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POLYMORPHISM FOR CHROMOSOMES CAPABLE OF INDUCING FEMALE STERILITY IN *DROSOPHILA*

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SUMMARY

Genetic polymorphism for factors capable of inducing female sterility have been discovered in two wild populations of *Drosophila melanogaster*. The polymorphisms exist both on the 2nd and 3rd (and possibility the X) chromosomes, and were detected by backcrossing chromosomes from the population into a multiply marked tester stock. The effect is only apparent in certain cytoplasms.

1. INTRODUCTION

IN recent papers, Picard and others describe investigations into female sterility in *Drosophila* arising from crosses among certain laboratory stocks and lines derived from the wild (Picard, 1976; Bucheton, Lavige, Picard and L'Heritier, 1976). Such sterility appears to arise from a nuclear cytoplasmic interaction and to show certain effects akin to paramutation.

Exactly similar results have been obtained and been under investigation in this laboratory since the discovery in 1973 of a genetic polymorphism, in two independent populations, for chromosomes capable of inducing female sterility (Williams, 1973; Allen, 1974).

The present paper describes some of the results of these studies and summarises the evidence that the phenomena described are analogous to those of Picard and his associates.

2. MATERIALS AND METHODS

(i) *Populations*

Texas, originating from 30 inseminated females caught in Austin, Texas, in 1965. Inhaca, originating from 10 inseminated females caught at Inhaca, Mozambique, in 1965. Both populations have been subsequently maintained as cage populations.

Fourteen inbred lines maintained by brother/sister mating from flies extracted from the Texas population some 180 generations previously, were also used.

(ii) *Multiply marked inbred stocks*

Two long inbred stocks (A, B) carrying the following recessive markers on the three main chromosomes:

y = yellow (body); X—0·0,
bw = brown (eye); 2—104·5,
st = scarlet (eye); 3—44·0.

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As well as having a different origin, stock B also contains the marker p^p, which since it is not relevant to what follows will be ignored in future discussion.

N.B. Flies homozygous for *bw* and *st* have white eyes.

(iii) *Procedures used for extracting autosomes from population (fig. 1)*

A large number of males from the two populations were crossed, individually, to the *y.bw.st.* tester stock A. One son was chosen at random

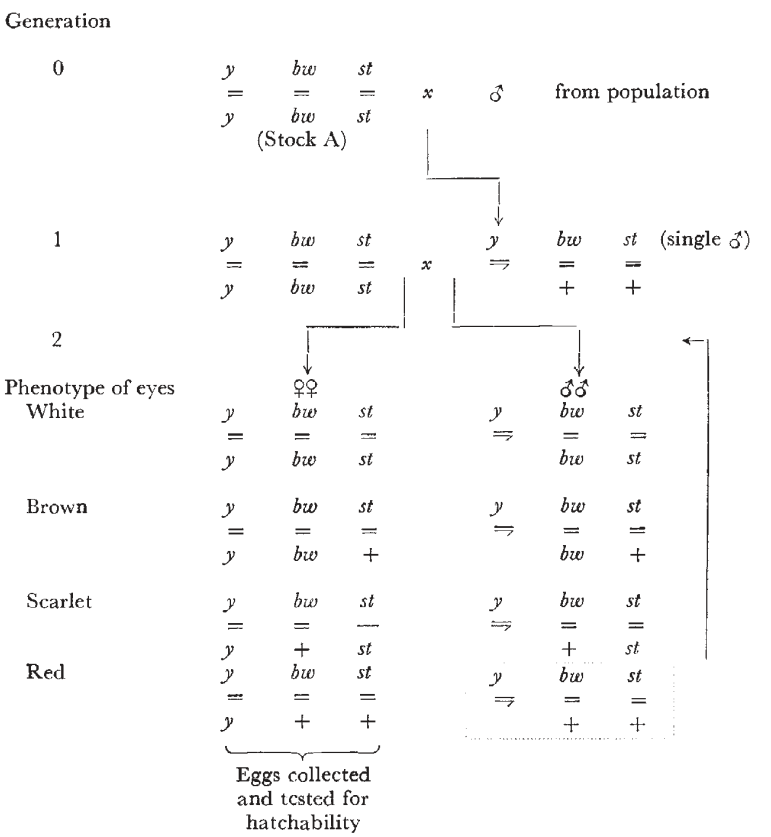
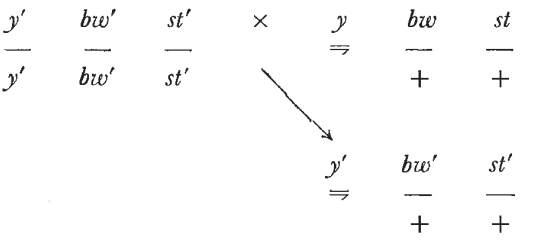


FIG. 1.—Crossing scheme to extract autosomes from the two populations (" + ") indicates an autosome from the population).

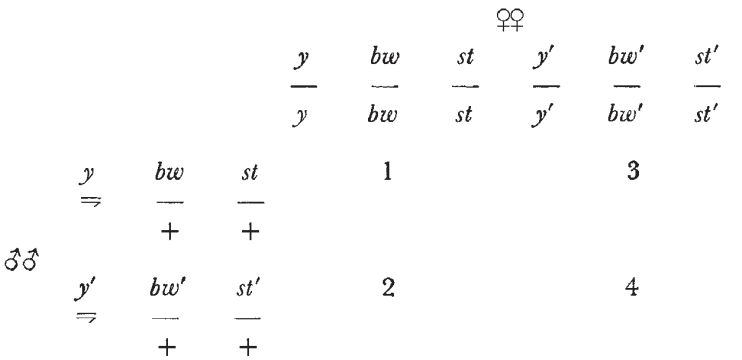
from the progeny of every male and backcrossed to stock A. Such a son would contain one second and one third chromosome from his father, and by backcrossing to the *y.bw.st.* stock, their "+" chromosomes can be maintained and replicated. As indicated in fig. 1, such a backcross will produce 4 ♀♀ and ♂♂ genotypes which can be recognised on the basis of their eye colour. All flies of a given phenotype are genetically identical for the three main chromosomes, and ♂♂ with wild type (red) eyes are identical to their father in generation. Such ♂♂ were used to maintain the extracted chromosomes by backcrossing to stock (A) as indicated in fig. 1.

(iv) *Substitution of tester chromosomes*

Tester chromosomes from stock A can be replaced by those from stock B simply by crossing the red-eyed males described in (iii) to stock B ♀♀. Thus, if we use the notation y′, *bw*′, *st*′ to describe the stock B chromosomes then:



Crossing the two types of male to both stocks A and B yields four sets of progeny as follows:



The genotypes of these four sets of progeny are shown in table 1.

TABLE 1

Genotypes of the four sets of ♀♀ *derived from backcrossing to stock A* (y; bw; st) *and B* (y′, bw′, st′) (*see materials and methods, iv*)

Eye colour	Set 1	Set 2	Set 3	Set 4
White	*y bw st* / *y bw st*	*y bw st* / *y′ bw′ st′*	*y′ bw′ st′* / *y bw st*	*y′ bw′ st′* / *y′ bw′ st′*
Brown	*y bw st* / *y bw +*	*y bw st* / *y′ bw′ +*	*y′ bw′ st′* / *y bw +*	*y′ bw′ st′* / *y′ bw′ +*
Scarlet	*y bw st* / *y + st*	*y bw st* / *y′ + st′*	*y′ bw′ st′* / *y + st*	*y′ bw′ st′* / *y′ + st′*
Red	*y bw st* / *y + +*	*y bw st* / *y′ + +*	*y′ bw′ st′* / *y + +*	*y′ bw′ st′* / *y′ + +*

(v) *Hatchability*

Hatchability was recorded at 25°C by collecting eggs from mass matings. In most experiments to be described the hatchability is that of the eggs from the four types of ♀♀ (with white, brown, scarlet or red eyes) produced from the backcrosses to the tester stocks (table 1). Eggs were collected from 3- to 8-day-old females.

3. Results

One second and one third chromosome was extracted from 54 different Inhaca males and 37 different Texas males, as described in (iii) above. The effect of these chromosomes on hatchability was assessed in females of the genotypes shown in table 1 (set 1), *i.e.* from backcrosses to stock A.

These 91 extractions yielded four different patterns of results (table 2). In some extractions, class D, hatchability was uniformly high from white, brown, red and scarlet eyed females. In class B on the other hand, those females containing a second chromosome from the population (those with

Table 2

Mean hatchability of the four classes of extraction from the Texas and Inhaca populations (%)

		White	Brown	Scarlet	Red	
		y — — / *y* *bw* *st*	*y* — — / *y* *bw* +	*y* — — / *y* + *st*	*y* — — / *y* + +	No. of lines
Class						
A	In.	67	5	3	4	(17)
	Tex.	76	2	7	5	(25)
B	In.	68	70	3	3	(9)
	Tex.	86	44	1	5	(2)
C	In.	69	6	72	8	(20)
	Tex.	72	8	68	12	(6)
D	In.	69	67	78	68	(8)
	Tex.	77	58	57	38	(4)

scarlet or red eyes) produced eggs with low hatchability. Similarly, in class C, hatchability was low from those females with third chromosomes from the population, while lines of class A exhibited low hatchability from all females except those with white eyes. Table 2 also shows the number of lines from each population in every class.

Females of the four phenotypes (white, brown, etc.) were mated, separately, to their male sibs of each phenotype. Apart from an overall depression of hatchability resulting from white males, the hatchability pattern remained unchanged. Thus hatchability depression is confined to females and is independent of the genotype of the male used to fertilise the eggs.

Clearly, both populations are polymorphic for second and third chromosomes which affect hatchability in the background of *y.bw.wt* stock A. The frequencies of low second and third chromosomes can be estimated from table 2 as:

	II	III
Inhaca	0·48	0·69
Texas	0·73	0·84

The effects are consistent and repeatable over generations of backcrossing. Different " low " chromosomes do, however, vary somewhat in their depressing effect on hatchability.

In the Inhaca population there is no evidence of significant association between low second and third chromosomes (Contingency $\chi_1^2 = 0{\cdot}23$, $P = 0{\cdot}631$), while in Texas, low chromosomes are associated ($\chi_1^2 = 5{\cdot}71$, $P = 0{\cdot}01$).

Examination of table 2, class A, shows that the presence of second and third low chromosomes together in the same ♀♀ (red) has no greater depression on hatchability than the two singly (brown, scarlet). Thus they behave like duplicate genes, and show dominance.

TABLE 3

Effect of " low " second and third chromosomes on female fertility in different backgrounds. Each figure is the mean hatchability (%) of five replicates. This table should be read in conjunction with the genotypes shown in table 1

	Set			
Phenotype	1	2	3	4
White	91·0	94·0	95·4	86·0
Brown	5·6	25·0	90·2	78·4
Scarlet	1·6	13·2	94·0	79·4
Red	2·2	14·0	92·0	80·6
Source of cytoplasm	Stock A		Stock B	

Certain Inhaca extractions, typical of each of the four hatchability classes (A-D) shown in table 2, were selected for further study. From these lines, females of sets 2, 3 and 4 (table 1) were produced and their egg hatchability measured. For simplicity the results for class A alone will be given (table 3).

Several points can be noted from this table. Firstly, the low chromosomes do not manifest their effects in sets 3 and 4 at all. These sets have cytoplasms from stock B. Secondly, the " low " chromosomes have less of an effect in set 2 than set 1, although the hatchability is still low. These two sets share the same cytoplasm and, in the case of the red-eyed ♀♀, the only distinction between the two sets is in the zygosity of the X-chromosome.

Thirdly stock B chromosomes do not cause low hatchability since the white-eyed females in every set have high hatchabilities. In particular the white-eyed females in sets 2 and 3, which share the same genotype in different cytoplasms have nearly identical fertility. The corresponding females in sets 1 and 4 are somewhat less fertile, due probably to their homozygosity alone.

The extracted chromosomes are maintained through the male line and kept heterozygous. Hence, it is not normally possible to produce identical female genotypes reciprocally. The Texas inbred lines do, however, allow

such females to be produced and in all such crosses so far examined hatchability is reduced *only* when stock A is the female parent.

Although there is a marked qualitative difference between " high " and " low " chromosomes, variability is still detectable among " low " chromosomes. This can be clearly seen from the hatchability data of brown females carrying different extracted chromosomes in sets 1 and 2, thus:

	Extraction			
	2	45	16	6
Set 1	7·6	3·6	2·0	7·2
Set 2	12·8	37·2	47·2	65·2

These data are the pooled results of five replicates scored in different generations. Analysis of variance of these data, transformed to angles, shows very highly significant effects of " extractions ", " sets " and their interaction. Tukey's test on the means of the four extractions (See Snedecor, 1961, p. 251) shows that three distinct groups exist, 2; 45 and 16; 6. The last two groups are particularly interesting in that they have achieved medium to high hatchability in set 2, which still has the cytoplasm of stock A.

A similar analysis of scarlet fails to reveal the same effects on chromosome 2.

4. Discussion

The major autosomes from both populations studied are polymorphic for a factor (or factors) which can drastically lower egg hatchability when present in the genetic and cytoplasmic background of the *y.bw.wt.* stock A. Although no data are presented here, we have evidence of a similar polymorphism on the X chromosome.

These " low " factors are dominant and behave like duplicate genes in the sense that hatchability is as low with one as with two " low " chromosomes.

Similar genotypes in other cytoplasmic backgrounds do not exhibit the reduction in hatchability. This is clearly illustrated both by the reciprocal differences found in crosses between stock A and Texas inbred lines, and by the reversion to normal high hatchability when " low " chromosomes are placed in the stock B background (see table 3).

We have no reason to believe that the " low " chromosomes have any effect on hatchability in the cytoplasm of the populations from which they were taken. Indeed, inbred lines homozygous for both second and third low chromosomes, have normal hatchability.

This pattern of behaviour is similar in all respects to that described by Picard and his associates (Picard, 1976).

From their studies of the fertility of F_1 ♀♀ in crosses among inbred strains of *Drosophila* they have recognised three classes of strain: Inducer, Reactive and Neutral. Crosses between ♀♀ from a " reactive " strain and ♂♂ from an " inducer " strain yield daughters (termed SF ♀♀) whose eggs have low hatchability. Daughters from the reciprocal cross (RSF ♀♀) on the other hand, lay eggs that hatch normally. The inducing factors show normal Mendelian inheritance when transmitted through the male line, and are

associated with the three major chromosomes. They report serious departures from Mendelian inheritance, however, when the " inducing " chromosomes are passed through SF or RSF females; a departure akin to paramutation.

A single case of a " neutral " strain is reported which appears to have none of the properties of the other two.

In our particular case, the *y.bw.st* stock A is the " reactive " strain while certain chromosomes from the Texas and Inhaca populations are responsible for inducing low hatchability in the " reactive " cytoplasm. The other *y.bw.st.* stock B appears to be " neutral " in that it neither induces low hatchability when crossed to stock A ♀♀ (see table 3, white ♀♀ of the four sets) nor does it respond to the low, inducing chromosomes from the populations.

The phenomena described here resemble those of Picard *et al.* in several other ways. The adults which emerge from the few eggs that do hatch appear to be segregating normally for the recessive markers. The sterility is only manifest in females never males, is readily affected by the environment, and is independent of the genotype of the male used to fertilise the eggs.

We have no experimental evidence on the effects of transmitting low chromosomes through SF or RSF females. However, it has been noted that attempts to affect chromosome substitutions between Texas lines using " cross-over suppressors " have frequently been defeated by progressive loss of fertility as the breeding programme progresses. Such programmes have employed Muller 5 and Cy stocks, stocks which have been shown by Picard to be reactive.

The results presented here thus confirm the findings of Picard and associates and provide evidence of extensive genetic polymorphism for the factors concerned. The existence of the " cryptic " polymorphism requires explanation but will have to await some knowledge of the action of the factors involved.

It does however, suggest that neutral strains should be more common than has been previously reported.

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