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'Exceptional sons' from *Drosophila melanogaster* mothers carrying a balancer *X* chromosome

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Summary

This study reports on exceptional males which are obtained by using *Drosophila melanogaster* mothers carrying the balancers *In(1)FM6* or *In(1)FM7* as one of their *X* chromosomes. The phenomenon was first observed in interspecific crosses between *D. melanogaster* females and males of its closest relatives which normally produce unisexual female hybrid progeny. Whereas hybrid sons from these crosses die as third instar larvae, the presence of the particular *X* balancers in the mother allows a low percentage of sons to survive. Similar sterile males are also observed among non-hybrid flies. Data are presented which suggest that the males thus generated could be hyperploid for part of their *X* chromosome as a result of a meiotic event in their mothers or else they could start life as female zygotes and change sex through a mitotic event at an early stage.

1. Introduction

Crosses between *Drosophila melanogaster* females and males of each of its sibling species, *D. simulans*, *D. mauritiana* or *D. sechellia* normally produce hybrid daughters exclusively, which are sterile (Lachaise *et al.* 1986). Hybrid sons die as third instar larvae or pseudopupae (Sturtevant, 1920; Lemeunier *et al.* 1986; Lachaise *et al.* 1986). Nevertheless, about one male for every 2000 females survives to adulthood. Such males result from the fertilization of non-disjunctional, nullo-*X* *D. melanogaster* eggs by *X*-bearing sperm from the sibling species; thus, they are of *XO* constitution and are expected to be sterile. Two mutations have been discovered which allow the otherwise inviable regular male hybrids between the above species to survive. The first mutation (*Lhr*; 2–95) was reported by Watanabe (1979) in *D. simulans*. It rescues the lethal hybrids between *D. melanogaster* and *Lhr* *D. simulans*. The second mutation (*Hmr*; 1–31.84) found by Hutter & Ashburner (1987) in *D. melanogaster* has the same phenotypic effect in hybrid progeny from crosses between *Hmr* *D. melanogaster* and either *D. simulans*, *D. mauritiana* or *D. sechellia*. All rescued hybrids remain sterile.

This study focuses on the observation of exceptional male hybrid survivors produced by crossing *D. melanogaster* females heterozygous for certain *X* chromosome balancers to males from their sibling species, although such sons can equally be generated in non-hybrid (*D. melanogaster*) flies. The surviving hybrid males appear with a frequency some forty times higher than *XO* non-disjunctional males, which suggests that they result from a genetic event independent of non-disjunction.

2. Materials and methods

Crosses were done in units of ten 1-day-old virgin females and fifteen 5-day-old virgin males. Crosses were set up at 25 °C for 1–2 days followed by culture at 19 °C (Lee, 1978). Yeast-glucose medium was used for crosses nos 5, 6 and 9 whereas all other experiments were carried out on a richer medium (cornmeal, wheatmeal, oatmeal, dried yeast). The various mutations as well as the four balancer chromosomes referred to in the text and in the Tables are described by Lindsley & Grell (1968) or Lindsley & Zimm (1985–7). The mutations used are visible markers easy to score as they affect physical structures such as the shape and the colour of the eyes or the shape of the wings.

3. Results

Table 1 shows that, although the *X* chromosome balancers *In(1)Basc* and *In(1)FM1* do not yield any exceptional hybrid males, the balancers *In(1)FM6* and *In(1)FM7* (referred to as *FM6* and *FM7* below) consistently produce about 2% of such males. Remarkably, these survivors invariably express some of the maternal as well as some of the paternal *X*-

Table 1. *Progeny of crosses between* D. melanogaster *females carrying balancer* X *chromosomes and males from its sibling species* (D. simulans *and* D. mauritiana)

D. melanogaster mother	Percentages of males obtained/total number of females: Sibling sp. father*	Hybrid daughters‡	Non-disjunctional hybrid sons†	%	Exceptional hybrid sons and markers	%
1 *Basc/Df(1)527*‡	*D. maur.* S-7	3190	49+	1·54	0	0
2 *FM1/Df(1)527*	*D. maur.* S-7	1187	7+	0·60	0	0
3 *FM6/Df(1)527*§	*D. maur.* S-7	1655	16+	0·97	33 *Hw B*	1·99
4 *FM6/Df(1)522*‡	*D. maur.* S-7	242	2+	0·83	5 *Hw B*	2·07
5 *FM6/Df(1)N110*	*D. maur.* S-7	926	5+	0·54	4 *Hw B*	0·43
6 *FM6*/Canton-S	*D. maur.* S-7	569	14+	2·46	7 *Hw B*	1·23
7 *FM6/w cv sn*	*D. maur.* S-7	1452	6+	0·41	10 *Hw B*	0·69
8 *FM7/Df(1)527*‖	*D. maur.* S-7	1281	26+	2·03	30 *Hw B*	2·34
9 *FM7c/Df(1)HC133*	*D. maur.* S-7	703	4+	0·57	5 *Hw B*	0·71
10 *FM6/Df(1)527; Gla/Cy0*	*D. maur.* S-7	498	62 *Bal*¶	12·45	9 *Hw B*; *Bal*	1·80
11 *FM6/Df(1)527*	*D. sim. y w*	341	3 *y w*	0·88	7 *y Hw w B*	2·05
12 *FM6/Df(1)527*	*D. sim. w f*	254	2 *w f*	0·79	6 *Hw w B*	2·36
13 *FM6/Df(1)527*	*D. sim. y v f*	629	4 *y v f*	0·64	12 *y Hw v B*	1·91
14 *FM6/Df(1)527; Gla/Cy0*	*D. sim. w f*	302	22 *w f*; *Bal*	7·28	6 *Hw w B*; *Bal*	1·99
15 *FM6/Df(1)527; Gla/Cy0*	*D. sim. y v f*	460	28 *y v f*; *Bal*	6·08	8 *y Hw v B*; *Bal*	1·73

* *D. maur.* = *mauritiana*; *D. sim.* = *simulans*.
† These males are presumably *XO* due to fertilization of nullo-*X* ova by *X* sperm from the sibling species.
‡ *Df(1)522* and *Df(1)527* are small deletions in the 9D region.
§ *FM6* carries the following markers: $y^{31d}sc^{8}$ (*Hw*, referring here to the *Hw* effect) *dm B*.
‖ *FM7* carries the following markers: $y^{31d}sc^{8}$ (*Hw*) w^{a} sn^{z2} v^{of} $g^{4}B$.
¶ *Bal* = balancer chromosome (*Gla* or *Cy0*).

linked markers. A similar observation has been made by Steinmann–Zwicky (personal communication) in a cross between *D. melanogaster* females carrying *FM6* and *D. simulans* wild-type males. In all the experiments the exceptional males tended to emerge earlier than the non-disjunctional *XO* males.

As shown in Table 1 the exceptional males always express two markers carried by the *X*-balancer: the *Hw* effect [on $In(1)sc^{8}$] and *B* when their mothers are heterozygous for *FM6*, *FM7* or *FM7c*. However, they never express any of the other maternal *X*-linked (recessive) markers carried by these balancer chromosomes. Indeed, none of the exceptional males produced expressed either *y*, *w*, *dm*, *sn*, *v* or *g* carried by the balancers (see bottom of Table 1), suggesting that part of this chromosome is either absent or complemented in the exceptional males. Moreover, when mutations homologous to either *y*, *w* or *v* are carried by the sibling paternal *X* chromosomes, these mutations are expressed by the exceptional males. All the exceptional males are sterile, showing on dissection strongly reduced testes, accessory glands and ejaculatory ducts. They are also flightless, whereas *XO* non-disjunctional males do fly. The exceptional males usually have extra bristles on the vertex and the first and second segments of the antenna. The anterior ocellus is always very reduced or absent. The flies often have missing bristles on the mesonotum, as do normal species hybrids (Biddle, 1932).

Since at first sight the above phenomenon seems to be best interpreted on the basis of some abnormal segregation of maternal *X* chromosomes at meiosis [e.g. in a way somehow resembling the effect of the mutation *pal* (Baker, 1975) which causes loss of entire chromosomes], meiotic non-disjunction was artificially increased by introducing autosomal inversions, to see whether the two events were related. In the crosses nos 10, 14 and 15 (Table 1), using the second chromosome balancers *In(2LR), Gla* heterozygous with *In(2LR)O, Cy*, the frequency of non-disjunction of the maternal *X* chromosomes was considerably increased, resulting in an about 10-fold higher frequency of *XO* non-disjunctional males. However, this effect was not paralleled by an increased frequency in the appearance of the exceptional males. Although this observation by no means rules out the possibility that the origin of these males is a direct consequence of some meiotic event, the expressivity of the dominant markers (*Hw* effect and *B*) carried by the balancers under study cannot exclude the possibility that the source of the phenomenon might also be sought at the zygotic stage (see Discussion).

When either the *FM6* or the *FM7* balancer chromosomes are used in heterozygous condition over a deletion (Table 1), the average frequency of exceptional males is close to 2% (disregarding crosses nos 5, 6 and 9 which were carried out on a less rich medium as mentioned above). This is more than twice the frequency at which non-disjunctional *XO* males were recovered in these experiments. The frequency of exceptional males appears to be increased when a maternal *X* chromosome carries a deficiency het-

Table 2. *Progeny of crosses between* D. melanogaster *females, heterozygous for* Df(1)527 *and* In(1)FM6, *and males carrying various markers*

D. melanogaster mother	*D. melanogaster* father	Daughers	Regular sons (*FM6*)	Non-disjunctional sons	%	Exceptional sons	%
FM6/Df(1)527	*y v f*	464	207	4 *y v f*	0·86	2 *y Hw v B*	0·43
FM6/Df(1)527	*w cv sn*	612	273	3 *w cv sn*	0·49	0	0

Table 3. *Progeny of crosses between females heterozygous for* In(1)FM6, *and Canton-S males*

(*a*) Progeny of 156 isofemale lines. Genotype of the mothers = *FM6/Df(1)527*

Df(1)527/+ females	*FM6/+* females	*FM6/Y* males	Non-disjunctional males	%	Exceptional males	%
2891+	2812 *B*	2351 y^{31d} *Hw dm B*	21+	0·37	43 *Hw B*	0·75

(*b*) Progeny of 5 sublines selected from (*a*) (see text). Genotype of the mothers = *FM6/+*

+/+ females	*FM6/+* females	*FM6/Y* males	+/*Y* males	Exceptional males	
2566+	2355 *B*	1756 y^{31d} *Hw dm B*	2379+	7 *Hw B*	0·14

erozygous to the balancer *X*, as compared to the situation where an *X* chromosome free of structural aberrations is combined with one of the balancer *X*s in question (crosses nos 6 and 7).

Although it was first discovered in hybrids the above phenomenon is clearly not restricted to hybrids since it also occurs in *D. melanogaster* sons from mothers carrying *FM6* chromosomes. In a first experiment, *FM6/Df(1)527* females were crossed to both *y v f* and *w cv sn D. melanogaster* males (Table 2). The data indicate that with *y v f* fathers, *D. melanogaster* sons emerged phenotypically corresponding to the exceptional hybrid males reported above, although the former were produced at a substantially lower frequency. A second experiment consisted in setting up 156 isofemale lines from the *FM6/Df(1)527* stock, whose F_2 females were crossed to wild-type Canton-S males, in a search for abnormal sex ratios and/or phenotype among the progenies. There were 147 fertile lines, out of which 19 (12·9%) yielded *XO* non-disjunctional males and 33 (22·4%) yielded *Hw B* exceptional males which were all sterile (Table 3*a*). These data suggest that *D. melanogaster* exceptional males could be produced at a lower frequency (0·75%) as compared to what is observed in hybrids, although this discrepancy might largely be accounted for by inbreeding depression. In relation to this it is worth pointing out here that the *FM6* chromosomes were introduced into both *Df(1)522* and *Df(1)527* stocks from a single male about 12 months before the experiments started. This strongly suggests that the effect under investigation is recurrent and reflects a property of the balancer chromosome to undergo abnormal division. I have also attempted to select flies for a high production of exceptional sons to find out whether the trait under study was genetically based. Indeed crosses nos 5 and 6 (Table 1) suggest that the frequency of non-disjunctional males might be strain-specific. For this purpose, 5 isofemale sublines were set up from those of the above 147 lines which produced the most *Hw B* exceptional sons. *FM6*/Canton-S females from the F_2 generation were crossed to their wild-type brothers (carrying a Canton-S *X* chromosome). For each of the 5 sublines 25 females were mated individually to a single male. Whereas all but 6 females produced progeny, only 7 females yielded each a single exceptional male, and Table 3*b* shows the numbers of flies obtained for all phenotypic classes. By and large these results appear to rule out a straightforward genetic basis for the generation of the exceptional males. Again these data suggest an adverse effect of inbreeding on the survival of the exceptional males.

4. Discussion

The frequency of exceptional males is clearly independent of the frequency of primary non-disjunction, and inversion-bearing chromosomes other than *FM6* and *FM7* do not give rise to exceptional males. Since the exceptional males carry markers from both *melanogaster* and sibling *X* chromosomes, they appear to start life as hypoploid *X melanogaster/X sibling* zygotic females. Because non-hybrid *melanogaster* males analogous to the exceptional hybrid males can also be obtained (at least from mothers carrying *FM6*), the phenomenon does not simply fall into the general class of chromosome abnormalities in hybrid zygotes as first described by Boveri (1904). With respect to this the complete sterility of the exceptional *melanogaster*

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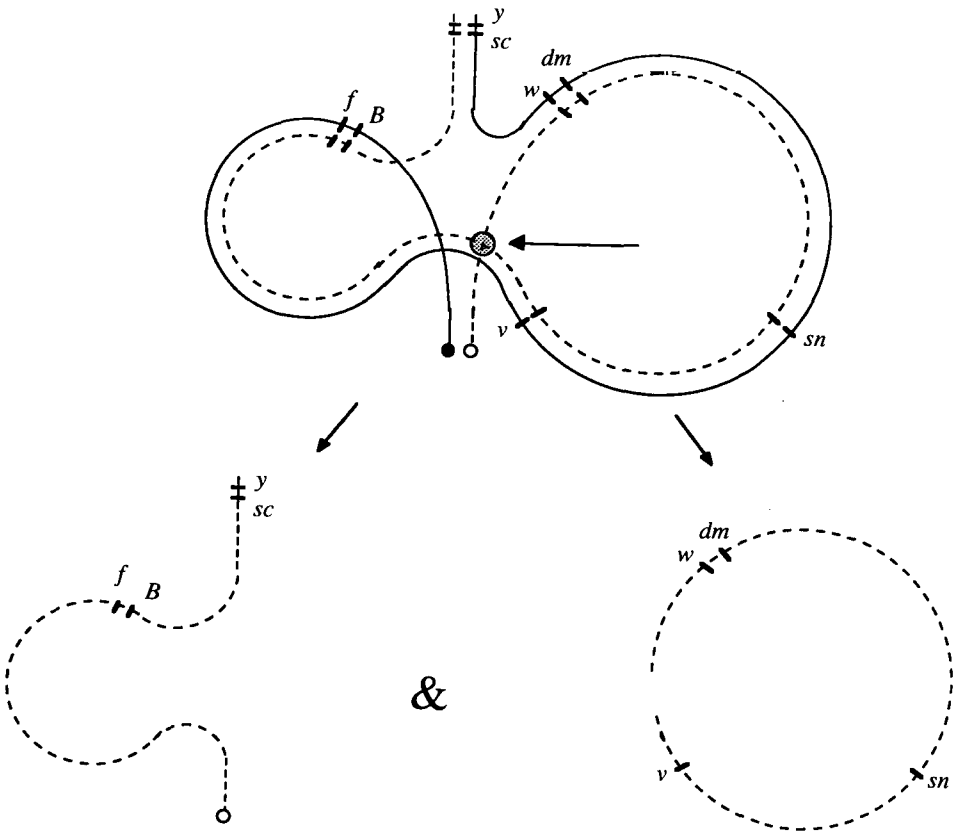

Fig. 1. Simplified scheme showing a possible meiotic event leading to the generation of exceptional males. The arrow indicates where an illegitimate crossing-over might occur. ●——, Wild-type chromosome; ○----, *In(1)FM6*.

males is therefore expected to result either from a lack of a *Y* chromosome or from a hyperploidy for part of the *X* chromosome. In order to find out whether this sterility is not solely caused by the absence of the *Y* chromosome, *Df(1)527/FM6* females were crossed to conspecific males carrying attached *X–Y* chromosomes carrying the markers *y w* and *f* on their *X* chromosomes. Eleven *y w Hw B* exceptional males (presumed to have inherited the attached *Y* chromosome) were recovered and individually crossed to Canton-S females. All males were sterile (data not shown).

The above observations do not provide any critical clue as to whether the exceptional males result from a meiotic phenomenon rather than from some mitotic recombination coupled with chromosome loss during nuclear cleavage divisions, although the former possibility seems more plausible. Indeed, mitotic recombination would be hard to reconcile with the fact that no mosaics were observed in my experiments. The simplest explanation to account for the exceptional phenotype would then require an illegitimate meiotic crossing-over between the inverted maternal *X* chromosomes as outlined in Fig. 1. This would lead to the formation of an acentric chromosome and an incomplete chromosome including at least the regions covering the loci of *Bf* and the *Hw* effect, whilst deficient for at least the loci of *w* and *v*. The above interpretation is consistent with the observation that $sc^8/0$ males are known to be nearly lethal in *D. melanogaster* (Lindsley & Grell, 1968), whereas in a heterozygous condition the effect would no longer be deleterious.

It must be stressed, however, that the above interpretation of the exceptional phenotype solely based on a meiotic phenomenon is not fully compatible with my data. Indeed in the presence of an entire paternal *X* chromosome heterozygous to the deficient *X* balancer, the above males would be expected to be heterozygous for the dominant markers *B* and the *Hw* effect, which does not appear to be the case. Unless one postulates that the deficient *X* balancer becomes hyperactivated through dosage compensation a hemizygotic condition for part of the *X* chromosome of the exceptional males is suggested from the invariably strong phenotypes of both *Hw* and *B* in these males. There is no *a priori* reason why *B* males carrying a duplication of the B^+ region should not display a phenotype typical of a female (B/B^+) heterozygous condition.

Considering the eight breakpoints carried by *In(1)FM6* it is possible to speculate that mitotic recombination within inversions might occasionally produce fragmented chromosomes at the zygotic stage followed by loss of most of the *melanogaster X* chromosome in subsequent nuclear divisions. It is worth pointing out that the restricted chromosome pairing (more pronounced in hybrids) coupled with the effect of the multiple rearrangements of the

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balancer chromosomes might favour abnormal exchanges. This would require at least two such recombination events if small duplications/deletions are tolerated. At first sight this situation would somewhat resemble the effect of mutations such as the *X*-linked mutation *mit* in *D. melanogaster* (Gelbart, 1974) which induces somatic chromosome loss.

Still, if it is to be envisaged that part of the paternal *X* chromosome is eliminated through some exceptional mitotic exchanges in early zygotic females at the first division of cleavage, the way in which some of the mitotic recombinants would then be eliminated (again no mosaics or gynandromorphs were obtained) remains obscure. Whatever the process generating these males may be it will be worth trying to identify the chromosomal factor(s) responsible for the appearance of the exceptional males by analyzing the several components of the *FM6* and *FM7* balancer chromosomes.

If the actual genotypic constitution of the exceptional males cannot be inferred from the present data it must, however, be emphasized that the survival of the exceptional hybrid males can readily be explained by their carrying the Hmr^+ locus (situated in *D. melanogaster* between the loci of *sn* and *v*, 1·16 cM away from *v*) from *D. simulans* or *D. mauritiana*, which would be consistent with the model proposed by Hutter *et al.* (1990) or the genetic basis of the inviability of these hybrids. On this model the hybrid males should die because they carry Hmr^+ which acts as a lethal normally suppressed within *D. melanogaster* by recessive suppressor alleles on the autosomes. The lethal effect would no longer be suppressed in hybrids where the suppressor becomes heterozygous to a mutant allele from the sibling species. According to the above model the sibling species should also carry a mutant allele of the above *X*-linked lethal gene which is fully viable. Therefore if the exceptional males inherit this locus from their sibling father they are expected to be viable. The model thus accounts for the intriguing observation that the exceptional hybrid males survive to adulthood, whereas their brothers carrying an entire *melanogaster X* chromosome and a *sibling Y* chromosome invariably die as third instar larvae. The viability of hybrid males from reciprocal crosses between *D. melanogaster* and *D. simulans* at least, is known to be independent of the origin of the cytoplasm (Orr, 1989).

5. Concluding remarks

The results reported here are not easy to relate to previously described cases of illegitimate crossing-over or partial chromosome loss in *Drosophila*. If a somatic event is involved at the zygotic stage the nearest known example would perhaps be the *Rex* mutation discovered by Robbins (1981) in *D. melanogaster*. This mutation, localized in the heterochromatin of the *X* chromosome acts maternally to induce an exchange-like event between heterochromatic elements of an attached *X–Y* chromosome during the first embryonic mitosis. More precisely the *Rex* event occurs during G1 of the first mitotic division which is presumably the time when the exceptional males should be generated. Swanson (1987) has established that the site of the *Rex* action is the ribosomal RNA gene cluster of the *bb* locus. From this parallel it can be argued that the reason why the *X* chromosome balancers *FM1* and *Basc* do not behave as *FM6* or *FM7* might be connected with a difference at a gene analogous to either *Rex* or its responding site, unless the crucial difference would reside in the number of breaks of these chromosomes (4 and 6 on *FM1* and *Basc* respectively versus 8 on *FM6*). If the effect reported in this paper is equally due to a mutation which acts maternally it would mean that its gene product is active in germinal tissue and persists to early mitotic cleavages in the embryos.

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