Studies on the sterility induced by the male recombination factor 31.1 MRF in *Drosophila melanogaster*

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SUMMARY

The sterility which is associated with male recombination induced by 31.1 MRF was studied genetically and cytologically. In all crosses it was found that female sterility mainly involves failure of the heterozygous females to lay eggs because their ovaries are atrophic. Under the optical microscope, the atrophic ovaries were seen to contain only germaria in their ovarioles. It was also found that in some cases 31.1 MRF affects only one of the two ovaries of the same female. This observation suggests that defective development of atrophic ovaries is not due to influences from the rest of the body but should be attributed to the inability of the germ cells to differentiate. Moreover, various stocks as well as homologous chromosomes were found to react differently to 31.1 MRF with respect to female sterility. In their effect on male sterility it was observed that some strains behave as neutral and others as reactive when mated with $31.1/Cy\ L^4$ males.

1. INTRODUCTION

A second chromosome (designated 31.1 MRF) isolated from a large natural population of Southern Greece was found to induce male recombination in both chromosomes II and III (Yannopoulos & Pelecanos, 1977). The phenomenon had the following characteristics: (*a*) its expression was temperature-sensitive in the larval stage, (*b*) the 31.1 MRF expressed its effect independently of sex when inherited from females deriving their cytoplasm from stock other than $31.1/Cy\ L^4$ stock (Yannopoulos, 1978*a*), and (*c*) male recombination was always associated with chromosome breakage which mainly occurs during male meioses. In addition, a gradual acquisition of cytoplasmic resistance against the activities of 31.1 MRF was found when the factor was introduced by outcrossing into the cytoplasm of a laboratory stock (Yannopoulos, 1978*b*). Our previous data favour the hypothesis that the reciprocal-cross effect is due to a cytoplasmic factor.

The aim of the present study was to investigate the sterility which is associated with male recombination induced by 31.1 MRF. In particular, we have tried to determine whether the complete sterility is due to inability of the eggs to develop, or to the failure of the adult females to produce eggs. Furthermore, if the sterility is due to the latter cause, we have thought it of interest to investigate the stage in which the differentiation of the ovaries is arrested.

<div align="center">2. MATERIALS AND METHODS</div>

The following strains of *Drosophila melanogaster* maintained at $25 \pm \frac{1}{2}$ °C were used. (For a description of mutants and balanced stocks, see Lindsley & Grell, 1968.)

(1) A second and third chromosomal line with the mutants *dp b cn bw*; *ve*.

(2) A balanced stock *Sp*/SM 5 = {In(2 LR)SM 5, *al² Cy ltᵛ cn² sp²*} designated below as *Sp/Cy*.

(3) Canton-S (Canton-Special), a wild type stock, designated below as Canton.

(4) A balanced stock *Pm/Cy L⁴* = In(2L+2R)*Cy, Cy L⁴ Sp²*.

(5) A second chromosome (symbol 31.1) bearing the 31.1 MRF factor (Yannopoulos, 1978*a*).

An 'Instant' *Drosophila* medium (Philip Harris Biological Ltd, England) was used throughout the present experiments. Parents were 1–3 days old when crosses were set up; in the male recombination tests, progeny were scored until the 17th day after setting up matings. All cultures were maintained in vials kept at $25 \pm \frac{1}{2}$ °C.

In the crosses in which daughters were examined for atrophic ovaries, four virgin females were mated with two males and were allowed to lay eggs for 5 days. Female progeny were collected twice a day for 4 days and were transferred to vials containing fresh food. Four days after the day of collection these females were dissected in insect saline and the two ovaries of each female were classified according to their gross morphology as follows: normal+normal (N+N), normal+reduced (N+R), normal+atrophic (N+A), reduced+reduced (R+R), reduced+atrophic (R+A), and atrophic+atrophic (A+A).

Ovaries without any egg chambers were classified as atrophic and those with at least one egg chamber, including those up to one third of the normal size, as reduced; all the others were considered to be normal.

In the crosses in which females were tested for sterility a 1-day-old female was mated to five males. All vials in which the females died within 5 days were excluded, regardless of the presence of larvae. Females that did not produce any adult progeny were considered as sterile.

When testing for male sterility, one male was mated to four virgin females from the *dp b cn bw*; *ve* strain. Again males that did not produce any offspring were considered as sterile.

Feulgen stain

Whole atrophic ovaries were fixed in 1:3 glacial acetic acid:ethanol, washed with distilled water and hydrolyzed in 1 M-HCl at 60 °C for 20 min. After a further wash in distilled water the ovaries were stained with Schiff's reagent. They were then washed in three changes of freshly prepared bisulphite wash (sodium meta-bisulphite ($Na_2S_2O_5$) 10% 5 ml, 1 M-HCl 5 ml, distilled water 90 ml) and were mounted in water.

3. RESULTS

(i) *Female sterility*

Various crosses were made in order to determine whether the complete female sterility, which is associated with male recombination induced by 31.1 MRF (Yannopoulos & Pelecanos, 1977; Yannopoulos, 1978*a*, *b*), is mainly due to defective development of the ovaries; and to test whether various strains react differently when mated with individuals bearing 31.1 MRF. Both the crosses and the classification of the female progeny according to the size of their ovaries are presented in Table 1.

In cross 1 (Table 1) high frequencies of heterozygous 31.1/*dp b cn bw*; *ve*/+ and *Cy L*⁴/*dp b cn bw*; *ve*/+ female progeny were found to have both ovaries atrophic (57 and 64 %, respectively). Female progeny of the same phenotypes with one ovary normal or reduced and the other atrophic were also observed. In contrast, in the reciprocal cross (cross 2, Table 1) all 31.1/*dp b cn bw*; *ve*/+ and *Cy L*⁴/*dp b cn bw*; *ve*/+ female progeny examined were found to have normal ovaries. Virgin 31.1/*dp b cn bw*; +/*ve* females from cross 1 were individually crossed to *dp b cn bw*; *ve* males. From the 68 females crossed, only 28 gave at least one progeny; thus, the sterility found was 59 %, a frequency which is similar to that of their sisters with both ovaries atrophic (see cross 1). This finding means that the female sterility is due mainly to defective development of the ovaries. Furthermore, the reproductivity (the number of adults produced per female) of these 28 females was low (26 ± 24 s.d.). Female progeny of the above mentioned 28 females, except the *dp b cn bw* ones, were also examined for atrophic ovaries. The results, which are presented in cross 3 (Table 1), appear similar to those found in cross 1 (Table 1). In contrast, when 58 heterozygous 31.1/*dp b cn bw*; +/*ve* virgin female progeny from cross 2 were individually crossed to *dp b cn bw*; *ve* males, only one did not give progeny (sterility 1·72 %). The reproductivity of the other 57 females was high (143 ± 46 s.d.). In addition, all daughters of these females examined were found to have normal ovaries (cross 4, Table 1).

No difference was found in the hatchability of the eggs between those laid by the heterozygous 31.1/*dp b cn bw*; *ve*/+ females produced by cross 1 (Table 1) (359 eggs hatched out of 405 examined, i.e. 88·6 %) and those from cross 2 (Table 1) (284 eggs hatched out of 324 examined, i.e. 87·6 %). In order to estimate hatchability, virgin 31.1/*dp b cn bw*; *ve*/+ females separately collected from crosses 1 and 2 were mass crossed. Hatchability was estimated in the eggs which had been laid for three days starting one day after setting up the crosses. These results show that the low reproductivity observed in the 31.1/*dp b cn bw*; *ve*/+ females produced from cross 1 as compared with that of the reciprocal cross 2 (Table 1) was mainly due to the small number of the eggs laid by these heterozygous females and not to the inability of the eggs to hatch.

In order to test whether the heterozygous 31.1/*dp b cn bw*; *ve*/+ and *Cy L*⁴/*dp b cn bw*; *ve*/+ males produced from crosses 1 and 2 both induced atrophic ovaries in the next generation, the crosses 5–8 were set up. In each of

Table 1. *Female sterility due to atrophic ovaries induced by 31.1 MRF*

Type of mating* (♀♀ × ♂♂)	Genotype of ♀♀ examined	No. of ♀♀, classified according to size of ovaries†						Total no. of ♀♀ screened
		N+N	N+R	N+A	R+R	R+A	A+A (%)	
(1) dp; ve × 31.1/Cy L⁴	31.1/dp; ve/+	15	7	16	6	16	81 (57)	141
	Cy L⁴/dp; ve/+	13	10	7	6	18	98 (64)	152
(2) 31.1/Cy L⁴ × dp; ve	31.1/dp; ve/+	140	0	0	0	0	0	140
	Cy L⁴/dp; ve/+	134	0	0	2	0	0	136
(3) 31.1/dp; ve/+ × dp; ve (♀♀ from 1)	All ♀♀ except dp b cn bw	15	5	11	6	20	100 (64)	157
(4) 31.1/dp; ve/+ × dp; ve (♀♀ from 2)	All ♀♀ except dp b cn bw	143	2	0	1	0	0	146
(5) dp; ve × 31.1/dp; ve/+ (♂♂ from 1)	31.1/dp	28	16	24	17	26	180 (62)	291
(6) dp; ve × Cy L⁴/dp; ve/+ (♂♂ from 1)	Cy L⁴/dp	22	10	10	16	48	204 (66)	310
(7) dp; ve × 31.1/dp; ve/+ (♂♂ from 2)	31.1/dp	34	52	44	26	106	270 (51)	532
(8) dp; ve × Cy L⁴/dp; ve/+ (♂♂ from 2)	Cy L⁴/dp	14	12	22	4	52	238 (70)	342
(9) dp; ve × Cy L⁴/Pm	Cy L⁴/dp; ve/+	122	2	0	6	0	0	130
	Pm/dp; ve/+	108	1	0	5	0	0	114
(10) Canton × 31.1/Cy L⁴	31.1/Canton	4	3	7	2	12	114 (79)	142
	Cy L⁴/Canton	3	1	3	0	15	131 (86)	153
(11) 31.1/Cy L⁴ × Canton	31.1/Canton	143	1	0	1	0	0	145
	Cy L⁴/Canton	154	1	0	2	0	0	157
(12) dp; ve × Canton	Canton/dp; Canton/ve	165	0	0	4	0	0	169
(13) Canton × dp; ve	Canton/dp; Canton/ve	140	0	0	2	0	1 (0·70)	143
(14) Cy/Sp × 31.1/Cy L⁴	31.1/Cy	17	7	21	31	30	48 (31)	154
	31.1/Sp	120	3	3	7	1	1 (0·74)	135
	Cy L⁴/Sp	120	4	7	8	1	2 (1·41)	142
(15) dp; ve × 31.1/Cy (♂♂ from 14)	31.1/dp	28	3	26	0	15	66 (48)	138
(16) dp; ve × 31.1/Sp (♂♂ from 14)	31.1/dp	26	1	15	2	7	43 (46)	94
(17) dp; ve × Cy L⁴/Sp ♂♂ from 14)	Cy L⁴/dp	26	4	17	4	13	61 (49)	125
(18) 31.1/Cy × dp; ve (♀♀ from 14)	31.1/dp	5	3	12	10	12	83 (66)	125
(19) 31.1/Sp × dp; ve (♀♀ from 14)	31.1/dp	25	7	9	11	13	68 (51)	133
(20) Cy L⁴/Sp × dp; ve	Cy L⁴/dp	16	2	20	6	5	69 (58)	118
(21) Stock 31.1/Cy L⁴	31.1/Cy L⁴	110	1	0	1	0	0	112

* dp stands for the whole dp b cn bw chromosome. † For details, see materials and methods.

these crosses the $31.1/dp\ b\ cn\ bw$; $ve/+$, $31.1/dp\ b\ cn\ bw$; ve/ve, $Cy\ L^4/dp\ b\ cn\ bw$; $ve/+$ and $Cy\ L^4/dp\ b\ cn\ bw$; ve/ve female progeny were examined separately in order to determine whether the third chromosome from the $31.1/Cy\ L^4$ stock would have any effect on the frequency of females with atrophic ovaries. As the segregation of *ve* did not influence atrophy in these crosses the data on ve/ve and $ve/+$ were pooled. The results of these crosses show that both the heterozygous 31.1 and $Cy\ L^4$ males from crosses 1 and 2 retain the ability to induce atrophy in the next generation. It can be seen from Table 2 (lines 1 and 2), that the third chromosome of the $31.1/Cy\ L^4$ stock also did not influence male recombination frequencies. In these crosses $31·1/dp\ b\ cn\ bw$; $ve/+$ (line 1) and $31.1/dp\ b\ cn\ bw$; ve/ve (line 2) males were separately collected from cross 5 (Table 1) and were individually mated with $dp\ b\ cn\ bw$; *ve* virgin females; the progeny of these crosses were then scored for recombinants.

Evidence that the $Cy\ L^4/Pm$ strain (the original one with which the 31.1 second chromosome was isolated from one wild male) does not produce atrophic ovaries is presented in cross 9 (Table 1).

In order to determine whether similar results would be observed with a wild-type stock, Canton virgin females were crossed to $31.1/Cy\ L^4$ males (cross 10, Table 1). Heterozygous 31.1/Canton and $Cy\ L^4$/Canton female progeny with both ovaries atrophic were found in high frequencies (79 and 86 %, respectively). Again, in the reciprocal cross (cross 11, Table 1) all female progeny dissected had normal ovaries. When males or virgin females from the Canton strain were mated to $dp\ b\ cn\ bw$; *ve* virgin females or males, all female progeny examined were found to have normal ovaries (crosses 12 and 13, Table 1). Moreover, from the 69 virgin Canton/$dp\ b\ cn\ bw$; Canton/*ve* females of cross 12 (Table 1) which were mated with $dp\ b\ cn\ bw$; *ve* males, only two (sterility 2·90 %) did not give any progeny. The reproductivity of the remaining 67 females was high (152 ± 57 s.d.). Furthermore, heterozygous Canton/$dp\ b\ cn\ bw$; Canton/*ve* males from cross 12 (Table 1) when individually mated with $dp\ b\ cn\ bw$; *ve* virgins, showed very low male recombination frequency, i.e. 0·02 % (line 3, Table 2).

The cytoplasm of the Sp/Cy stock does not suppress either the male recombination or the chromosome breakage induced by $31.1/Cy\ L^4$ (Yannopoulos, 1978*a*), and the effect of second chromosomes of this stock on the induction of atrophic ovaries is analysed in Table 1, crosses 14–20. When Sp/Cy females were mated to $31.1/Cy\ L^4$ males, F_1 females showed a high frequency of atrophic ovaries unless the Sp second chromosome was present (cross 14). When progeny of either sex from this cross are mated to $dp\ b\ cn\ bw$; *ve*, female progeny carrying either 31.1 (crosses 15, 16, 18, 19) or $Cy\ L^4$ (crosses 17, 18, 20) all had a high frequency of atrophic ovaries similar to the frequencies observed in cross 1. These results indicate that the Sp chromosome itself must prevent ovarian atrophy in cross 14.

Evidence was provided (Yannopoulos, 1978*a*, *b*) that the responsibility for the non-induction of male recombination and chromosome breakage when 31.1 MRF is inherited through $31.1/Cy\ L^4$ females should be attributed to the cytoplasm of the $31.1/Cy\ L^4$ strain. The results of crosses 3 and 18–20 when compared with

those of cross 2 (Table 1) are in good agreement with this hypothesis. No atrophic ovaries were found in the 112 females of the inbred $31.1/Cy\,L^4$ strain examined (line 21, Table 1).

The atrophic ovaries from the above crosses were of small size and were always connected to the oviduct (cf. B with A in Plate 1, fig. 1). Moreover, no differences in external morphology were observed among females possessing normal, reduced or atrophic ovaries. In order to determine in which stage the differentiation of the atrophic ovaries was arrested, whole atrophic ovaries from females aged 3 to 10 days were Feulgen-stained, and seventy pairs of atrophic ovaries selected at random from all crosses were examined. Seen through the optical microscope these ovaries appeared to contain only germaria in their ovarioles (Plate 1, Fig. 2).

Table 2. *Second chromosome male recombination in 31.1/dp b cn bw; + /ve (line 1), 31.1/dp b cn bw; ve/ve (line 2) males produced from cross 5, Table 1, and Canton/dp cn bw; Canton/ve (line 3) produced from cross 12, Table 1*

No. of males tested	No. of progeny	Male recombination frequency (%)
(1) 22	2652	2·19
(2) 20	2514	2·31
(3) 27	4652	0·02

(King's, 1970 terminology is used to describe ovarian development). Mitotic divisions of germ cells were observed in the anterior region of these germaria (Plate 2), but cystocyte complexes were not observed in their central or posterior region. Instead, the posterior portion was full of follicle cells which seem to have degenerated in the narrow posterior end. This observation suggests that the atrophic ovaries may be the result of either a blockage of development prior to cystocyte formation, or to the same phenomenon at some stage during subsequent cystocyte cluster development. The observed mitoses would then represent mitotic divisions which occurred prior to cystocyte cluster formation, i.e. stem cell–cystoblast divisions or cystoblast–cystoblast divisions, respectively. It should also be noted that reduced ovaries possessed normal ovarioles as well as ovarioles containing only germaria.

(ii) *Male sterility*

Male sterility was examined in the progeny of crosses 1, 2, 10, 11, 13, and 14 of Table 1 and the results are given in Table 3. The findings of lines 1–4, 9 and 10 (Table 3) compared with those of line 11 (control) show that 31.1 MRF has no effect upon heterozygous $31.1/dp\,b\,cn\,bw; + /ve, Cy\,L^4/dp\,b\,cn\,bw; + /ve, 31.1/Cy$ and $31.1/Sp$ males with respect to male sterility in the reciprocal crosses. In contrast, heterozygous 31.1/Canton and $Cy\,L^4/$Canton (lines 5 and 6, Table 3) which inherited 31.1 and $Cy\,L^4$ chromosomes from their fathers, showed high male sterility (72 and 81%). However, in the reciprocal cross (lines 7 and 8) male fertility was normal. These results indicate that the various strains react
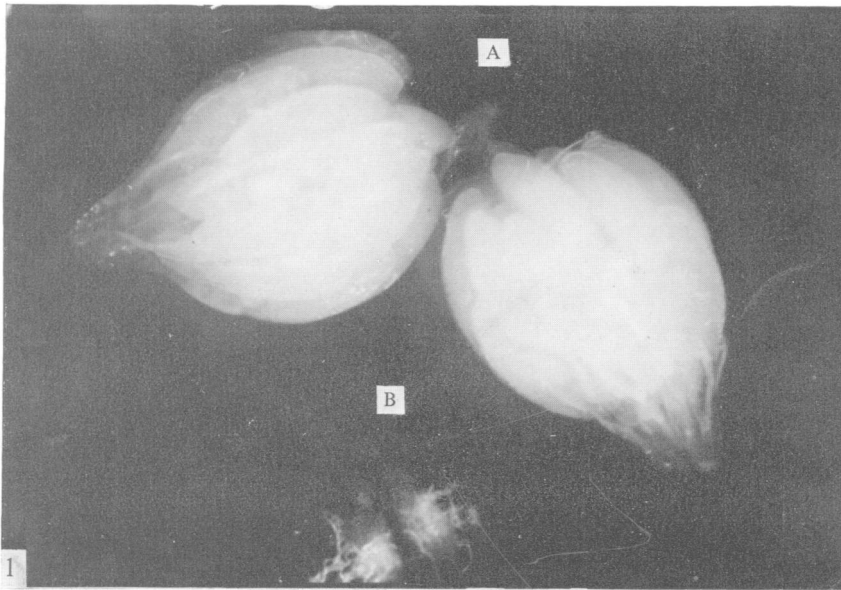
Fig. 1. A, pair of normal ovaries from 4-day-old female ($31.1/dp\ b\ cn\ bw;\ ve/+$) coming from cross 2 (Table 1) and B, pair of atrophic ovaries from 4-day-old female ($31.1/dp\ b\ cn\ bw;\ ve/+$) coming from cross 1 (Table 1) (magnification $\times 50$).

Fig. 2. Photomicrograph of Feulgen-stained pair of atrophic ovaries containing only germaria, from 5-day-old $31.1/dp\ b\ cn\ bw;\ ve/+$ female produced by cross 1 (Table 1) (magnification $\times 200$).

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Table 3. *Sterility of heterozygous males produced from the crosses shown in Table 1*

Genotype of males examined*	No. of males examined	Sterility (%)
(1) 31.1/dp;† +/ve (1)	68	4·4
(2) $Cy\ L^4/dp$; +/ve (1)	56	5·3
(3) 31·1/dp; +/ve (2)	75	2·7
(4) $Cy\ L^4/dp$; +/ve (2)	73	4·1
(5) 31.1/Canton (10)	100	72·0
(6) $Cy\ L^4$/Canton (10)	78	81·0
(7) 31.1/Canton (11)	78	3·8
(8) $Cy\ L^4$/Canton (11)	80	3·7
(9) 31.1/Cy (14)	75	4·0
(10) 31.1/Sp (14)	66	3·0
(11) Canton/dp; Canton/ve (13)	75	4·0

* Numbers in parentheses correspond to the cross numbers in Table 1.
† dp stands for the whole $dp\ b\ cn\ bw$ chromosome.

differently to 31.1 MRF with respect to male sterility. Dissection of 30 31.1/ Canton and 20 $Cy\ L^4$/Canton sterile males showed they possessed smaller and thinner testes while their paragonia were normal.

4. DISCUSSION

The results presented in this paper clearly show that the female sterility associated with male recombination induced by 31.1 MRF mainly involves failure of the female progeny to produce eggs, as their ovaries are atrophic. Furthermore, it was found that the atrophic ovaries contain only germaria. The observation that these germaria do not contain germarial cystocyte complexes suggests that the defective development of the ovaries is due either to the inability of the cytoblasts to divide, or to their inability to form cystocyte-clusters. Moreover, the finding that in some females one of the two ovaries was normal or reduced, while the other was atrophic, may be considered as evidence that the failure of the germ line to become functional is not due to influences from the rest of the body. However, whether the previous finding is due to the fact that 31.1 MRF acts during different stages of the development of the ovaries, that is earlier or later than the migration of precursor oogonial cells (pole cells), is still unknown.

The observation that both 31.1 and $Cy\ L^4$ chromosomes of the 31.1/$Cy\ L^4$ strain have shown, so far, the same properties, while neither $Cy\ L^4$ nor Pm chromosomes of the $Cy\ L^4/Pm$ stock have affected the ovaries, may be explained by assuming some kind of 'transmission' of the 31.1 MRF factor from the wild

PLATE 2

Fig. 3. Germaria showing mitotic division (arrow) in the central region with their posterior end full of follicle cells (magnification × 1600).

chromosome to the $Cy\ L^4$ chromosome. The possibility that the $Cy\ L^4/Pm$ stock had this property in the past and has become free of it is low, for the factor has not become lost from the $31.1/Cy\ L^4$ strain since 1971. Moreover, other results (unpublished data) show an ability of the factor to be transmitted to other chromosomes. Whether this transmission should be considered to be transposition (see review by McClintock, 1965), paramutation (see review by Brink, 1973) or chromosomal contamination (Picard, 1976), remains obscure.

Comparisons of the results of the present study with those found previously (Yannopoulos & Pelecanos, 1977; Yannopoulos, 1978a, b) concerning male recombination and chromosome breakage, allow us to conclude that all these phenomena are associated with each other and are probably induced by the same factor.

The finding that various stocks as well as the two homologous chromosomes of the same strain react differently to 31.1 MRF, at least as regards female sterility, suggests that chromosomal agents may affect (regulate) the extent of action of the *male recombination* factors. The results reported by Sved (1976) also seem to show different reactions by various stocks to MRF factors.

Regarding the male sterility associated with male recombination, Kidwell & Kidwell (1975) observed reciprocal differences, while Woodruff & Thompson (1977) did not observe reciprocal differences. Furthermore, Sved (1976) did not find male sterility associated with male recombination. Our results show that some strains (e.g. *dp b cn bw*; *ve* and *Cy/Sp*) do not react to the 31.1 MRF, in affecting male sterility (neutral strains), while others (e.g. Canton) do (reactive strains), when crossed to $31.1/Cy\ L^4$ males. The observation that sterile males had smaller and thinner testes suggests that 31.1 MRF induces some damage in testes which blocks normal development. Experiments are now in progress to test whether the reactivity of the Canton strain is due to any specific gene. Our findings provide evidence that the different results reported by previous workers are at least partially due to the different laboratory stocks used. However, in attempting to explain such differences neither the MRF factors themselves, nor the residual genotype of the various MRF stocks can be ignored.

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