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Chromosomal Contamination for Male Recombination in *Drosophila melanogaster*

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Abstract

It has previously been shown that the ability to induce male recombination may be passed from one chromosome to another, not necessarily homologous, chromosome. This phenomenon is investigated, using strains known to interact in the P–M system of hybrid dysgenesis, and is shown to be analogous to the phenomenon of 'chromosomal contamination' in the I–R system of hybrid dysgenesis, in that the passage of properties from one chromosome to another is correlated with other manifestations of dysgenesis. Unlike the I–R system, contamination can occur in males as well as in females in the P–M system, a result which is consistent with other differences between the two systems. The contaminated chromosome acquires only a portion of the dysgenic properties of the original chromosome, and the inheritance is unstable over more than a few generations.

Introduction

Picard (1976) introduced the term 'chromosomal contamination' to describe an event in which genetical properties are acquired by one chromosome from another, not necessarily homologous, chromosome, the frequency of its occurrence being close to 100% in some circumstances. The genetical properties studied by Picard relate to the ability to induce female sterility of a particular kind. The genetic system has been designated as the I–R system of hybrid sterility (see Bregliano *et al.* 1980 for a review of this system).

Kidwell *et al.* (1977) introduced the term 'hybrid dysgenesis' in an attempt to group several genetic phenomena, including sterility, high mutation rates and male recombination, into a single syndrome. The terminology was further refined by Kidwell (1979), who argued that hybrid dysgenesis could be divided into two discrete systems, which she designated the 'P–M' and 'I–R' systems respectively. The principal properties of the former system are gonadal sterility in both females and males, as well as male recombination. By contrast, the I–R system involves female sterility only, manifested by reduced egg hatchability, and does not involve male recombination. Elevated mutation rates and non-disjunction are found in both systems. The two systems are also very similar in their patterns of inheritance. In both cases, dysgenesis is produced when a chromosome of one type (I or P depending on the system) is present in a cytoplasm, or cytotype (Engels 1979), of the other type (R or M respectively).

Substantial evidence already exists that a phenomenon analogous to I–R chromosomal contamination occurs for male recombination. Slatko (1978) showed that chromosome III could acquire male recombination properties from chromosome II,

although the exact timing of the acquisition was not clarified. Yannopoulos (1979) showed that a male recombination factor could be passed in males from one chromosome II to an homologous chromosome II. Kidwell *et al.* (1981) showed that stocks produced from mixtures of M and P stocks acquired the characteristics of the P stocks after several generations, although chromosomal contamination could not be directly implicated in this process.

More precise information exists for the I–R system of dysgenesis (Picard 1976, 1978). It has been shown that the genetical properties may be passed from almost any chromosome to any other chromosome, homologous or non-homologous, although with different probabilities. Furthermore, this passage occurs primarily in females under conditions of dysgenesis. It seems important to test whether an analogous phenomenon occurs in the P–M system of dysgenesis. Preliminary evidence that this is so comes from the study of Yannapoulos (1979), who found a negligible rate of homologous transfer in reciprocal hybrids that lack male recombination. The purpose of the present paper is to investigate the phenomenon of chromosomal contamination in the P–M system of dysgenesis. The experiments reported here confirm that Picard's overall conclusion holds for male recombination in the P–M system of dysgenesis, although some differences have been found which are revealing of overall differences between the two systems.

Materials and Methods

Strains

Three newly collected wild-type strains were used:

HA = Harwich—a P strain obtained from Dr M. G. Kidwell.

PW = Para Wirra—an Australian strain obtained from Dr D. Angus, also classified as a P strain.

HV = Hunter Valley—a strain made from collections in the Hunter Valley district in 1975. This strain possesses some of the properties of a P strain, in being able to induce male recombination. However, it has not been found to be associated with gonadal sterility, and therefore cannot be classified as a P strain. It appears to correspond closely to the strains classified by Engels and Preston (1981a) and Kidwell (1981) as Q strains, a classfication originally described as 'neutral' in the P–M system, but perhaps more accurately described as 'weak P'.

al = *al cn bw*—a strain containing markers that span chromosome II, classified as M in the P–M system.

BLT = *B; Cy/Pm; Ubx/Sb; pol*—a multiple balancer stock obtained from Dr R. Frankham, classified as an M strain.

CS = Canton-S—a standard laboratory wild-type strain, classified as an M strain.

All experiments were carried out at 25±2°C, and all stocks were raised using a standard yeast–semolina–molasses medium.

Experimental Design

All experiments reported in this paper follow a design closely related to that shown in Fig. 1. The purpose of the design is to produce a genotype (the female of cross 3), that is dysgenic through the receipt of P chromosomes X and III but whose wild-type chromosome II is of M origin. Later generations of the pedigree are designed to remove all chromosomes of P origin. The male in cross 5 is then backcrossed, and any recombination resulting must be due to the receipt by an original M second chromosome of P properties from other chromosomes.

The key individual in Fig. 1 is the female of cross 3, since this is the presumptive individual in which the contamination event actually takes place. It is convenient to have a special designation for this individual for easy reference, and it will be referred to in later discussion as the 'test individual'.

It is part of the purpose of this paper to rule out the possibility that the contamination event occurs in individuals other than the test individual (e.g. the male in cross 4). This is done by utilizing variations of the procedure of Fig. 1. First, experiments have been carried out in which either or both of the P chromosomes, X and III, are replaced by marked non-P chromosomes. The X is replaced by using the reciprocal of cross 1, and chromosome III is replaced by using *Ubx* progeny in cross 3. Secondly, the test individual has been produced by the reciprocal of cross 2, thereby giving it the same chromosomal constitution, but ensuring that it is only minimally dysgenic (Engels 1979). The reciprocal cross of this type is referred to as a 'B cross', in contrast to the A cross shown in Fig. 1 which produces dysgenic offspring. Thirdly, the reciprocal of cross 3 (rather then cross 2) has been used, so that the test individual is male rather than female. In this case, only chromosome III can be of P origin, since the X chromosome will be donated by the M stock female parent. In all the above designs, the Canton-S and *al cn bw* stocks in crosses 2 and 4 can be interchanged, so that the *al cn bw* marker chromosome is used as the contaminated chromosome in some experiments.

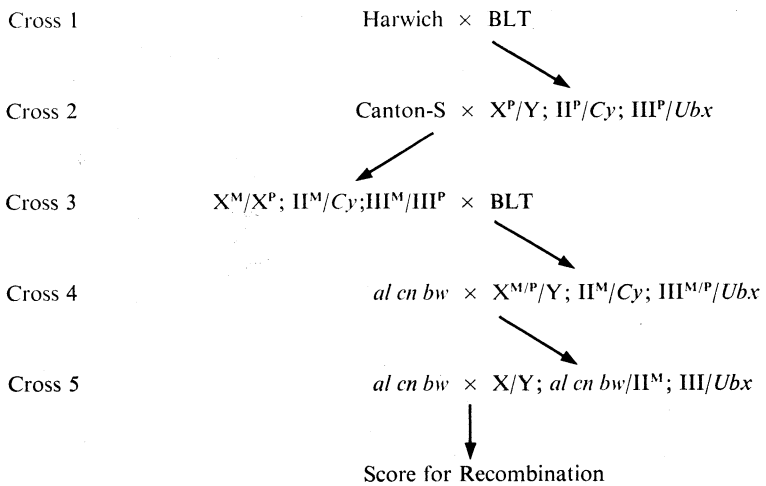

Fig. 1. Example of crossing program used to produce chromosomal contamination and to measure its effect. The female is written first in all cases. The 'test individual' in this case (female of cross 3–see text) is produced by an A cross in which both X chromosome and chromosome III are of P origin. The remainder of the pedigree ensures the removal of all chromosomes of P origin and leads to an estimate of male recombination induced by the presumptive contaminated second chromosome, designated as II^M.

Results

Table 1 summarizes the results for all crosses of the form given in Fig. 1, in which the test individual is produced by an A cross between an M female (either Canton-S or *al cn bw*) and a hybrid male carrying P chromosomes (either Harwich or Para Wirra). The results are given as total recombination percentage per experiment, and also as minimum recombination percentage, each potential cluster of recombinants being counted as a single event. The latter statistic, although biased as a measure of the amount of male recombination, nevertheless seems to smooth out some of the fluctuations due to the occurrence of large clusters of recombinant gametes (see e.g. Kidwell *et al.* 1977).

In the present experiments, both measures showed a high amount of variability from one experiment to another. However, an analysis of variance shows that both chromosomes X and III contribute significantly to contamination. There was,

furthermore, a notable lack of recombination when the test individual received no P chromosome from the male parent. The level of recombination in this case is comparable to the background level found in experiments in which Canton-S replaced the P stock in cross 1, thereby excluding all P chromosomes from the pedigree. The absence of contamination in this class argues against the possibility that homologous rather than non-homologous contamination is involved in other classes. For this to happen, it would be necessary for the *Cy* chromosome to acquire the P properties in cross 2, and then pass them on to the wild-type M chromosome in cross 3. This explanation would need to be modified to explain why this transfer occurs in the presence of certain background chromosomes but not others.

Table 1. Summary of male recombination values given by the procedures outlined in Fig. 1
The test individual is female and produced by an A cross in all cases

P chromosomes	Expt No.	M stock	P stock	Total	Recombination (%) Actual	Minimum
Neither	1	CS	HA	2594	0·04	0·04
	1	*al*	HA	3367	0	0
	3	CS	HA	1506	0	0
	4	CS	PW	2501	0	0
				Mean	0·01	0·01
X	1	CS	HA	3423	0·26	0·20
	3	CS	HA	2902	0·07	0·07
	4	CS	HA	1724	0	0
	4	CS	PW	2685	0	0
				Mean	0·08	0·07
III	1	CS	HA	2550	0·04	0·04
	3	CS	HA	11742	0·37	0·26
	4	CS	HA	3030	0·43	0·39
	4	CS	PW	2991	0	0
				Mean	0·21	0·17
X and III	2	CS	HA	14578	1·01	0·65
	3	CS	HA	5176	0·46	0·35
	3	*al*	HA	4345	0·74	0·46
	4	CS	HA	4536	0·29	0·26
	4	CS	PW	1860	0·75	0·37
				Mean	0·65	0·41

The highest rate of contamination was found in an experiment that involved only contamination with chromosomes X and III. Experiment No. 1 was designed to include this class in combination with the three other classes. The sterility of the test individuals, a problem in many of the contamination experiments, unfortunately prevented the recovery of any chromosomes from the X+III class and necessitated a repeat of the experiment at a slightly later date.

Table 2 summarizes an equivalent series in which the test individual is a female produced by the B cross, in which low levels of dysgenesis would be expected (Engels 1979). All recombination values are low in this case, in keeping with the prediction from the I–R system results in which contamination occurs at low, although signifi-

cant, levels in non-dysgenic flies. The difference between dysgenic and non-dysgenic contamination is, however, significant only in the case of contamination by both chromosomes X and III.

Table 2. Male recombination induced when the test individual is female and produced by B cross
All results are from experiment 1

P chromosomes	M stock	P stock	Total	Recombination (%) Actual	Minimum
Neither	CS	HA	1637	0	0
	al	HA	1397	0	0
X	CS	HA	1262	0	0
	al	HA	1869	0·11	0·11
III	CS	HA	1703	0·12	0·12
	al	HA	1775	0·17	0·11
X and III	CS	HA	1297	0	0
	al	HA	3745	0·03	0·03

The differences between the levels of recombination in Tables 1 and 2 points to the importance of the test individual in determining the level of contamination. Should the contamination event occur in the cross 4 male hybrid, no such large difference in contamination levels would be predicted.

Table 3. Male recombination induced when the test individual is male
All P properties in this case must be from chromosome III (see text)

Hybrid type	M stock	P stock	Total	Recombination (%) Actual	Minimum
A	CS	HA	3682	0·38	0·35
A	*al*	HA	5352	0·43	0·26
B			6371	0·05	0·03
A	*al*	PW	7531	0·50	0·23
B			6343	0·09	0·05
A	*al*	HV	2709	0·07	0·03
B			3192	0·03	0·03
A	CS	HV	9119	0·01	0·01
B			10494	0·01	0·01

Table 3 summarizes the results from crosses in which the test individual is male rather than female. There is again seen to be an appreciable level of contamination when the test individual is produced by an A cross but not when it is produced by a B cross. The finding of contamination in males is in contrast to the I–R system where the phenomenon occurs only in females. However, the result is predictable based on the presence of dysgenic manifestations in males in the P–M system and

its absence in the I–R system. Also, as mentioned previously, Yannopoulos (1979) showed that homologous contamination can occur in males, in a system that is presumably analogous to the P–M system.

Two other sets of results of interest were obtained. First, despite extensive experiments using the Hunter Valley strain, only trivial levels of contamination could be demonstrated. This is shown from the male-contamination experiment of Table 3. The low level of contamination observed here is in contrast to the high levels of homologous contamination found by Yannapoulos (1979). Low levels of contamination were also found in a female contamination experiment involving Canton-S and Hunter Valley, in which a single recombinant was produced in 10000 progeny when both chromosomes X and III were of Hunter Valley origin. Based on the ability of the Hunter Valley strain to induce male recombination when crossed to *al cn bw*, it could be argued that passage of these properties to Canton-S might be expected. However, the Canton-S × Hunter Valley cross shows no dysgenic manifestations, judged by the results of sterility tests (Sved and Colgan, unpublished data), a result that is consistent with the 'weak P' status of the Hunter Valley stock. Thus the absence of contamination in this case is again in agreement with Picard's contention that contamination occurs only under conditions of dysgenesis.

Table 4. Change in male recombination levels induced by contaminated chromosome at various times after initial tests

Experiment No.	Generation	Total	Recombination (%) Actual	Minimum
X and III, expt 2	1	14578	1·01	0·65
	3	8845	1·16	0·62
	5	6816	0·25	0·19
	7	16224	0·32	0·18
X and III, expt 3	1	4345	0·74	0·46
	11	10446	0·27	0·17
Male contamination	1	12883	0·47	0·24
	2	11728	0·04	0·03

The final set of results is given in Table 4 and refers to the re-testing of contaminated chromosomes in succeeding generations. Where this was attempted, the recombination levels fell, most strikingly in the male-contamination experiment, in which only low levels were found after the first generation. In all cases it seems that the loss of dysgenic properties could be a comparatively sudden event.

Discussion

A notable feature of the contamination process was the dependence of the process on the particular stocks used. The Canton-S and *al cn bw* stocks used in the experiments of this paper were lost after the completion of these experiments. A new Canton-S stock was brought into the laboratory, and a new *al cn bw* stock of similar background to the original was synthesized. Although these stocks were M stocks as judged by the criteria of female sterility and male recombination, we were not

able to recover contamination levels comparable to those found in the earlier experiments.

The contamination observed was apparently incomplete, in the sense that the male recombination produced by the contaminated chromosomes was not accompanied by male sterility, in contrast to the situation in hybrid males produced by M × P crosses in which male fertility was normally severely reduced. Tests for female sterility were also made in the first experiment, although at 25°C, which is below the optimum for expression of sterility (Kidwell *et al.* 1977), and these also proved negative. The stocks with the highest level of male recombination were unfortunately not tested for female sterility. Contamination for female sterility has, however, been achieved at a low level by M. G. Kidwell (personal communication), using transfer from autosomes to the X-chromosome.

The incomplete contamination in the experiments of the present paper is in contrast to the complete contamination found in the I–R system (Picard 1976, 1978). This difference between systems is in some respects analogous to the observed fall in the level of manifestation of the contamination that we found, again in contrast to the I–R system where such a fall has not been reported. Also chromosomal contamination in the I–R system is the most sensitive indicator of dysgenesis (Bregliano *et al.* 1980), whereas the present results indicate that contamination frequently fails to occur in the P–M system despite the occurrence of other manifestations of dysgenesis.

A likely physical basis for the contamination phenomenon has been provided by the recent discovery by G. M. Rubin, M. G. Kidwell and P. M. Bingham (personal communication) that there is a particular insertion sequence, appropriately termed the 'P factor' (Engels and Preston 1981*b*), asociated with the P–M difference. The analogy with IS insertion in bacteria provides a highly plausible explanation for the ability of the factor to insert into, and thereby 'contaminate', another chromosome. However, the variable and incomplete nature of the contamination found in the present paper suggests that the relationship between the level of dysgenesis and the location and number of P factors is not likely to be a simple one.

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