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Studies of the species barrier between *Drosophila subobscura* and *D. madeirensis*. III. How universal are the rules of speciation?

MAHNAZ KHADEM & COSTAS B. KRIMBAS
Department of Genetics, Agricultural University, Iera Odos 75, Votanicos, 118-55 Athens, Greece

The prevalence of Haldane's rule and the large effect of the X chromosome are examined by analysing the genetics of seven abnormal characters in interspecies hybrids between *D. subobscura* and *D. madeirensis*. In two cases Haldane's rule is not obeyed (viability and abnormality of the head). In all characters except the abnormal abdominal tergites, an important effect of the X chromosome is observed. There is an asymmetry in the importance of the effect of the X chromosome. The X of *D. madeirensis* origin induces abnormality mostly in a mixed background. The Y chromosome plays an important role in determining male sterility.

Keywords: *Drosophila*, Haldane's rule, hybrid abnormality, hybrid sterility, hybrid viability, sex chromosomes.

Introduction

Interspecific hybrids provide a good opportunity to study the process of speciation, providing that at least some of the F_1 hybrids are fertile and viable. During the last decade, following the pioneering work of Dobzhansky (1936), we have witnessed a slowly growing number of genetic analyses of species differences (Coyne, 1984, 1985; Vigneault & Zouros, 1986; Lumme & Heikken, 1990; Khadem & Krimbas, 1991a), which has been accompanied by theoretical advances (Zouros, 1986; Charlesworth *et al.*, 1987; Coyne & Orr, 1989; Frank, 1991a; Read & Nee, 1991).

Generally it is believed that two rules are held in interspecific hybridization (Coyne & Orr, 1989):

1 It is generally the heterogametic sex that suffers most from sterility/inviability (Haldane's rule) (Haldane, 1922);

2 The X chromosome has a disproportionate effect compared to that of the autosomes on hybrid sterility/inviability. The large effect of the X chromosome was observed for both sexes in all the cases that have been studied (Dobzhansky, 1936; Patterson & Griffen, 1944; Coyne, 1984, 1985; Orr, 1987, 1989; Khadem & Krimbas, 1991a). Although the mainstream of the theoretical generalization is in support of the rules (Coyne *et al.*, 1991; Frank, 1991b) the significance of Haldane's rule has been questioned by some authors (Read & Nee, 1991). On the other hand, among the opponents of the rule there is disagreement as to its causation (Coyne *et al.*, 1991; Frank, 1991a). In the present paper we report on the prevalence of Haldane's rule, the role of the sex chromosomes and of the maternal effect in the genetic analysis of seven different characters in the hybrids between *Drosophila subobscura* and *Drosophila madeirensis*: (i) female fertility; (ii) testes size; (iii) sperm motility; (iv) viability; (v) extra sex combs; (vi) abnormal head shape; (vii) abnormal abdominal tergites. Hybrid male sterility, extra sex combs and abnormal head shape have been examined in detail only in one type of cross, i.e. F_1 females from *madeirensis* mother crossed to male *subobscura* (Khadem & Krimbas, 1991a,b). Extra sex combs have been also analysed by Papaceit *et al.* (1991) in the same type of cross. All the characters are included again in the present study, because for the first time we were able to analyse them in all the crosses. Does the same mechanism(s) also hold in the case of the reciprocal cross?

Materials and methods

Strains

One strain of vermilion flies was used. *Vermilion* (*ve*) is a marker on the X chromosome of *Drosophila subobscura*. As the originally described *vermilion* is lost we were unable to perform allelism tests between the two *vermilion* strains. Our strain was constructed from a cross between *D. madeirensis* females and *D. subobscura* males; the mutation occurred spontaneously in the backcross males. These males were sterile so their female sisters were crossed to *D. subobscura* males for seven generations. Therefore the strain was cleansed of *D. madeirensis* material and consists mainly of *D. subobscura* chromosomes, except for one cytological marker on the E chromosome that remains polymorphic. All the characteristics of the strain are those of *D. subobscura* (small body size, dark body colour, dark antennae, number of the sex combs' teeth). (The *vermilion* strain is called *D. subobscura* throughout this paper.) Another marker, also located on the X chromosome, controls the colour of the antennae (Khadem & Krimbas, 1991a). A single strain of *Drosophila madeirensis* was used. This species has light coloured antennae; the colour being darker in females.

Crosses

Two reciprocal crosses were performed:

1 *D. madeirensis* females were crossed to *D. subobscura* males and the F_1 females were backcrossed to either of the parental species.

2 *D. subobscura* females were crossed to *D. madeirensis* males and the F_1 females were backcrossed to either of the parental species.

The single strain of *D. madeirensis*, used in the present study, differs from the two strains that were used in our previous work (Khadem & Krimbas, 1991a,b). Using the new strain we were able to perform the cross between *D. subobscura* females and *D. madeirensis* males and obtain fertile and viable female progeny. Further explanations are given later in the text.

Abbreviations

The abbreviations *mad* and *sub* are sometimes used instead of *D. madeirensis* and *D. subobscura*. The origin of the maternal species is specified in brackets, for example F_1 [mad]. The crosses are all between F_1 females and males of the two parental species, therefore the male sign is dropped. Thus F_1 [mad] × mad indicates a backcross between F_1 females (from mad females and sub males) to mad males.

Results

Female sterility

A female was considered sterile when each of the ovaries had only one or no ovariole, semi-normal when they had between two and five ovarioles and normal when they had more than five ovarioles. Semi-normal females are considered fertile. In Tables 1 and 2 the numbers of parental, F_1 and B_1 females of each category are given.

The results show that a combination of madX/subX has a significant effect on the sterility of the females, when the original mother is *madeirensis*. A *madeirensis* X chromosome, combined with some foreign autosomal factor(s), fares better in foreign cytoplasm than in its own cytoplasm. A significant difference is also detected when the female progeny of F_1[mad] × mad and F_1[sub] × mad or F_1[mad] × sub and F_1[sub] × sub are compared ($\chi^2 = 53.3$ and 18.9, respectively for 2 d.f., $P < 0.001$). This is a strong indication of the importance of the maternal effect in determining female sterility in both the F_1 and the backcross females.

Testes size

F_1 males from a *madeirensis* mother have testes of almost normal size (67 normal, 3 one normal + one small testis and 4 small). When the mother is *subobscura*, about half of the F_1 males have normal or almost normal and the other half have small testes (33 normal — 36 small). The difference between the two reciprocal crosses is significant ($\chi^2 = 37.36$ for 1 d.f., $P < 0.001$). Note, however, that the results in this second cross vary greatly from one repetition to another, and thus the difference between the two crosses should be treated with some caution.

From Tables 3 and 4 it is clear that in all possible crosses, the compatibility of the sex chromosomes leads almost always to individual backcross males with normal testes size. On the other hand incompatibility of the sex chromosomes always results in small and empty testes. There are some exceptional cases, which are marked with an asterisk on the left of the Tables. The exceptional cases are 20 out of 304 and 8 out of 222 in Tables 3 and 4, respectively.

Sperm motility

In general, males with testes of normal size have sperm and the ones with small testes do not. However, sperm can be either motile or immotile. Males with motile sperm are considered to be fertile (regardless of the percentages of their motile sperm). Sterile males are the ones that either do not have any sperm or their sperm is immotile. When the sex chromosomes are compatible, about half of the backcross males with subcytoplasm and two-thirds with mad cytoplasm are fertile (see Tables 3 and 4).

Table 1 Number of sterile (S), semi-normal (S-N) and normal (N) females in parental species, F_1 hybrids and hybrid backcrosses

Generation	Cross	S	S-N	N	χ^2 (2 d.f.)	Percentage†
P	mad	9	18	87		8
P	sub	0	7	53		0
					6.61*	
F_1	[mad]	8	0	20		29
F_1	[sub]	6	12	20		16
					10.66**	
B_1	F_1 [mad] × mad	73	19	24		63
B_1	F_1 [mad] × sub	65	27	55		44
					10.53**	
B_1	F_1 [sub] × mad	28	8	79		24
B_1	F_1 [sub] × sub	9	6	38		17
					2.25	

mad = *D. madeirensis*, sub = *D. subobscura*, F_1 [mad] is from mad females crossed to sub males, F_1 [sub] is from sub females crossed to mad males. F_1 females of the two kinds are crossed to mad or sub males.

†The percentage of sterile females is given in the last column.

Each chi-square test examines homogeneity of the progeny for the two associated crosses. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 2 Number of sterile (S), semi-normal (S-N) and normal (N) backcross females classified according to the origin of their X chromosomes. Chi-square tests examine the significance of the effect of the X chromosome in determining female sterility

Generation	Cross	X origin	S	S-N	N	χ^2 (2 d.f.)	Percentage
B_1	F_1 [mad] × sub	madX/subX	47	6	13		71
		subX/subX	18	21	42		22
						36.16***	
B_1	F_1 [sub] × sub	madX/subX	7	2	18		26
		subX/subX	2	4	20		8
						3.52	

See footnote to Table 1 for definition of crosses and percentage.
$*P < 0.01$, $**P < 0.01$, $***P < 0.001$.

Viability

Female viability is compared to male viability in F_1 and backcross generations (viability is measured by the number of emerged flies). F_1 progeny from a *madeirensis* mother have a normal sex ratio and those from a *subobscura* mother an abnormal sex ratio (Table 5). In an earlier work this cross was reported to produce an extreme sex ratio in favour of the males (Khadem & Krimbas, 1991a; Papaceit *et al.*, 1991). Our current results differ from those in an earlier work, in the sense that the ratio of the females to males increased significantly. It varies from 1:2 to 1:1 in different performances of the same cross. Preliminary experiments indicate that the stock of *D. madeirensis* used in the present work is the cause of the increased number of females in the F_1 generation.

Lower viability is also observed in the case of backcross females of F_1[sub] × mad (Table 5). It is possible that some chromosomal factor(s) of *madeirensis*, including those of the X chromosome, interact with the *subobscura* cytoplasm causing this inviability. We cannot be more specific about females' viability because in some crosses we were unable to distinguish madX/

Table 3 Testis size and the presence or absence of sperm in backcross males. All individuals have *madeirensis* cytoplasm and different combinations of X and Y chromosomes. The origin of the X chromosome is detected by eye and antennae colour. The origin of Y is known from the type of backcross. Incompatible combinations are those bearing X and Y from a different origin and they display small testes, usually empty. Exceptions to this rule are indicated by an asterisk (*) on the left of the table

Eye colour	Antennae colour	X	Y	Testis size	Motility	*n*	Inc.
F_1[mad] × mad							
* ve	Dark	sub	mad	Normal	Immotile/absent	9	Yes
ve	Dark	sub	mad	Small	Absent	78	Yes
* ve	Light	sub/mad	mad	Small	Absent	7	Yes/no
+	Light	mad	mad	Normal	Motile	37	No
+	Light	mad	mad	Normal	Immotile	19	No
F_1[mad] × sub							
* +	Light	mad	sub	Normal	Immotile/absent	9	Yes
+	Light	mad	sub	Small	Absent	38	Yes
ve	Dark	sub	sub	Normal	Motile	65	No
ve	Dark	sub	sub	Normal	Immotile	40	No
* ve	Dark	sub	sub	Small	Absent	2	No

n = number of flies examined, Inc. = incompatibility of the sex chromosomes.

Table 4 Testis size and the presence or absence of sperm in backcross males. All individuals have *subobscura* cytoplasm and different combinations of X and Y chromosomes. The origin of the X chromosome is detected by eye and antennae colour. The origin of Y is known from the type of backcross. Incompatible combinations are those bearing X and Y from a different origin and they display small testes, usually empty. Exceptions to this rule are indicated by an asterisk (*) on the left of the table

Eye colour	Antennae colour	X	Y	Testis size	Motility	*n*	Inc.
F_1[sub] × mad							
* ve	Dark	sub	mad	Normal	Absent	2	Yes
ve	Dark	sub	mad	Small	Absent	50	Yes
* ve	Light	sub/mad	mad	Small	Absent	1	Yes/no
+	Light	mad	mad	Normal	Motile	21	No
+	Light	mad	mad	Normal	Immotile	23	No
+	Light	mad	mad	1s/1n	Motile	1	No
F_1[sub] × sub							
* +	Light	mad	sub	Normal	Immotile	4	Yes
+	Light	mad	sub	Small	Absent	39	Yes
ve	Dark	sub	sub	Normal	Motile	37	No
ve	Dark	sub	sub	Normal	Immotile	39	No
ve	Dark	sub	sub	1s/1n	Motile	2	No
* ve	Light	sub/mad	sub	Small	Absent	2	Yes/no
* ve	Light	sub/mad	sub	Normal	Immotile	1	Yes/no

n = number of flies examined, Inc. = incompatibility of the sex chromosomes.

madX flies from those of madX/subX constitution. The case of males' viability is more clear: backcross males with a madX may have their viability reduced when they carry a subY and the majority of autosomes are of sub origin (Table 6) (the origin of Y coincides with that of the majority of autosomes).

Extra sex combs

F_1 males from the cross of *madeirensis* females to *subobscura* males have extra sex combs, in almost all cases, on the second and sometimes on the third pair of legs. However, the trait's expressivity differs in the repetitions of this cross. Female *subobscura* crossed to *madeirensis* males produced males mainly with normal phenotype.

From the results given in Table 7 and summarized in Fig. 1, it seems that the appearance of extra sex combs mainly depends on the origin of the X chromosome. Males bearing a madX/subY and a majority of sub autosomes almost always (80–91 per cent) display esc regardless of the origin of their cytoplasm. Males with madX/madY and a majority of mad autosomes display the character less frequently; the percentage depends on the origin of the cytoplasm. The appearance of esc phenotypes in the F_1 of the two reciprocal crosses fits well with the results explained above.

Table 5 Female viability is compared to male viability in F_1 and backcross generations in the two reciprocal crosses. Chi-square testing departure from a 1:1 ratio (1 d.f.) and its significance are given in the last column

Generation	Cross	n ♀♀	n ♂♂	χ^2 (1 d.f.)
F_1	[mad]	109	89	2.02
B_1	F_1[mad] × mad	340	364	0.818
B_1	F_1[mad] × sub	514	372	22.76***
F_1	[sub]	296	360	6.24**
B_1	F_1[sub] × mad	233	290	5.99*
B_1	F_1[sub] × sub	484	424	3.96

Table 6 Viability of flies with a different genetic constitution to the backcross progenies, classified according to the origin of their X chromosome. Chi-square tests examine the effect of this chromosome on the hybrid viability

Sex	Chromosome	X origin	Y origin	n	χ^2 (1 d.f.)
♂	F_1[mad] × mad	mad	mad	265	
		sub	mad	300	2.04
♂	F_1[mad] × sub	mad	sub	84	
		sub	sub	288	111.9***
♀	F_1[mad] × sub	mad/sub	—	239	
		sub/sub	—	275	2.52
♂	F_1[sub] × mad	mad	mad	142	
		sub	mad	148	0.12
♂	F_1[sub] × mad	mad	sub	56	
		sub	sub	121	23.1***
♀	F_{21}[sub] × sub	mad/sub	—	74	
		sub/sub	—	97	2.83

**$P < 0.01$, ***$P < 0.001$, n = total number of emerged flies.

Abnormal head shape

Several individuals in the F_1 and the backcross generations display an abnormal head shape. It is clear from the results given in Table 8, that the cytoplasm does not play a role in determining this abnormality in hybrids (e.g. backcross females with madX/subX in a mad cytoplasm do not differ from madX/subX in a sub cytoplasm). The origin of the X chromosome(s), however, has a strong effect on the presence of the head abnormality. Females madX/subX show the abnormality much more frequently than those of subX/subX.

In the case of the backcross males the results in Table 8 are summarized in Fig. 2. Head abnormality is displayed more often in individuals with a madX in combination with a Y and half or the majority of autosomes of *subobscura* origin.

Abnormalities of abdominal tergites

These abnormalities comprise all anomalies observed in A1–A8 segments (abdominal tergites). They include the presence of incomplete tergite(s), the fusion of two or three tergites, or simply the absence of one or more tergites. It appears from the results given in Table 9 that the X chromosome does not play any important role in causing this abnormality. Cytoplasmic factor(s) are probably involved. The percentage of abnormal flies increases when mad cytoplasm is substituted by sub cytoplasm. The highest percentages of abnormal tergites occur in the backcross individuals with a sub cytoplasm and the majority of mad chromosomes. This is an indication of a cytoplasm–chromosome interaction.

Discussion

Seven abnormal characters have been analysed here, six of which are found only in the hybrids; the seventh (abnormal abdominal tergites) is also present in the parental species but with a lower frequency. The existence of these anomalies in the hybrids is an indication of some aberrant developmental process. First we will

Table 7 Extra sex combs and normals in backcross males are classified according to the origin of their cytoplasm, Y and X chromosomes. Chi-square tests compare the difference between the two types of X chromosome in inducing extra sex combs anomaly in different genetic make ups

Cross	Cytoplasm	X	Y	Auto.	Esc	*n*	Percentage esc	χ^2 (1 d.f.)
F_1[mad] × mad	mad	mad	mad	mad	19	107	18	
	mad	sub	mad	mad	10	128	8	5.03*
F_1[mad] × sub	mad	mad	sub	sub	45	56	80	
	mad	sub	sub	sub	0	165	0	161.95***
F_1[sub] × mad	sub	mad	mad	mad	5	164	3	
	sub	sub	mad	mad	2	150	1	0.7
F_1[sub] × sub	sub	mad	sub	sub	72	79	91	
	sub	sub	sub	sub	2	196	1	13.5***

Auto. = majority of autosomes, Esc = extra sex combs, n = total number of flies $^*P < 0.05$, $^{***}P < 0.001$.

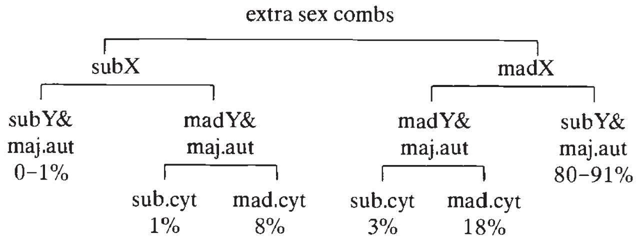

Fig. 1 A classification of the effect of the sex chromosomes, the majority of autosomes and cytoplasm on the extra sex combs phenotype.

discuss the prevalence of Haldane's rule and the effect of the X chromosome on these characters. Then we will explain the asymmetry observed in the role of the two different X chromosomes (madX and subX).

Both Haldane's rule and the X chromosome rule are concerned with sterility/inviability of the hybrids (i.e. fitness). Hybrid sterility agrees with the two rules.

1 F_1 male sterility is absolute (all males are sterile) but females are partially sterile. This illustrates a partial barrier, i.e. species that have not reached a complete genetic isolation, at least under laboratory conditions.

2 Male sterility appears whenever there is an incompatibility between the sex chromosomes. This type of sterility in the backcross progenies is characterized by the presence of abnormally small testes, usually empty of sperm. When the sex chromosomes are compatible (homospecific) the testes are large and the male may be sterile or fertile, depending on autosomal factors. We have reported on them in detail by examining the backcross males produced by F_1 [mad] crosses to *subobscura* males (Khadem & Krimbas, 1991a). On the other hand, sperm mobility is studied in backcross individuals with a normal testis size, i.e. with compatible sex chromosomes. Thus the detection of sex chromosome influence is methodologically excluded. It is quite possible that there are factors on the X chromosome that determine sperm motility. The near absence of individuals with large testes and incompatible sex chromosomes in the backcross progeny precludes the study of the influence of the X chromosome on the motility of sperm.

The effect of the X chromosome was observed in the case of hybrid inviability but Haldane's rule was sometimes violated. Lower viability of the homogametic sex was detected in the F_1 hybrids from *subobscura* mothers and also in the progeny of the latter females crossed to *madeirensis* males. It is mentioned earlier that the extreme case of female lower viability has somehow been modified in the present work. The two stocks of *D. madeirensis* (used in previous studies), when crossed to female *subobscura*, produced inviable progeny that died as embryos. Males from another stock of *D. madeirensis*, which were used in this study, were crossed to *subobscura* females that showed a recovery of the F_1 hybrid females (the sex ratio changed from 5 to 25 or even 50 per cent); the total number of progeny also increased considerably. This system is analogous to the hybrid rescue genes described in the species of the *D. melanogaster* subgroup (Hutter & Ashburner, 1987; Hutter *et al.*, 1990). We assume that our new strain of *D. madeirensis* probably carries an allele analogous to *Hmr* which enables female hybrids to be rescued. Note that if this assumption is correct, then the gene also rescues some of the hybrid males.

The heterogametic sex suffers a lower viability, when the F_1 females from a *madeirensis* mother were crossed to *subobscura* males. The large effect of the X chromosome and deviation from Haldane's rule has been also reported in a cross between *D. montana* and

Table 8 Flies with abnormal head shape, classified in F_1 and the backcross generation according to their cytoplasm and sex chromosomes. Chi-square tests examine the significance of the origin of the X chromosome in determining the shape of the head in different types of genetic background

Sex	Cross	Cytoplasm	Y	X	A	I	χ^2 (1 d.f.)	Percentage
♂	F_1[mad]	mad	sub	mad	59	89		66
♂	F_1[sub]	sub	mad	sub	6	62	49.19***	10
♀	F_1[mad]	mad	—	mad/sub	73	85		86
♀	F_1[sub]	sub	—	mad/sub	74	79	2.39	94
♂	F_1[mad] × mad	mad	mad	mad	28	214		13
		mad	mad	sub	84	257	23.9***	33
♂	F_1[mad] × sub	mad	sub	mad	72	111		65
		mad	sub	sub	25	293	147.1***	9
♂	F_1[sub] × mad	sub	mad	mad	35	178		20
		sub	mad	sub	59	178	8.4**	33
♂	F_1[sub] × sub	sub	sub	mad	69	114		60
		sub	sub	sub	2	222	149.66***	1
♀	F_1[mad] × sub	mad	—	mad/sub	138	176		78
		mad	—	sub/sub	3	213	247.93***	1
♀	F_1[sub] × sub	sub	—	mad/sub	87	114		76
		sub	—	sub/sub	4	158	160.80***	3

**$P < 0.01$, ***$P < 0.001$, A = number of abnormal individuals, T = total number of flies examined.

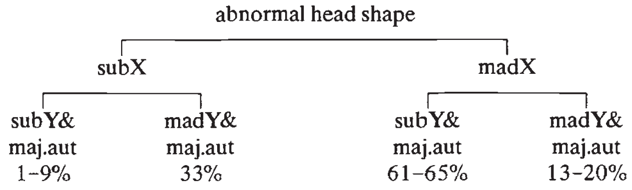

Fig. 2 The effect of the sex chromosomes and the majority of autosomes on head abnormality.

D. americana texana males (Patterson & Griffen, 1944).

Previously we reported (Khadem & Krimbas, 1991b) that the two characters of extra sex combs and abnormal head shape are positively correlated and that there is a close link between these two characters and the sterility/inviability of hybrids. Regardless of the applicability of this to other types of cross, our present data show that the X chromosome has a strong effect on the determination of these two characters. On the other hand, Haldane's rule is not obeyed with regard to the abnormality of the head shape (extra sex combs is specifically a male character). A head shape abnormality appeared in all the progeny of one of the crosses (F_1 progeny of *madeirensis* mother), and only in F_1 females of the reciprocal cross (of *subobscura* females). Curiously it is the same cross in which hybrid viability also deviates from Haldane's rule.

The last character, abnormal abdominal tergites, is rare and it is premature to decide whether the effect of the X chromosome is really so little, as one of the cases might suggest. Furthermore, because this abnormality has little influence on hybrid fitness (except in a very few cases where many segments are involved) it may not be considered as an exception to the two rules.

In addition to the strong effect of the X chromosome on some characters (female sterility, male viability, extra sex combs and head shape abnormality), our data show the existence of an asymmetry between the influence of the two Xs (see Figs 1 and 2). In all these cases the madX chromosome has a greater tendency to induce abnormalities. Our unpublished results show that an autosomal factor which affects head and extra sex combs anomalies, interacts with subX in homozygous and with madX either in the homo- or heterozygous state. Therefore, there are more abnormal individuals with madX than there are with subX. How can this asymmetry be explained? One might consider that it is due to chance: when two populations diverge during the speciation process, the chromosome(s) of one population might accumulate those changes that render it (them) incompatible with the genetic background of another. The converse may not be true. Alternatively the original split between the two species may be asymmetric, i.e. only one population of a restricted size isolates itself from other large group of

Table 9 Percentage of flies with abnormal abdominal tergites in parental species, F_1 and backcross hybrids. Chi-square tests show whether the origin of the sex chromosome in determining this anomaly is significant

Generation	Cross	X	Y & majority of autosomes	A	T	χ^2 (1 d.f.)	Percentage
♀ P mad	mad	mad/mad	—	2	129		1
♂ P mad	mad	mad	mad	2	200		1
♀ P sub	sub	sub/sub	—	3	55		5
♂ P sub	sub	sub	sub	3	69		4
♀ F_1	[mad]	mad/sub	—	1	103		1
♂ F_1	[mad]	mad	sub	2	110		2
♀ B_1	F_1[mad] × mad	mixed	—	19	251		8
♂ B_1	F_1[mad] × mad	mad	mad	7	112		6
		sub	mad	9	144	0.00	6
♀ B_1	F_1[mad] × sub	mad/sub	—	3	76		4
		sub/sub	—	3	109	0.563	3
♂ B_1	F_1[mad] × sub	mad	sub	3	29		10
		sub	sub	4	88	2.9	4
♀ F_1	[sub]	mad/sub	—	8	55		14
♂ F_1	[sub]	sub	mad	15	106		14
♀ B_1	F_1[sub] × mad	mixed	—	29	130		22
♂ B_1	F_1[sub] × mad	mad	mad	20	103		16
		sub	mad	22	82	4.04*	27
♀ B_1	F_1[sub] × sub	mad/sub	—	1	20		5
		sub/sub	—	3	40	0.00	7
♂ B_1	F_1[sub] × sub	mad	sub	4	13		31
		sub	sub	3	35	3.30	9

A = abnormal abnominal tergites, T = total number of flies examined, *$P < 0.05$.

populations that occupy an important geographical area. In this case populations of the larger area would change little and would retain most of their original genetic variability. The new species, following its isolation, could undergo a reorganization of the small amount of genetic variability that it contains and be subjected to selective pressures which ensure a fine tuning between its components. It would therefore less easily tolerate foreign genetic material. Here, *D. madeirensis* is the species with the restricted distribution (it is found only in the Madeira island) and is apparently monomorphic for chromosomal inversions, while *D. subobscura* has a much wider geographical distribution and is one of the richest species in inversion polymorphism in its natural populations.

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