Heredity (1979), **43** (1), 71-77

ABSENCE OF DOSAGE COMPENSATION FOR A SEX-LINKED ENZYME IN BUTTERFLIES (*HELICONIUS*)*

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Received 5.iii.79

Summary

6-phosphogluconate dehydrogenase (6PGD) is sex-linked in *Heliconius* butterflies. Within each of two species tested, the specific activity of 6PGD in males (the homogametic sex) is approximately twice that in females. This confirms that sex-linked genes in lepidoptera, as in birds, are not dosage-compensated. This absence of dosage compensation may be the basis for the frequent female-limitation of mimicry, and explains the peculiarity that the loci involved are never sex-linked, whereas male-limited sexual characters can be both sex-linked and autosomal.

1. Introduction

The heterogametic sex has only half as many copies of each X-linked gene as has the homogametic sex of the same species. This difference in gene dosage is " compensated " in mammals and in *Drosophila*, so that males and females produce equal amounts of enzymes from such sex-linked genes. In male *Drosophila* compensation is achieved by transcription of X-linked genes at twice the female rate (Lucchesi, 1973); in mammals, one of the X-chromosomes in every somatic cell of the female is inactivated, so that females have the same effective gene dose as males (Lyon, 1972). Neither of these mechanisms has been demonstrated to occur in other organisms.

Indeed, dosage compensation itself is not universal. Cock (1964) cited several examples of the absence of dosage compensation in birds, along with two possible such cases in moths. There was apparently no compensation for a sex-linked gene for melanism in the moth *Lymantria monacha* (Goldschmidt, 1921), but this observation was hard to interpret, as there were indications that the two sexes differed in their general sensitivity to melanisation. Less equivocal was Stehr's (1959) interpretation of haemolymph colours in *Choristoneura* species as resulting from the interaction of an autosomal locus and a sex-linked modifier locus without dosage compensation, which he extended to account for the wide occurrence of sex-limited polymorphism in lepidoptera, thereby implying that the absence of dosage compensation in these organisms is widespread. In this paper we present the first direct evidence for the absence of dosage compensation of a sex-linked locus in lepidoptera.

* Contribution number 247 from the Program in Ecology and Evolution at the State University of New York at Stony Brook.

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2. Materials and methods

Several species of butterflies of the South American genus *Heliconius* are polymorphic for the enzyme 6-phosphogluconic dehydrogenase (6PGD; E.C. 1.1.1.43), as revealed by starch-gel electrophoresis (Turner *et al.*, 1979). We have confirmed segregation of the 6PGD variants in *Heliconius melpomene*. Indications of sex-linkage of 6PGD were tested by electrophoresis of laboratory stocks of *H. melpomene* originating from Belém, Brasil, and of wild-caught samples of *Heliconius erato* from Trinidad and Panamá. Variation in α-glycerophosphate dehydrogenase (αGPD; E.C. 1.1.1.8) was also assessed for the same samples. The enzymes were detected essentially as described by Brewer (1970).

Activities of 6PGD and αGPD were determined for adults from laboratory stocks of *H. melpomene* originating from Belém, and of *H. erato* originating from São Paulo and Rio de Janeiro. Only the head and thorax were used, 20 to 40 mg of tissue (two or three butterflies) being homogenised in 0·1 ml distilled water per mg tissue. Homogenates were incubated for 20 min. in an ice bath, then centrifuged for 15 min. at 4000xg to remove debris. For *H. melpomene*, six preparations from males and two preparations from females were made. For *H. erato*, two extracts from females and one from males were made.

The reaction mixture for the 6PGD assay was 0·05 ml extract, 1 mg NADP, 8 mg sodium 6-phosphogluconate, and 2 mg $MgCl_2$ in 2 ml 0·1 M Tris (hydroxymethyl) aminomethane-HCl buffer, *p*H 8·0. The reaction mixture for αGPD was 0·02 ml extract, 10 mg sodium α-glycerophosphate, and 2 mg NAD, in 2 ml 0·1 M sodium phosphate buffer, *p*H 7·0. All co-factors and substrates were from Sigma Chemical Co. (Saint Louis, Missouri).

The reactions were followed as an increase in absorbance at 340 mμ, using a Beckman DB spectrophotometer. Reaction rates were measured at 25°C for 2 min., the rates being linear during this period. In each case, the reference cuvette contained the assay mixture, excluding the substrate. Protein concentrations of the extracts were determined by ultraviolet absorbance (Layne, 1963), and enzyme activities were expressed as μmoles NADPH (or NADH) converted per min. per mg protein.

Table 1

Comparison of numbers of homozygotes and heterozygotes for 6PGD in males and females of Heliconius melpomene *and* H. erato. *Allozymes are labeled alphabetically in decreasing order of electrophoretic mobility*

	Homozygotes			Heterozygotes		
H. melpomene		CC	DD	BC	BD	CD
Males		13	2	5	5	8
Females		15	3	0	0	0
H. erato	AA	CC	EE	AC	AE	
Trinidad males	39	27	0	43	3	
females	29	33	0	0	0	
Panamá males	13	9	5	13	2	
females	18	5	2	0	0	

3. Results

6PGD is X(Z)-linked, as shown by the absence of heterozygous females (the heterogametic sex), despite large numbers of heterozygous males in both *H. melpomene* and *H. erato* (table 1). No heterozygous females have been found in any species of *Heliconius* or the related genus *Dryas* (table 2), suggesting (although small sample sizes preclude certainty) that 6PGD is X-linked in the whole group. Heterozygotes of each sex were found for αGPD, confirming the autosomal inheritance of this enzyme.

Table 2

Comparison of numbers of homozygotes and heterozygotes for 6PGD in males and females of several species of Heliconius *and* Dryas

	Males		Females	
Species	Homozygotes	Heterozygotes	Homozygotes	Heterozygotes
H. atthis	9	3	3	0
H. cydno	3	1	2	0
H. ismenius	2	1	5	0
H. numata	15	3	6	0
H. sara	70	2	34	0
D. iulia	28	2	5	0
Total	127	12	55	0

In both *H. melpomene* and *H. erato* the specific activity of 6PGD is significantly greater in males than in females (table 3). The ratio of activity in females to activity in males in not appreciably different from one half. As there was a possibility of their being general differences in enzyme activities in the two sexes, we also determined activities of the autosomally encoded αGPD. No differences between males and females were found (table 3). Consequently, activities of each enzyme simply reflect gene dosage. As expected in the absence of dosage compensation, the ratio of 6PGD to αGPD activities is twice as large in males as in females of the same species (Table 3—all probabilities are one-tailed; no heterogeneity of residual variances).

Table 3

Comparison of activities of 6PGD and αCPD in adult males and females of Heliconius melpomene *and* H. erato. *Values are mean ± standard deviation μmoles NADPH (or NADH) converted per min. per mg protein*

	Males	Females	t	d.f.	P
H. melpomene					
6PGD	3·24±0·80	1·32±0·28	3·18	6	0·0095**
αGPD	9·88±1·63	8·61±0·23	1·04	6	0·17
6PGD/αGPD	0·33±0·05	0·15±0·03	4·90	6	0·0014**
H. erato					
6PGD	7·25	3·36±0·16	19·50	1	0·016*
αGPD	10·46	9·36±0·31	2·89	1	0·11
6PGD/αGPD	0·69	0·36±0·03	9·27	1	0·034

* Significant 5 per cent; ** Significant 1 per cent

4. Discussion

(i) *Evolution of dosage compensation*

The scarcity of information on sex-linked genes has prevented an evaluation of the prevalence of dosage compensation in groups other than mammals, birds (which lack compensation), and *Drosophila* (Cock, 1964). Indeed, 6PGD in *Heliconius* is only the third example of an X-linked locus in butterflies (Silberglied and Taylor, 1973; Grula and Taylor, 1979), and only the second X-linked enzyme in a lepidopteran (May, Leonard and Vadas, 1977). The approximately two-fold difference in 6PGD activities of males and females is a clear case of the absence of dosage compensation. While it could be that by chance we have encountered one of the few uncompensated loci in an otherwise compensated X chromosome, or a locus with sex-limited expression (*e.g.* Freyvogel *et al.*, 1968) which is, fortuitously, sex-linked, this seems unlikely in view of the evidence for the absence of dosage compensation in *Lymantria* and *Choristoneura* (Goldschmidt, 1921; Stehr, 1959).

In some cases, such as the determination of sex, the absence of dosage compensation is to be expected. Similarly, if natural selection favours female-limited colour polymorphism in butterflies (Sheppard, 1961), the absence of dosage compensation could be adaptive (Stehr, 1959). However, the fact that the 6PGD variation in *Heliconius* does not underlie a visible polymorphism suggests that the absence of dosage compensation in lepidoptera may be general, rather than restricted to genes of special function.

That mechanisms exist for dosage compensation of individual genes or small portions of chromosomes has been shown in *Drosophila* (Bowman and Simmonds, 1973; Lucchesi, 1973; Strobel *et al.*, 1978). Consequently, our results are counterintuitive: there are no obvious genetic or evolutionary reasons for X-linked genes in general to be uncompensated in lepidoptera. Indeed, from the apparent selective importance of genetic variation in enzyme activities (Koehn, 1969; Merritt, 1972; Day *et al.*, 1974; Miller *et al.*, 1975; McDonald and Avise, 1976; Devonshire, 1977), one would predict either selection for dosage compensation of most sex-linked genes, or selection for translocation of portions of the sex chromosomes to the autosomes.

We do not understand why dosage compensation occurs in some organisms, but not in others. Why, for example, is it necessary for *Drosophila* males and females to have the same activity of sex-linked enzymes, but not *Heliconius*? There does appear to be some regularity in the presence or absence of dosage compensation. In both *Drosophila* and mammals, the two groups known to have dosage compensation, males are the heterogametic sex; in contrast, both lepidoptera and birds, which lack compensation, have heterogametic females, and apparently Y(W)-chromosomes which are inactivated in the somatic tissues (Smith, 1945; Cock, 1964; Clarke *et al.*, 1976).

Charlesworth (1978) has postulated that in evolutionary time, the inactivation of the Y chromosome is an active process, accompanied in *Drosophila* and mammals by a compensating increase in the activity of the X to restore normal gene dosage. Lepidoptera demonstrate the first process very clearly; as is shown by those females of *Papilio glaucus* which have an active Y chromosome (Clarke *et al.*, 1976), the Y, far from being

genetically inert has potentially functioning genes (not involved in sex determination or fertility), and is "switched off" in the somatic tissues by being condensed into a heteropycnotic body, like the X chromosome in mammals. Another curious regularity relates to the fact that it is only females which inactivate chromosomes, whether X or Y, in this way. It is possible, but unlikely, that lepidoptera inactivate the Y chromosome because there is some adaptive advantage in having a sex-difference in dosage for some of the sex-linked genes. If, on the other hand, the Y is inactivated as Charlesworth suggests, to nullify the effects of accumulated mutational damage, we need to find some explanation for their failure to develop dosage compensation at the same time. The metabolism of the active and inactive Y females of *Papilio glaucus* would be of great interest.

(ii) *Genetic architecture of sex-limitation in lepidoptera*

If the whole, or most, of the X chromosome in butterflies lacks dosage compensation, then this probably does provide the metabolic base for the limitation of expression to the female sex, which is a common feature of mimicry, and of some non-mimetic polymorphisms, in butterflies (*e.g.* Turner, 1978). For an autosomal allele to be sex limited in its expression, it is not necessary that it interact with an X-linked locus with felicitously adjusted dosages, as in Stehr's original model; interaction with the metabolic products of *any* X-linked locus could provide an opportunity for limiting expression to one sex or the other.

Moreover, it is now possible to answer the question raised by Sheppard (1961); if female-limitation is produced by the interaction of a pair of loci, why do we always find that the alleles controlling the polymorphism are on the autosome? One would expect the polymorphism to be controlled in roughly half the species from the X-linked member of the pair.

Consider an allele at the autosomal locus; for its expression to be limited to females it must interact with the X-linked locus in such a way that a single dose permits, and a double dose suppresses, expression. This seems perfectly feasible. But for an X-linked allele to be female-limited it is necessary that it be expressed in a single dose, but that its interactions with the autosome are somehow arranged that expression should disappear when the dose is doubled in a male. Although such interaction may not be impossible, it is perhaps rather rare, and this would account for our failure to find any female-limited X-linked polymorphisms.

Interestingly enough, this restriction does not apply to male-limitation. An X-linked allele will be male-limited if expressed in a double dose in the XX male, but not in a single dose in the XY female; an autosomal allele will be so if a single dose from its X-linked partner does not permit expression, which is enhanced to the point of penetrance by a double dose of the X-linked product (the opposite kind of interaction from that required for female-limitation). As both these styles of gene action seem quite likely, roughly 50 per cent of male-limited characters in butterflies (and just possibly in birds like ducks and pheasants) should turn out to be X-linked. This is exactly what has been found in the only investigation so far made of the repertoire of secondary sexual characters in a male butterfly; on hybridising the closely related species *Colias eurytheme* and *C. philodice*, it was found that both ultraviolet reflectance and hydrocarbon pheromones (the

secondary male characters of *eurytheme*) were X-linked, and that the n-hexyl esters which are the male pheromones of *philodice* were controlled by autosomal genes (Silberglied and Taylor, 1973; Grula and Taylor, 1979). Thus the linkage of the pheromone and reflectance genes on the X chromosome is not necessarily evidence for the evolution of a supergene (as suggested by Grula and Taylor, 1979), but is an outcome of Stehr's hypothesis that sex-limitation in lepidoptera results from the interaction of autosomal genes and uncompensated X-linked genes. Female-limited characters will be predominantly autosomal, and of the genes controlling male-limited characters, roughly half will be scattered among the autosomes and the rest will be linked together on the X chromosome.

Acknowledgments.—We thank Dr R. K. Koehn and Professor S. D. Bradshaw for facilities provided, Dr W. F. Eanes for assistance, and Dr B. Charlesworth and Dr. L. M. Cook for reading the draft. The late Professor P. M. Sheppard, F.R.S., provided the specimens for the determination of enzyme activities. This work was supported by NIH Grant 3R01GM20702 to J.R.G.T. and research funds from the University of Western Australia to M.S.J.

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