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Suppressor genes with gender differences in activity in natural populations of *Drosophila robusta*: another approach to wild-type

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Summary

Homozygous or hemizygous expression of an X-linked wing mutant of *Drosophila robusta* varies from a rudimentary wing that does not reach the tip of the abdomen (called 'club') to forms with full-sized but curled or crumpled wings (called 'curly'). Homozygous club females crossed to flies from natural populations or laboratory stocks derived from wild flies invariably produce significantly less club male progeny than the 100% expected, most of them exhibiting less severe phenotypes: 'curly' forms and wild-type. The male progeny from similar crosses using curly females tend to be predominantly normal. By contrast, the male progeny of outcrossed females homozygous for an X-linked eye colour mutant, *vermilion*, are all vermilion. The data indicate that natural populations of *D. robusta* contain suppressors of the wing mutant but not of the eye colour mutant studied. Activity of the suppressors differs by gender: in experiments in which genetic theory expects similar results in the two sexes, males consistently show stronger effects of the suppressors than females.

1. Introduction

In 1915, five years after T. H. Morgan found the white-eyed male that launched *Drosophila* as a major player in the development of genetics, one of his associates, C. B. Bridges, found flies with normal body colour that were homozygous or hemizygous for the X-linked mutant *sable* (*s*). When he finally published a note about it, he ascribed the reversal to an insertional duplication of normal alleles (Bridges, 1919). Bonnier (1926) demonstrated, however, that the cause was a suppressor gene. He referred to it as suppressor of vermilion eye colour (a frequent pleiotropic effect of these suppressors), but it is generally known as *su*(*s*). Since then many suppressors of *Drosophila melanogaster* laboratory mutants, including at least seven *su*(*s*)'s, have been described (Lindsley & Grell, 1967), and the field has burgeoned with the discovery that suppressor genes play critical roles in *Drosophila melanogaster* early development (e.g. DiNardo *et al*., 1994; Leptin, 1995) and that many human neoplasias can be attributed to mutations in, or structural damage to, genes that normally act as suppressors in the regulation of cellular multiplication and growth (e.g. Malkin, 1994; Riley *et al*., 1994).

The data in this paper indicate that the genomes of *Drosophila robusta* from widespread natural populations contain modifier genes that suppress the action of an X-linked wing mutation but apparently none to modify an X-linked eye colour gene. This appears to be the first report of suppressors from native woods-inhabiting *Drosophila* and suggests that such genes may play a role in maintaining wild-type in natural populations. The results would support theoretical models of the evolutionary roles played by suppressor genes and other regulatory elements and by the factors that often induce them – for example the work of McDonald and collaborators (reviewed by McDonald, 1993, 1995). And since the modifiers cause the flies to show an entire spectrum of wing development between extremes, the data strengthen the hypothesis that many modifiers existing in the natural population are responsible for quantitative variation, as has been invoked in the genetic-transilience model of speciation (see Templeton, 1996).

Preliminary data concerning the suppressor system in this study were reported by Levitan (1990).

2. Materials and methods

Drosophila robusta Sturtevant 1916, a relatively large black fly, is one of the more common species that inhabit the deciduous woods of North America east of the Rocky Mountains and north of central Florida.

The haploid number is 4, consisting of a large metacentric sex chromosome, the large heterochromatic Y being approximately the same size as the X; two nearly metacentric autosomes, one approximately the same size as the sex chromosomes, the other somewhat smaller; and a dot chromosome. Natural populations contain extensive chromosomal variation, largely the result of paracentric inversions (reviewed by Levitan, 1992).

The X-linked mutants that are the central focus of this report exhibit wing abnormalities that vary from the extreme of being rudimentary, usually non-eclosed stumps stuck to the back of the thorax, but sometimes free and resembling severe forms of the 'vestigial' mutants of *Drosophila melanogaster*, to the other extreme of being of normal length, variably whole or somewhat crumpled, but curved in some way: most commonly the wings are bent at a sharp angle dorsally; in some, however, they are curved ventrally in a rounded, rather than bent, way, or they are almost normal, quite flat but wavy. By analogy with an X-linked trait in *D. melanogaster* with similar variation of wing expressivity, the *clw* (club) trait described by Golubovsky & Zakharov (1980), Yurchenko *et al.* (1984*a*, *b*), and Zakharov & Yurchenko (1984), those with such severely abnormal wings that do not reach the tip of the abdomen we have dubbed 'club' (cb), and those with the longer, though often quite crumpled, wings as 'curly' (cy). A few flies have had one wing normal and one wing club or curly, or one wing curly and one club; they are counted as 0·5 in each appropriate column of the data. Neither club nor the more crumpled forms of curly can fly, though the flightless forms can 'skip' several centimetres at a time.

The curly form appeared in our laboratory first, in 1982, during routine transfer of a stock founded in 1963 from the siblings of larvae that were heterozygous for a pericentric inversion of the largest autosome (Ipe(2)47 of Levitan, 1985) in several samples from a population cage. The cage had been started by pooling strains derived from females carrying the chromosome breakage factor reviewed by Levitan & Verdonck (1986). The ancestral female of the inducer line was collected in Tibbetts Brook Park, Yonkers, Westchester County, New York in 1948; after inbreeding, her descendants gave rise to the first strain of this species that was homokaryous for all the Standard gene arrangements, and hence very useful for analyses of gene arrangements in natural populations. The founders of the aforementioned population cage derived from crosses of the inducer line to flies from Ohio, Indiana, North Carolina and Virginia populations annotated by Levitan (1992). The club form appeared during inbreeding attempts to develop a true-breeding curly line.

Much of the data of this report is derived from crosses of club or curly females to flies collected in nature. The wild flies used in these studies were from two localities in Arkansas (Fayetteville, Washington County, and Mount Magazine, Logan County), two in New Jersey (Englewood and Paramus, Bergen County), three in New York (Deerfield, Oneida County, Riverhead, Suffolk County, and Ithaca, Tompkins County) and two in Pennsylvania (Clarion, Clarion County, and Philadelphia, Philadelphia County).

Club and curly females were also crossed to males from laboratory stocks of two types: (1) wild stocks descended from inseminated females from Woodbury, Washington County, Minnesota, Ledgewood, Morris County, New Jersey, Dayton, Montgomery County, Ohio, or Myrtle Beach, Horry County, South Carolina; and (2) several mutant stocks: two other X-linked recessive mutants, vermilion eye colour (*v*) and singed bristles (*sn*), and two autosomal recessive mutants, scalloped wings (*sd*) and scarlet eye colour (*st*), or combinations of them with club or curly. These mutants had also arisen here in strains descended from crosses involving the aforementioned chromosome breakage factor and collected (wild) flies. *D. robusta* singed (*sn*/*sn*) females are usually sterile, and *sn*/+ females exhibit forked bristles. Mates derived from the same stock or cross are referred to as 'sibs' in the tables though they did not necessarily have exactly the same parents.

The data are given as mean percentages ± standard errors. The standard errors are based on means and standard deviations calculated from angular transformations of percentage results in the replicates in each experiment category. Since this involved several conversions, with attendant roundings-off, the resultant percentages generally do not add up to 100%. (Corollary: the few data that do total 100% are from single sets, i.e. non-replicative.) The significance of differences between means can generally be inferred by noting the absence of overlap between the larger mean minus two standard errors and the smaller mean plus two standard errors. Where the samples being compared differed greatly in size, the *t*-test for determining the significance of the difference of two means was calculated, using the appropriate sample sizes and standard deviations (e.g. Simpson *et al.*, 1960, pp. 176–178).

3. Results

(i) *Relation of club and curly*

By inbreeding, including matings of descendants of outcrosses, a number of true-breeding club strains have been developed. As indicated by the cb × cb data in Table 1, parents from club stocks sometimes produce a small number of curly. When the mates of the club females include curly as well as club from the same stock (the cb × cb&cy data of Table 1), more curly, and even a few normal-winged, are produced. The club male frequency drops from 98% to 78%.

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Table 1. *Progeny of club-winged* (*cb*) Drosophila robusta *females mated to laboratory stock or wild males described in the text*

	Wing structure of progeny							
	Male				Female			
Experiment	*n*	Club	Curly	+	*n*	Club	Curly	+
cb × cb	933	98·42 ± 0·03	1·58 ± 0·03	0	1013	99·98 ± 0·002	0·02 ± 0·002	0
cb × cb&cy	275	78·27 ± 0·03	21·01 ± 0·03	0·34 ± 0·01	335	98·99 ± 0·03	1·01 ± 0·03	0
cb × cy	1066	34·41 ± 0·02	57·76 ± 0·02	4·55 ± 0·007	977	86·98 ± 0·05	8·99 ± 0·03	2·59 ± 0·02
cb × other stocks	1433	12·06 ± 0·03	74·67 ± 0·06	6·05 ± 0·22	397	48·39 ± 0·21	13·83 ± 0·25	29·52 ± 0·43
cb × wildI[a]	5847	6·61 ± 0·003	46·85 ± 0·05	42·49 ± 0·08	nc			100·00
cb × wildII[b]	759	39·18 ± 0·15	54·71 ± 0·15	1·14 ± 0·06	nc			100·00

Data are the mean percentages ± SD.
cy, curly; nc, not counted because all were +.
Female '*n*' of crosses to other stocks does not include + progeny from experiments in which the mutant mates, like the 'wild' mates in other experiments, were all +/Y.
[a] Using cb females from earlier homozygous stocks.
[b] Using cb females from more recent, further inbred, strains.

Table 2. *Progeny of curly-winged* (*cy*) Drosophila robusta *females mated to males from laboratory stocks or natural populations described in the text*

	Wing structure of progeny							
	Male				Female			
Experiment	*n*	Club	Curly	+	*n*	Club	Curly	+
cy × cb	253	15·75 ± 0·03	83·34 ± 0·02	0·35 ± 0·03	257	96·52 ± 0·05	3·48 ± 0·05	0
cy × cy$sibs	1218	0·03 ± 0·005	9·02 ± 0·03	90·84 ± 0·04	1291	1·97 ± 0·02	85·22 ± 0·05	10·91 ± 0·05
cy × other stocks	823	10·27 ± 0·08	32·15 ± 0·04	54·43 ± 0·03	792	34·72 ± 0·06	34·65 ± 0·52	16·99 ± 0·33
cy × sibsI[a]	410	3·95 ± 0·23	33·39 ± 1·04	58·33 ± 1·15	326	54·58 ± 0·06	15·18 ± 0·40	21·51 ± 0·23
cy × sibsII[b]	1749	0·12 ± 0·004	8·40 ± 0·01	91·00 ± 0·01	1654	0·61 ± 0·003	50·01 ± 0·17	48·58 ± 0·18
cy × wild	7172	0·06 ± 0·001	2·72 ± 0·003	97·00 ± 0·003	nc			100·00

Data are the mean percentages ± SD.
cb, club; nc, not counted because all were +.
[a] The cy females and their mates were F_2 (parents $F_1+ \times F_1+$) from crosses of cb to wild males.
[b] The cy females and their mates were F_2 (parents $F_1+ \times F_1+$) from crosses of cy to wild males.

This trend is accentuated when the mates of the club females are all curly (the cb × cy data of Table 1): a larger number of curly and normal appear.

When curly females are mated to club males, over 95% of the female progeny are club (cy × cb data in Table 2), – similar in this respect to the cb × cb results in Table 1. This indicates that the curly females, too, are homozygous for the club gene.

(ii) *Crosses to other mutant stocks*

When club females are mated to other mutant males (cb × other stocks in Table 1) the club male progeny falls to about 12%. A similar percentage of club appears in the crosses of curly females to these mutants (cy × other stocks in Table 2), but the two sets of experiments differ in the non-club progeny: most of the males produced by the club females are curly, whereas most of the male progeny of the curly females are normal-winged.

(iii) *Crosses to wild males*

Experiments crossing club females to recently collected wild males or males from wild isofemale stocks (Table 1, cb × wild) fall into two groups. In the experiments using females from club stocks developed prior to 1994 less than 10% of the male progeny were club, with approximately equal numbers of curly and normal. Later experiments, using club females resulting from further inbreeding, produced many more club males, though the proportion was only about half of the 100% expected. In these experiments the curly male progeny were only slightly more frequent than in the previous set, but the normal-winged were much fewer in number.

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Table 3. *Progeny of F_1 normal-winged females derived from experiments in Tables 1 and 2*

		Wing structure of progeny							
		Male				Female			
P_1 source	Mates	*n*	Club	Curly	+	*n*	Club	Curly	+
cb × lab stocks	cb or cy	3233	18·19 ± 0·02	27·58 ± 0·02	50·77 ± 0·003	909	34·46 ± 0·03	0·14 ± 0·02	61·65 ± 0·16
cb × lab stocks	+	2614	15·65 ± 0·002	21·94 ± 0·001	61·57 ± 0·003	nc			100·00
cb × wildI	cb/cy 'sibs'	1398	9·99 ± 0·01	25·34 ± 0·02	62·99 ± 0·004	1580	26·78 ± 0·002	11·18 ± 0·006	60·77 ± 0·003
cb × wildII	+'sibs'	910	1·77 ± 0·02	11·05 ± 0·05	86·73 ± 0·07	971	5·88 ± 0·05	22·09 ± 0·04	69·33 ± 0·01
cy × wild	cb/cy 'sibs'	744	0·77 ± 0·03	7·47 ± 0·17	90·39 ± 0·25	820	3·00 ± 0·10	22·20 ± 0·02	68·20 ± 0·005
cy × wild	+'sibs'	4519	0·07 ± 0·002	4·27 ± 0·01	95·18 ± 0·01	5038	0·65 ± 0·009	29·57 ± 0·01	68·58 ± 0·01
cy × wild	cy$+	70	1·43 ± 1·42	0	98·57 ± 1·42	102	0	17·16 ± 3·73	82·84 ± 3·73
cy × wild	cb$cb	106	3·24 ± 0·14	26·88 ± 0	68·95 ± 0·09	116	30·06 ± 0·05	0	69·94 ± 0·05

Progeny percentages ± SD.
nc, not counted because all were +.

By contrast the crosses of curly females to wild males consistently produce over 90% normal-winged males (Table 2, cy × wild; note the low standard errors of the data). The same tendency persists in their curly granddaughters (Table 2, cy × sibsII). The progeny of curly females whose grandmothers were club (Table 2, cy × sibsI), on the other hand, more closely resemble the progeny of *their* grandmothers (Table 1, cb × wildI).

As expected, the female progeny of cb × wild and cy × wild are always normal-winged. Having one + allele, they are expected to produce 50% normal-winged and 50% club or curly male offspring. Table 3 shows that, no matter to whom they are mated, significantly more than 50% of their male offspring are normal-winged. Those F_1 + females whose mothers were curly resemble their mothers (cy × wild, Table 2) and daughters (cy × sibsII, Table 2) in having over 90% normal-winged sons, whereas the F_1 + from club mothers resemble *their* mothers (*cb* × wildI, Table 1) and daughters (cb × sibsI, Table 2) in producing a smaller proportion of normal-winged sons, though one set (F_1 + (from cb × wild) × + sibs, Table 2) comes close to the results from those with curly mothers.

The female offspring of these F_1 crosses are also more than 50% normal-winged. Substantially similar numbers of normal-winged females appear whether their fathers (the 'mates' of Table 3) are cb/cy or + sibs of their mothers – another indication that these sibs of their mothers have the same wing locus genotype (cb/Y) irrespective of phenotype. The experiments using cb/cy 'mates' in Table 3 tend to produce more club, and fewer curly, females than their counterparts using +'mates'.

(iv) *Vermilion experiments*

The parental females in some of the experiments in Tables 1 and 2 were also homozygous for vermilion eye colour. In addition 92 male progeny were observed from crosses of normal-winged vermilion females to wild males. All of the 919 male progeny from these matings (522 from the crosses to laboratory males and 397 from the crosses to wild males) had vermilion eyes.

(v) *Gender differences*

As noted above, some curly often appear in ostensibly club stocks, e.g. cb × cb in Table 1. Almost invariably, the curly are males; if some curly females do appear, they are much fewer than the curly males.

When the mates of the club females include curly as well as club (× cb&cy, Table 1), or they are all curly (× cy, Table 1), the percentage of male progeny that are club drops to as low as about 34%, but the percentage of club females remains in the 87–99% range. It follows that the frequencies of the milder phenotypes, curly and normal, in these experiments are consistently higher in the males than in the females.

In the crosses of club females to other mutant stocks in Table 1 the male parents were normal-winged, many of them +/Y; hence, the number of normal-winged female progeny is greater than among their male sibs. Although the relative numbers of +/Y and cb/Y fathers is not known, leaving unclear the exact proportions to be expected in the female offspring, it is noteworthy that the proportion of club females greatly exceeds that of the club males, whereas the male percentage of the less dramatic phenotype, curly, exceeds that of the curly females. Incidentally, the genotypes of some of these normal-winged mutant male parents had to be cb/Y to account for their having any club female progeny.

The cy × cb experiments in Table 2 produced over 90% club female progeny. Again, however, the male progeny tend to exhibit milder phenotypes, that is, much fewer club, 80% or more curly, and even some wild-type. This result and the anomalous results from the cb × cb&cy and cb × cy crosses of Table 1 are related to our consistent inability to produce a true-breeding curly stock. A number of stocks are referred

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to as 'curly stocks', however, because almost all the females exhibit the curly form (cy × cy$sibs in Table 2). Almost all the males in these stocks are normal-winged. Since they produce cb and cy daughters, these males must be predominantly, or entirely, hemizygous for the club mutation even though about 85% on average are normal-winged.

This trend persists in the rest of Table 2: male progeny are more likely to be normal-winged than their female siblings even though in every case 100% of the males are expected to inherit and express the club gene, whereas many of the females are expected to inherit a + gene from their fathers and are, therefore, not expected to have as large a percentage expressing the club gene they received from their mothers.

Similarly, in the Table 3 experiments the female progeny consistently contain more of the most dramatic phenotype, club, than the males. In some cases the males' predominance of the less dramatic forms invoves a greater number of curly than the females, but in most cases it involves a significantly greater frequency of normal wings.

4. Discussion

Although the first *Drosophila* suppressor is, as stated earlier, attributed to Bridges (1919), the first detailed study of one was the work of Payne (1920)* on what later came to be known as the Suppressor of scute [*Su*(*sc*)]. Scute is an X-linked recessive that results in absence of scutellar, and often other, large bristles. The wild-type number of scutellar bristles is four. Many of his observations resemble our own concerning the suppressors of club. He noted, for example, that even when he seemed to have a scute strain that was bristleless after many generations of inbreeding, a few flies would appear with one or two scutellar bristles. This is reminiscent of our encounter of a few curly in what had been thought to be true-breeding club stocks. Likewise, just as the curly lines were much more variable than the club lines, Payne found that scute lines which, upon selection, were able to increase the number of bristles to three or more (analogous to curly and normal in the wing situation), were much more variable than the lines with zero bristles, or, in a few, one bristle. Payne concluded that the ability to increase bristle number in indviduals that were homozygous or hemizygous for scute was due to at least two modifier loci that suppressed the action of the scute mutation.

Similarly, our data would be explained by assuming that modifier genes exist that can lessen the extreme effect of the X-linked recessive mutant club. Those with only one or a few modifiers appear as various degrees of the 'curly' phenotype with a differential ability to fly. In the presence of enough such modifiers, a wild-type wing is produced even though the individual is homozygous or hemizygous for the club gene. Wild-type in this view is the product of what Milkman (1970*a*) refers to as 'second-order genetic variation', that is, 'phenotypic variation assignable to a number of collaborative loci'. Unlike our postulated modifiers of club, however, the 'collaborative loci', *crossveinless* (*cve*) polygenes, that he observed in natural populations of *Drosophila melanogaster* (e.g. Milkman, 1965, 1970*b*), were based primarily on *cve*-like mutations at multiple, independent loci invoved in crossvein formation, rather than modifiers of a single locus, only secondarily on possible modifier polygenes that may play a role in variable penetrance and temperature sensitivity.

The concept that the curly wing phenotype represents a partial or intermediate stage towards wild-type has its counterpart in many other studies of suppressor genes. To cite but a few examples in various organisms: in *D. melanogaster*, *Lip* causes different degrees of suppression of a *white* variegation allele when it is heteroallelic compared with when it is heterozygous (Csink *et al.*, 1994), and while the authors state that it suppresses *white-ivory*, their figures show only partial suppression. Similarly, various mutations of the yeast *hrs* gene differ in the extent of their suppression of a hyper-deletion phenotype (Santos-Rosa & Aguilera, 1995). And in *Aspergillus nidulans* all the *sna* mutants described cause only partial suppression of the cytoplasmic dynein mutation, *nudA1* (Goldman & Morris, 1995).

Our data show that these suppressor modifiers exist in natural populations. When club females, which have none, or only a few, of the modifiers, are crossed to recently collected wild males or males from an isofemale wild stock (Table 1), the resultant increase in the number of modifiers causes a large proportion of the male progeny, all of whom are hemizygous for club, to be curly or normal. The variability may depend on variability in the number of modifiers carried by the wild males, and possibly also on which parental autosomes (which may differ in modifier content) they contribute to the progeny. A small frequency of the modifiers extant in the club stocks may also be a contributing factor. And when curly females are mated to wild flies (Table 2), the accumulation of modifiers coming from both parents results in a consistently overwhelming number of male progeny that have normal wings, that is, complete suppression of the club gene.

Although the data are more limited, the natural populations apparently do not carry similar suppressors for the vermilion gene. One could speculate that selection for suppressors of a wing mutation would be more critical to the species than suppressors of an eye colour mutant. It is noteworthy that studies of hidden variability of visible mutations in natural populations (Dubinin *et al.*, 1937; Stalker, 1945; Spencer, 1947, 1957) found many more eye colour

* Mistakenly listed as '1921' in Lindsley & Grell (1967).

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variations than severe wing abnormalities, apparently because the latter had been subject to greater selection pressure. Both Stalker and Spencer noted considerable incomplete penetrance among the variants, suggesting possible effects of modifiers.

The consistent uniformity in appearance of the members of animal species captured in nature has given rise to the concept of 'wild-type'. Earlier in the history of evolutionary genetics there was a general impression that the uniformity stems from the organisms having become homozygous ('fixed', in the language of population genetics) for the genes determining these external characteristics. In the words of Wright (1978), 'each wild species was assumed to be almost homoallelic at each locus for a "type" gene'. This concept was effectively demolished by extensive demonstrations that natural populations contained a great deal of hidden variability.

Although it was not generally noted at the time, many of these discoveries of variability provide additional evidence that the natural populations carry extensive arrays of suppressor genes. For instance, Dobzhansky and colleagues found that second, third and fourth chromosomes of *Drosophila pseudoobscura* collected at various localities contained many lethal genes, a similar number of semilethals, and a less easily quantified frequency of other deleterious mutations (often referred to as 'subvitals') that had been carried in the wild in heterozygous form (Dobzhansky *et al*., 1942, 1955; Dobzhansky & Spassky, 1963). A typical result (based on data of Dobzhansky & Spassky, 1963) was a weighted average of 13·9% lethals and 14·1% semilethals in 855 third chromosomes from 6 western United States localities; somewhat smaller percentages, 7·2 lethals and 11·1 sublethals, were found in a sample from Colombia. The studies used a technique which would result in 33·33% 'wild-type' homozygotes for a given wild chromosome if it lacked any deleterious alleles. If the frequency of this class was 0 to less than 4% of the progeny, the wild chromosome was assumed to carry a 'lethal'; from 4% to 16% was diagnostic of the presence of a 'semilethal'; and between 16% and a percentage less than two standard errors below 33·33% indicated the effect of a 'subvital'. (Sometimes 'subvital' was used to denote all the classes significantly less viable than wild-type.)

The intriguing parts of the data are the categories 'sublethal' and 'subvital' (and the 'lethal' situations with up to 4% wild-type progeny). Some homozygotes for these genes emerged as wild-type whereas others were lost. In the case of 'sublethals' up to half of the homozygotes for these genes survived and appeared completely normal; in the case of the subvitals a larger percentage survived. Since the external environments of the vials or bottles in each experiment were quite constant, it is highly probable that the difference between normal development and loss of viability in these cases depended primarily on the presence or absence of suppressor genes related to critical developmental functions.

The data show that when, on the basis of genetic theory, both of the sexes should express the club-wing gene in *Drosophila robusta* equally, a higher percentage of females do so than males. The males, on the other hand, are more likely to emerge normal-winged, that is, to suppress the mutant.

This is the opposite of what would be expected from dosage compensation in *Drosophila*, which appears to involve hypertranscription of the mutant gene in the male to compensate for its single dose compared with the female's double dose (see, for example, the review by Baker *et al*., 1994). Indeed, the males, by virtue of their X-chromosome hemizygosity, are most vulnerable to the deleterious effects of the club mutation, so that the reported suppressor system appears designed to protect them from the effects of dosage compensation by converting the phenotype to a milder wing variation or wild-type. True, Muller (1932) found that females that had only one dose of the mutant, because of a deletion on the other X-chromosome, exhibited a more severe phenotype than the males with a usual single dose. Such a deletion cannot be the cause of our observations, however, because it would demand viability not only for males hemizygous for the deletion but also for such club-winged females as the F_2 daughters of the F_1 normal-winged progeny when club or curly females are crossed to wild males. It would also not explain the basis of the greater suppression of the mutant in males than in females.

The extensive research on dosage compensation has nevertheless discovered unique properties of X-chromosomes that may help to uncover the mechanism of the phenomena described in this report. Certain *cis*- and *trans*-acting elements are now known to regulate specific genes or small groups of adjacent genes of the X-chromosome (see reviews by Baker *et al*., 1994; and Kelley & Kuroda, 1995). The *trans*-acting element *weakener of white* (Birchler *et al*., 1994) is particularly pertinent to our data, as it results in dosage effect suppression of an X-linked gene, analogous to the partial suppression of club in the curly phenotype.

And some of these elments act in a sex-specific manner. For instance, Miller *et al*. (1988) found that in *Caenorhabditis elegans* (where sexual differentiation depends on the X:autosome ratio in a manner very similar to that of *Drosophila*) the *xol-1* (XO lethal) gene represses certain X-linked genes when the X:A ratio is 0·5, as in XO males, but not when the X:A ratio is 1·0, analogous to the normal situation in *Drosophila* females.

Another clue may lie in the differences in chromatin structure of male and female *Drosophila* and their possible effects on gene action. The male X-chromosome has 'a more open chromatin structure as evidenced by its more diffuse appearance and increased width in salivary gland polytene squashes' (Baker *et*

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al., 1994). This may be critical if expression of the modifiers depends on differential dissociation from chromatin, as in the *Polycomb–zeste* suppression complex studied by Rastelli *et al*., (1993).

And the male has a much greater supply of heterochromatin by virtue of the Y-chromosome being totally heterochromatic. In *D. robusta* the metacentric Y-chromosome is as large as the X-chromosome (Carson & Stalker, 1947), but appears as a small clump of diffuse chromatin in polytene preparations because of its heterochromaticity. The pioneer of heterochromatin cytology, Emil Heitz, recognized early on that 'genes which lie within the heterochromatin … intervene in the developmental process of an organism' (Heitz, 1934, as translated and quoted by Zacharias, 1995). Recent work has implicated euchromatin–heterochromatin interactions in a number of *Drosophila melanogaster* modifiers, such as *suppressor of forked* (Mitchelson *et al*., 1993), *Suppressor 2 of zeste* (Wu & Howe, 1995), and hetrochromatin–heterochromatin interactions in the variable suppressor expressions of bw^{D} (reviewed by Talbert *et al*., 1994) and *rolled* (Eberl *et al*., 1993). These appear to involve X-chromosome centric heterochromatin, much less work having been done on the activity of Y-chromosome heterochromatin. The latter is clearly a factor, however, in partial or complete suppression of *abo* (abnormal oocyte) mutants located in second chromosome euchromatin (Pimpinelli *et al*., 1985).

A number of possible mechanisms have been advanced to explain these phenomena, and more than one mechanism may be involved (Tartof & Henikoff, 1991). A paricularly attractive one is that heterochromatin may contain normally silent allelic counterparts of critical euchromatic loci that can under certain circumstances, substitute for mutated genes of the euchromatic locus. This hypothesis would explain much of our data, but it awaits experimental testing.

A number of human pathological conditions exhibit puzzling differences of expression in the two sexes that are not attributable to differences in gene dosage (see discussion and references in McKusick *et al*., 1994). For example, in kindreds of Alport syndrome (hereditary nephritis and deafness) with male-to-male transmission (therefore not X-linked), affected males exhibit earlier and more severe symptoms than females. Affected males have affected progeny of both sexes in equal numbers, but affected females have almost exclusively female affected progeny, the males presumably being so severely affected that they are lost *in utero*. In Wildervanck syndrome (cervico-oculo-acusticus: fused cervical vertebrae, abducens palsy and deafness) and Spiegler–Brooke multiple tumour syndrome there are marked deficits of affected males. Simlarly in Graves' disease (thyrotoxicosis) the male:female ratio of those affected is about 0·2:1. Although in some cases the opposite of our results, in which the females are the more severely affected – as is perhaps to be expected since the basis of sexual differentiation is different – the human data could also be explained by gender differences in the expression of modifiers.

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