these markers is presently under way using molecular techniques.

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