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Genetic analysis of the sexual dimorphism of glass in *Drosophila melanogaster*

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SUMMARY

A modifier locus is described that alters the level of phenotypic expression of the third chromosome mutant glass in a sex specific manner. Alternative alleles either confer a sexually dimorphic level of pigment in glass mutants, with the male being greater, or cause similar expression in the two sexes. The alleles are indistinguishable in females but produce the respective phenotypes in males. The gene maps to the tip of the *X* chromosome at position $0{\cdot}96 \pm 0{\cdot}11$. Cytologically, the locus is present between polytene bands 3A6-8 and 3C2-3 as determined by its inclusion in translocated *X* segments in $w+Y$, $Dp(1;2)w^{70h31}$ and $Dp(1;3)w^{67k27}$. The dimorphic allele is dominant to the nondimorphic condition in males heterozygous for an insertional translocation carrying the dimorphic allele and a normal chromosome carrying the nondimorphic form. The dimorphic allele in two doses in males does not exhibit a dosage effect. The modifier phenotype is unaffected in two *X* flies by the presence of the transformer mutation.

INTRODUCTION

It has been known for some time that certain examples of autosomal loci in *Drosophila melanogaster* exhibit a sexual dimorphism in quantitative expression with the male showing higher levels (see Smith & Lucchesi, 1969; Yim, Grell & Jacobson, 1977). An example is the third chromosomal locus, glass, that exhibits a sexually dimorphic level of pigment in most backgrounds (see Lindsley & Grell, 1968). However, some stocks of glass mutants show a nearly equal level of pigment in males and females. This difference is not due to some property of glass alleles *per se*, as evidenced by the fact that a single allele may or may not show a sexual dimorphism depending on the genetic background (Smith & Lucchesi, 1969).

The experiments described below were designed to characterize the nature of the genetic factor(s) involved in determining this difference. The results indicate that alternative alleles of a trans-acting locus determine whether the glass mutants exhibit a sexual dimorphism.

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MATERIALS AND METHODS

Stocks of glass mutations were obtained from the Mid-America Drosophila Stock Center, Bowling Green University, Bowling Green, Ohio. First chromosome mutant stocks are maintained in the Oak Ridge collection and the *X*; autosome insertions were obtained from B. Judd at the National Institute of Environmental Health Sciences, Research Triangle Park, N.C. Descriptions of mutants can be found in Lindsley & Grell (1968).

Flies were grown on standard cornmeal-agar medium or instant medium (Carolina Biological Supply) at 25 °C.

RESULTS

Initially, two stocks of glass, representing the extremes of dimorphism, were examined. The first, homozygous for the *gl* allele, shows a strong sexual dimorphism, the male having a brick red eye colour and the female lemon orange. The second, gl^{60j9}, is only very weakly dimorphic with both sexes showing the lighter colour.

To analyse the basis of this difference, the *gl* and gl^{60j9} stocks were mated reciprocally. The F_1 from the cross in which the females were from the *gl* stock were strongly dimorphic with the expression in each sex resembling the original *gl* line. The results from the reciprocal cross (gl^{60j9} stock being maternal) gave offspring that were only weakly dimorphic. The progeny in both cases resembled the maternal stock with regard to the presence or absence of the sexual dimorphism. While the *gl* and gl^{60j9} alleles show slightly different eye texture phenotypes, they are interchangeable in tests of dimorphism.

At least two possibilities could explain these observations. First, the effect could be maternally inherited such that the trait is conferred to all of the F_1. Secondly, the two lines could possess different alleles of an *X*-linked locus that only produces a recognizable difference in males. These alternatives were distinguished with the following crosses.

Heterozygous females having one *X* chromosome from each of the two parents, and either *gl* or gl^{60j9} mothers, were individually crossed to F_1 males having *X* chromosomes from either the *gl* or gl^{60j9} parents. Results of these four crosses are shown in Table 1 (crosses 3–6). All of the crosses gave lightly coloured daughters but two distinct classes of sons. In no case was there a significant deviation from a 1:1 ratio of the two male classes. Thus, the presence or absence of sexual dimorphism is independent of maternal genotype and is consistent with segregation of a single *X*-linked locus.

To confirm the presence of this gene on the *X*, crosses were made to test for linkage to *X*-chromosome markers. Toward this end, males of the *gl* and gl^{60j9} stocks were mated to *y cv f* females, The F_1 progeny of each cross were allowed to mate *inter se*. Among the F_2 were glass homozygotes. All of the F_2 males from the *gl* crosses were strongly pigmented and did not allow mapping; however, the descendents from the gl^{60j9} cross had two classes of males. These were classified according to the level of pigment and subsequently for *y*, *cv* and *f*. These crosses placed the modifier between *y* and *cv*.

Table 1. *Phenotypes of progeny from reciprocal crosses*

Cross	Origin of female *X* chromosomes*	Origin of cytoplasm*	Origin of male *X* chromosomes*	Glass alleles of progeny	Female progeny phenotypes: Lemon orange	Female progeny phenotypes: Red	Male progeny phenotypes: Lemon orange (nondimorphic)	Male progeny phenotypes: Red (dimorphic)	χ^2	*P*
1	*gl*;*gl*	*gl*	gl^{60j9}	gl^{60j9}/gl	All	None	None	All	—	—
2	gl^{60j9}; gl^{60j9}	gl^{60j9}	*gl*	gl^{60j9}/gl	All	None	All	None	—	—
3	*gl*; gl^{60j9}	*gl*	*gl*	gl/gl or gl^{60j9}/gl	All	None	125	96	3·79	> 0·05
4	*gl*; gl^{60j9}	*gl*	gl^{60j9}	gl/gl^{60j9} or $gl/^{60j9}/gl^{60j9}$	All	None	184	166	0·92	> 0·05
5	*gl*; gl^{60j9}	gl^{60j9}	*gl*	gl/gl or gl^{60j9}/gl	All	None	126	121	0·88	> 0.05
6	*gl*; gl^{60j9}	gl^{60j9}	gl^{60j9}	gl/gl^{60j9} or gl^{60j9}/gl^{60j9}	All	None	217	230	0·38	> 0·05

* The origins refer to the stock from which the *X* chromosome or cytoplasm was derived irrespective of the allelic constitution at glass.

Table 3. *Cytological localization, dominance test and dosage study of* msd(gl)

Type of cross	Duplication	Cytology*	Phenotypes of male progeny: Dimorphic	Phenotypes of male progeny: Nondimorphic	No. tested for insertional translocation	Presence/absence of insertional translocation: +	Presence/absence of insertional translocation: −
y dimorphic ct^6;gl^{60j9}	$Dp(1;3)w^{67k27}$	3A4-6;3E8-3F2;86	152	0	18	8	10
Males	$Dp(1;2)w^{70h31}$	3A6-8;3C2-3;31	40	0	14	7	7
X	$Dp(1;3)w^{49a}$	3A9-B2;3E2-3;81	35	0	9	5	4
C(1)DX,y wf/Y/*Dp;gl*	$Dp(1;4)w^{m65g}$	3B1-2;3C3-5;101	95	0	11	4	7
Females	$Dp(1;3)w^{N264\cdot58a}$	3B2-4;3D5-6;80	312	0	17	10	7
y nondimorphic *cv*;gl^{60j9}	$Dp(1;3)w^{67k27}$	3A4-6;3E8–3F2;86	52	78	70	31	39
Males	$Dp(1;2)w^{70h31}$	3A6-8;3C2-3;31	36	22	28	19	9
X	$Dp(1;3)w^{49a}$	3A9-B2;3E2-3;81	0	26	12	6	6
C(1)DX, y w f/*Y*/*Dp*; *gl*	$Dp(1;4)w^{m65g}$	3B1-2;3C3-5;101	0	111	17	5	12
Females	$Dp(1;3)w^{N264\cdot58a}$	3B2-4;3D5-6;80	0	164	7	5	2

* As described by Judd, Shen & Kaufman (1972).

Another experiment was performed to more precisely define the location of this gene. Accordingly, a stock that was carrying the *y* and *cv* markers and that was nondimorphic when incorporated into the gl^{60j9} background was crossed to the *gl* sexually dimorphic stock to conduct the recombination test. The data are presented in Table 2. From a total progeny of 7799, the modifier of sexual dimorphism of *gl* (*msd*(*gl*)) maps $0{\cdot}96 \pm 0{\cdot}11$ (S.E.) units proximal to *y*. To establish

Table 2. *Genetic localization of* msd(gl)

(+ nondimorphic +; $gl\,♂$ *X y* nondimorphic *cv*/+ dimorphic +; $gl\,♀$)

Phenotype*	No. of male progeny	Genetic map
y nondimorphic *cv*	2119	
y nondimorphic +	353	
y dimorphic +	50	
		$y^{0{\cdot}96}msd(gl)^{7{\cdot}36}cv$
+ *dimorphic* +	5031	
+ *dimorphic cv*	221	
+ nondimorphic *cv*	25	
Total progeny	7799	

* Phenotypes of male progeny from the cross of nondimorphic glass males by females heterozygous for a *y* nondimorphic *cv* and a + dimorphic + chromosome.

that the presumptive crossovers that separate *y* from *msd*(*gl*) were indeed such, representative individual recombinant males were mated to *C*(*1*)*DX*, *yf*/*Y*; gl^{60j9} females and the F_1 scored. Thirteen *y* dimorphic cv^+ and eight y^+ nondimorphic *cv* stocks were established. Each confirmed the original classification.

The presence of this modifier gene on the *X* chromosome between *y* and *cv* and its sexual difference in expression suggested the possibility that the zeste locus (Gans, 1953) was involved. Zeste is sexually dimorphic in expression in chromosomally normal flies; the females are mutant but the males are wild type. If a zeste mutation were present in the sexually dimorphic stocks, the results could be trivially explained as a combination of the two mutants, glass and zeste.

To examine this question, four tests were conducted. First, female flies from the above crosses that were segregating for homozygotes for each respective *X* chromosome from the original stocks and that were +/+ or +/*gl* for the third chromosome, did not exhibit a zeste phenotype. This observation, however, does no rule out the possibility that a cryptic allele (z^a) (Kaufman, Tasaka & Suzuki, 1973) of zeste is responsible. That is, some alleles have no phenotype of their own but do not complement the z^1 mutation. Moreover different alleles of zeste are responsible for the respective types of interactions with *w*, *bx* and *dpp* (Kaufmann, Tasaka & Suzuki, 1973; Jack & Judd, 1979; Gelbart & Wu, 1982). Therefore, the second test was to cross both the *gl* and gl^{60j9} stocks by *sc* z^1 *ec ct* females. The F_1 females were scored for the zeste phenotype. Neither type was zeste, an observation that rules against the possibility of z^a alleles being present in either chromosome.

The third observation that suggests that zeste is not involved is the following. When +/*z*; *gl* females were compared to *z*; *gl* males from the above cross, there

was no sexual dimorphism. Thus, this particular *z* chromosome carries an allele of *msd(gl)* that does not favor the dimorphic situation. The fourth line of evidence, presented below, is that the cytological location of *msd(gl)* is not coincident with that of zeste, as determined by its inclusion in insertional translocations that fail to cover *z*.

The genetic map position of *msd(gl)* suggests that the modifier would be included in the *X* material translocated to the *Y* in the *w+ Y* chromosome (Brosseau *et al.* 1961). This inclusion would allow a test of the dominance relationship of the *msd(gl)* alleles to each other. To establish this, a *C(1)DX, yf/w+ Y; gl*^{60j9} stock was constructed. The attached *X/w+ Y* females were crossed to *y cv; gl*^{60j9} males that have a nondimorphic allele at *msd(gl)* and in independent crosses to *y ct*^{6}; *gl*^{60j9} males that show sexual dimorphism. Recombination between this *y ct*^{6} chromosome and the *X* from the original *gl*^{60j9} stock confirmed that the dimorphic property of this line maps between *y* and *ct*^{6} at a position coincident with *msd(gl)*.

In the former case, the *y cv/w+ Y; gl*^{60j9} males exhibit a sexually dimorphic eye colour relative to the *C(1)DX, y f/ Y* females, in contrast to the phentoype found in a similar stock carrying a normal *Y*. When the *w+ Y* males were crossed to the compound *X* stock with a normal *Y*, the next generation males returned to the nondimorphic state. Thus, there is a correlation between the *w+ Y* and the dimorphic phenotype. However, the presence of *w+ Y* in females does not alter the eye colour.

In the case of the dimorphic *y ct*^{6} chromosome, the presence of the *w+ Y* does not change the phenotype. When this chromosome is replaced by a normal *Y* by crossing again to *C(1)DX, y f/ Y; gl*^{60j9} females, the phenotype remains dimorphic. The presence of *w+ Y* gave no evidence of a dosage effect on the intensity of the eye colour.

These observations indicate the following: (1) The modifier locus resides within the cytological limits of the portion of the *X* translocated to *w+ Y*. (2) The 'sexually dimorphic' allele is dominant to the 'nondimorphic' one. (3) The 'dimorphic' allele in two doses does not exhibit a visible dosage effect. (4) The presence of a 'dimorphic' allele in females does not change the phenotype.

The locus in question lies in a region of the genome that has been subjected to extensive investigation (e.g. Judd, Shen & Kaufman, 1972). Thus, a number of insertional translocations are available that relocate various segments of this portion of the chromosome into autosomal sites. These permitted a more precise cytological localization. Five such insertional translocations, whose breakpoints are listed in Table 3, were each transferred to stocks that were homozygous for *gl* and that carried *C(1)DX, y w f/ Y*. The presence of the insertion was followed in the females by its complementation of the *w* mutant. For each of the five, *C(1)DX, y w f/ Y* females heterozygous for the insertion and homozygous for *gl* were crossed independently to males from the *y cv* nondimorphic and from the *y ct*^{6} dimorphic stocks.

The origins of these rearrangements are diverse and the chromosomes from which the *X*-insertion originated might contain different alleles of *msd(gl)*. The failure of any particular insertion to alter the phenotype could be due to the fact that the *msd(gl)* locus is not included within it, or to the presence of an allele that does

not differ from the one located in the normal *X* chromosome. Only those cases that alter the phenotype can provide information on the action and cytological position of *msd(gl)*. The results are shown in Table 3. All crosses to the *y ct*^{6} stock resulted in dimorphic progeny. To insure the presence of the insertion in each case, representative males were crossed to *y w* free *X* females and the progeny were examined for *w* or *w*$^{+}$ males. The presence of the latter would indicate that the insertional translocation was carried by the paternal parent in the original cross. In each of the five cases, the insertion had been present (see Table 3).

In the crosses of the *C(1)DX, y w f/Y*, heterozygous insertion females to *y cv* nondimorphic *gl*^{60j9} males, a different spectrum of results was found. Both dimorphic and nondimorphic males were recovered in the *Dp(1:2)w*^{70h31} and and *Dp(1:3)w*^{67k27} crosses. When male progeny were classified into dimorphic and nondimorphic classes and individually testcrossed to *y w* free *X* females, the results indicated that the dimorphic phenotype was completely coincident with the presence of the respective insertions. The remaining three insertional translocations produced no recognizable phenotypic effect on glass expression; yet the progeny tests confirmed that each had been present in a fraction of the flies examined. Since the smallest cytological segment that influences the sexual dimorphism of glass is 3A6-8 to 3C2-3, *msd(gl)* must reside within this region of the *X* chromosome.

Since the *msd(gl)* locus has phenotypic consequences only in males, a test was conducted to determine the effect, if any, of the transformer (*tra*) gene on the expression of glass. The recessive allele at this locus, when homozygous, transforms genetic two *X* females into animals phenotypically resembling males (Sturtevant, 1946). Dosage compensating alleles of *X*-linked genes have an unaltered phenotype in transformed flies, rather than the elevation in function expected if transformer or sexual physiology were responsible for dosage compensation.

For this test, *C(1)DX, y f/Y*; females were mated to *ru tra p* males. The F_1 compound females were crossed to *TM3, ri p*^{p} *se bx*^{34e} *e*^{s}*/Pr Dr* males to recover a *ru tra gl* recombinant as a heterozygote with *TM3*. This was aided by a selection of *p*$^{+}$ females, a fraction of which carry a crossover between *gl* and *tra*. These flies were mated to *TM3/Pr Dr* males. From their progeny, males and females heterozygous for *TM3* were mated to produce homozygotes for the individual third chromosomes. Two types of comparison were made. In the first, *C(1)DX, y f/Y, gl tra*$^{+}$ females from certain culture vials were compared to *C(1)DX, y f/Y; gl tra* transformed females from other vials; they were phenotypically similar with regard to the intensity of *glass*. The second comparison is of the *C(1)DX, y f/Y; gl tra* transformed females to the +; *gl tra* males. The eye colours were typical of a sexually dimorphic stock, the males being darker than the *C(1)DX*, transformed females.

It could be argued that the *C(1)DX* chromosome does not contain a dimorphic allele of *msd(gl)* and thus would not be capable of exhibiting a dimorphic phenotype under any circumstances. In view of this possibility, one of the insertional translocations that had previously been demonstrated to carry a dimorphic allele was introduced into the *C(1)DX/Y, gl tra* stock. Accordingly, *C(1)DX, y w f/Y; Dp(1;2)w*^{70h31}*/+* females were crossed by *gl tra* homozygous males. The F_1 *C(1)DX, y w f/Y; Dp(1;2)w+/+; gl tra/++* females were backcrossed to *gl tra*

males. Those F_1 females that inherit the insertional translocation exhibit eye pigment. The compound *X* females homozygous for *gl* and *tra* were compared to *C(1)DX, y w f/Y*; *Dp(1;2)w+*^{70h31} flies that were homozygous for glass but heterozygous for transformer as a result of recombination between the two loci. The two were indistinguishable in phenotype although the glass males in the progeny exhibited the dimorphic phenotype. Thus it is confirmed that the dimorphic state of glass requires the normal male chromosomal constitution (1*X*; 2*A*).

DISCUSSION

The sexual dimorphism of the hypomorphic alleles of glass is determined at least in part by a gene located on the *X* chromosome at map position $0{\cdot}96 \pm 0{\cdot}11$ and cytologically between polytene bands 3A6-8 to 3C2-3. The effect of the gene is discernible only in males; there is no evidence that any response occurs in females. The allele producing the sexually dimorphic phenotype is dominant to the nondimorphic form. Two doses of the dimorphic allele do not exhibit a dosage effect on the level of eye pigment. The transformer gene does not change the female type of expression even in the presence of an allele previously shown to be dimorphic in males.

The two alleles have no obvious phenotypic consequences beyond those described. This suggests that their influence is at least reasonably specific rather than generally effective on many genes. Yet it seems unlikely that the sole function of this locus is to modify the sexual dimorphism of glass mutants; this is merely the means by which it was identified. The failure to observe a dosage effect for the dimorphic allele indicates that the product of this gene is not rate limiting for glass expression. Rather, only the presence of the dimorphic allele is required for this type of response to occur.

In general, the recessive allele at a locus is one which has lost its normal functioning. In the case of *msd(gl)*, the dimorphic allele would produce a functional product and the nondimorphic form would not. The basis of the interaction between the modifier and glass itself is obviously unknown but is intriguing in view of the fact that glass is exceptional in being an autosomal dimorphic locus. This would require that the functional allele produces the unusual phenotype.

An alternative view is that the nondimorphic allele is involved in preventing some autosomal genes from exhibiting a sexually dimorphic mode of expression. The dimorphic allele, then, might encode an altered product that interferes with this function at the glass locus. A mutational analysis of the two allelic forms might distinguish among these and other possibilities.

Yet another explanation might be that *msd(gl)* alters the pigment level by a metabolic process unrelated to the glass gene or its product. The reactions required for such 'metabolic suppression', however, would necessarily be limited to 1*X*; 2*A* males.

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