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Some new mutants of the Large Milkweed Bug *Oncopeltus fasciatus* Dall

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1. SUMMARY

Three new autosomal mutants of the milkweed bug, *Oncopeltus*, obtained by treatment with ethylmethane sulphonate, are described and compared with the two other previously known variants. Preliminary data are given on the results of testcrosses and of chromatographic analysis of some pteridene pigments.

2. METHODS

Ethylmethane sulphonate was dissolved in insect Ringer and 1 μl of a 0·4 M solution injected through the metathoracic leg of mature adult males. This dose (about 700 μg/g) is comparable to that used by Fahmy & Fahmy (1957) on Drosophila (about 500 μg/g).

(i) *Mutagenesis*

The insects used were a stock that has been inbred for over 10 years in the Department of Zoology, Cambridge. Each male was allowed to mate with several untreated virgin females and the resulting egg batches were isolated. Survivors (F1) from egg batches with a low hatching frequency were selected and isolated as single breeding pairs. From their progeny (F2) single pairs were selected and the progeny of these (F3) were screened for abnormal insects.

In the event of a mutagenic hit in a sperm the F1 individual developing from it would, if the mutation were dominant, show altered phenotype. Dominant mutations are rare, however, and further breeding is required to reveal recessive mutations: on mating F1 individuals will give rise to progeny, half of which will be heterozygous for the mutation. One quarter of F2 matings will segregate homozygous individuals (F3).

(ii) *Chromatography*

With the help of R. R. Jones and Dr R. Harmsen, some preliminary chromatographic studies were undertaken. One dimension paper chromatograms were run in sodium citrate and distilled water and examined under U.V. light.

3. RESULTS

(i) *Description of the mutants with proposed nomenclature*

(*a*) *white body* (*wb*)

Oncopeltus is normally a bright orange red, due to the presence of two pteridenes; the red erythropterin and the yellow YP2 (R. Harmsen, personal communication) in the

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epidermal cells. There are several other pteridenes, and these probably include xanthopterin, isoxanthopterin, leucopterin and pterin-7 carbonic acid (R. R. Jones and R. Harmsen, personal communication). Apart from local spots of melanin the cuticle is transparent. The *wb/wb* individuals lack, from hatching, erythropterin and YP2, and are whitish yellow in colour. The eyes are brown and there is a faint brownish tinge in the abdominal epidermis; this pigment is thought to be an ommochrome (R. R. Jones, personal communication).

(*b*) *red eye* (*re*)

Unlike the wild-type, which has dark brown eyes, this mutant strain has bright red eyes. Insects homozygous for both *re* and *wb* have white eyes, and no longer possess the brownish tinge over the body. *re/re* individuals, therefore, probably lack the ommochrome.

(*c*) *short antenna* (*sa*)

The distal segments of the antenna fail to grow properly, so that the adults often have exceptionally truncated antennae: the two distal segments are sometimes completely lost, although the pedicel, scape and first segment are not affected. The mutant has 100 % penetrance but variable expression. The phenotype can be identified from the third larval stage.

Two other mutant forms have been isolated and described by others; cultures were kindly sent to me by Drs Feir and Abbott.

(*d*) *cream body* (*cb*) (Smissman & Orme, 1969)

The phenotype is visually indistinguishable from *white body;* chromatography has revealed that erythropterin and YP2 are missing, and that, in addition and unlike *wb*, xanthopterin is also lacking (R. Harmsen, personal communication). Individuals homozygous for *cb* and *re* are very similar to *re/re, wb/wb* insects although there is some difference in eye colour.

(*e*) *melanic wing* (*mw*) (Abbott, 1968).

This is a melanic form: the larval stages have darkly melanized wing pads and thoraces, and in adults the normal striped wing pattern is almost completely obscured. The phenotype is identifiable with certainty from the 4th larval stage; *mw/+* adults have dingy wings and can thereby be distinguished from either *mw/mw* or *+/+* individuals.

(ii) *Pigmentation*

wb and *cb* are non-allelic; *wb/+*, *+/cb* individuals have the wild-type orange body colour. Grafting experiments between *cb/cb* and *+/+* and between *wb/wb* and *+/+* have shown that both mutant phenotypes are autonomously expressed by grafted cells. Intercellular complementation does not occur when *cb/cb* tissue is grafted onto *wb/wb* hosts.

(iii) *Genetic crosses*

Crosses made between wild-type and mutant individuals showed that all the mutants described above are autosomal recessives. The results of some test crosses are reported in Table 1, and contingency χ^2 tests showed that the data provided no evidence either for linkage or viability interactions between the genes concerned. If it is assumed that viability interactions do not occur the recombination frequency can be estimated (Table 1).

Viability effects due to particular genes vary from experiment to experiment and this could be due either to different genetic backgrounds or to varied culture conditions.

(iv) *The utilization of sperm by females*

It was noted that *re/re* females, when mated alternately with *re/re* and +/+ males almost always used the sperm from the most recent impregnation. Probably the new sperm displaces the old sperm further into the spermotheca (K. G. Davey, personal communication). This useful feature of *Oncopeltus* means that virgin females are not an essential prerequisite for genetic experiments. A more detailed quantitative study of this phenomenon is being undertaken by A. P. Economopoulos & H. T. Gordon (in preparation).

Table 1. *Results of testcrosses: progeny are from several pairs*

Test Cross	Progeny			
$\frac{sa\ wb\ re}{sa\ wb\ re} \times \frac{sa\ wb\ re}{+\ +\ +}$	+ + +	+ + *re*	+ *wb* +	*sa* + +
	141	142	92	104
	sa wb re	*sa wb* +	*sa* + *re*	+ *wb re*
	78	90	88	118
$\frac{mw\ sa}{mw\ sa} \times \frac{mw\ +}{+\ sa}$	*mw sa*	+ +	*mw* +	+ *sa*
	14	150	32	41
$\frac{cb\ sa}{cb\ sa} \times \frac{cb\ +}{+\ sa}$	*cb sa*	+ +	*cb* +	+ *sa*
	81	93	116	93
$\frac{cb\ mw}{cb\ mw} \times \frac{cb\ mw}{+\ +}$	*cb mw*	+ +	*cb* +	+ *mw*
	77	145	122	92
$\frac{cb\ re}{cb\ re} \times \frac{cb\ re}{+\ +}$	*cb re*	+ +	*cb* +	+ *re*
	85	178	137	137

Corrected recombination frequencies (%) between: *sa* and *wb* 48; *sa* and *re* 57; *wb* and *re* 48; *mw* and *sa* 44; *cb* and *sa* 54; *cb* and *mw* 50; *cb* and *re* 53.

4. DISCUSSION

There has been some interest in the development of pteridenes in *Oncopeltus* (Harris & Forrest, 1967; Bartel, Hudson & Craig, 1958; Hudson, Bartel & Craig, 1959), and the mutants described above should prove useful in any investigations on their biosynthesis.

Oncopeltus is an excellent experimental animal (Dingle, 1968; Lawrence, 1968) with a remarkably short life cycle for such a large insect (4–5 weeks at 29 °C). The holokinetic nature of its chromosomes (e.g. LaChance & Degrugillier, 1969) adds to the interest of *Oncopeltus* genetics. *Oncopeltus* has a diploid chromosome number of 16 (Wilson, 1912).

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