Postlethwait, J.H. University of Oregon, Eugene. A quantitative juvenile hormone assay on Drosophila.

The Cecropia juvenile hormone (JH) has been shown to have a number of morphogenetic effects on Drosophila (1-4). These include on the abdominal tergites and sternites a reduction in bristle size, abnormal bristle morphology, a reduction in bristle number, regions without any hairs (trichomes), regions without any pigment, failure of male genitalia to rotate and decrease in eclosion frequency. In order to provide a useful JH assay, these parameters have here been quantified.

Oregon RC animals were cultured as usual and individual animals were collected as white prepupae, either before or after JH treatment, to provide animals of known age at the time of hormone application. JH was dissolved in acetone and delivered topically to animals in 0.3 µl drops. Eclosed or uneclosed animals were fixed in 70% ethanol 7 days after pupariation.

The abdominal cuticle was mounted between two cover glasses and examined under 400X magnification. The 5th tergite of the male and the 6th tergite of the female were scored, as were the three posterior most sternites. A total of 1071 JH treated animals provide the data for Figures 1 and 2. An additional 359 animals provide the data in Table 1.

Table 1. Relative abilities of 24 JH analogues to block metamorphosis.

Analogue	% aberrant bristles per tergite	Cecropia JH equivalent*
Epoxygeranyl sesamole	95	3,400
Iso C_{17}JH	55	120
C_{16}JH	39	56
C_{18}JH	36	34
C_{18}JH	19	19
C_{18}JH + C_{16}JH	19	19
C_{18}JH + sesamex	13	16
C_{17}JH	18	20
Epoxygeranyl-p-ethyl denzene	19 19	19 19
Epoxygeranyl-p-proply benzene	8	10
Geranyl sesamole	5	6
Chloromethyl C_{16}JH	4	4
C_{16}episulfide	4	5
Bishomofarnesoate	1	2
Williams-Law	0.4	2
Ethyl dichloro farnesoate	0.2	1
Farnesenic acid	0.1	1
Farnesol	0	1
C_{18}imino JH	0.1	1
C_{16}imino JH	0	<1
C_{17}aldehyde	0	<1
Geranyl-Methyl-benzoate-dihydro chloride	0	<1
Epoxy geranyl-o-p-bensoic acid methyl ester ether	0	<1
Geranyl-o-p-benzoic acid (methyl ester) ether	0	<1
Sesamex	0	<1
Acetone	0	<1

*Amount of cercropia JH required to cause the same degree of abnormality.

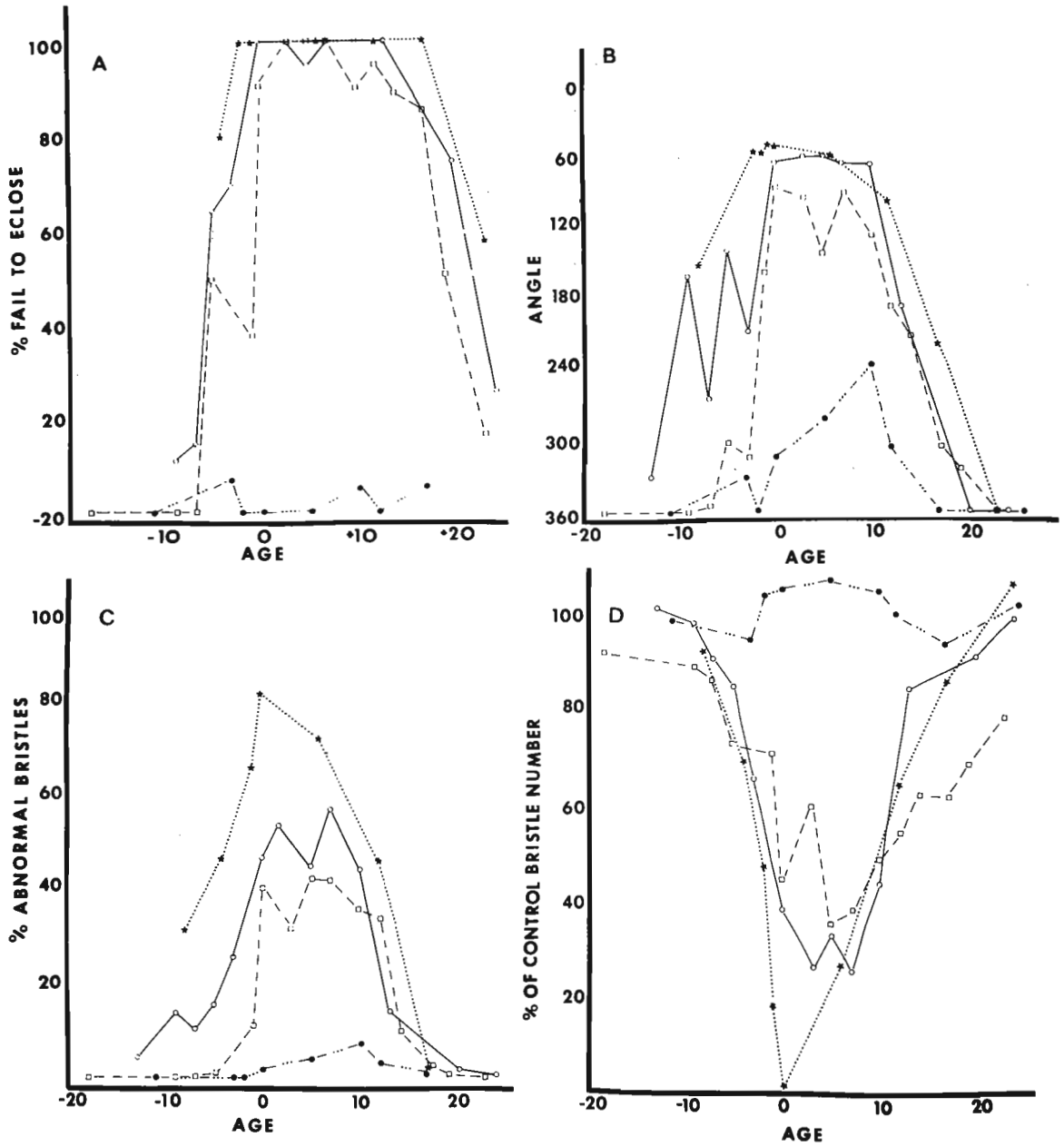

Figure 1. Delineation of the JH sensitive period. A. % of treated animals that fail to eclose by seven days after pupariation. B. The angle of rotation of male genitalia. C. % abnormal bristles on the tergite. D. Bristle number on the tergite as % of control. Stars: 3,400 μg/g; Empty circles: 340 μg/g; Squares: 34 μg/g; Filled circles: 3.4 μg/g.

To insure a sensitive assay the most sensitive developmental stage must be found. Figure 1 shows how four parameters - eclosion frequency, angle of rotation of male genitalia, fre-

quency of abnormal tergite bristles, and bristle number - vary with age. Each point represents 10 - 20 animals. The most sensitive stage includes the first 10 hours after eclosion.

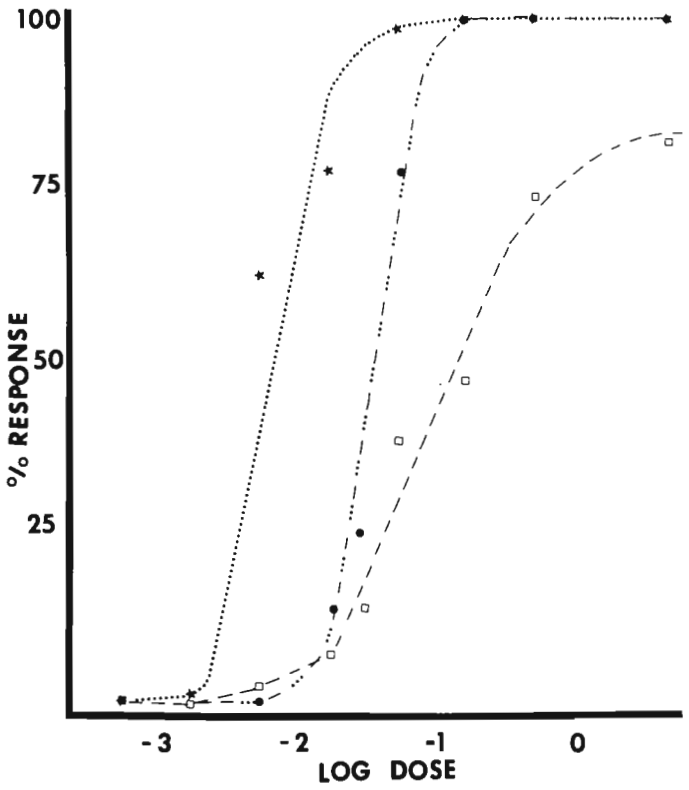

Figure 2 shows the dose-response curve for sternite and tergite bristle number, and eclosion frequency. The threshold response for the posterior sternites is 0.002 µg/individual. Figure 3 shows the sternites from a graded series of JH doses, as well as normal adult cuticle and normal pupal cuticle.

Figure 2. Dose-response curve for JH applied to white prepupae. Filled circles: failure to eclose; Squares: % aberrant bristles on tergite; Stars: % aberrant bristles on sternite.

Several JH analogues were tested using this assay, and the results are given in Table 1. All analogues were delivered at 34 µg/gm animal, and the results are recorded as the dose of cecropia JH required to give quantitatively similar results.

The dose resulting in 50% morphological inhibition of metamorphosis ($I.D._{50}$Morph.,5) is 5 µg/g live weight. The $I.D._{50}$Morph. for topically applied Cecropia JH is about 8 µg/g for Tenebrio and 25 µg/g for Pyrrhocoris (5,6). So Drosophila is about as sensitive as some other insects. The JH sensitive phase is during the period the abdominal histoblasts are dividing most rapidly (7-10).

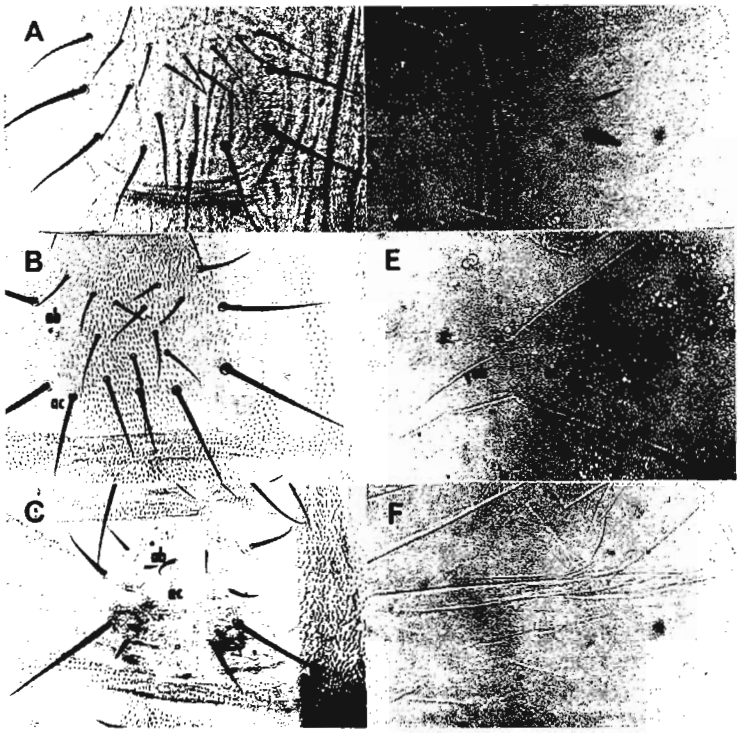

Figure 3. Response of sternites to graded dosages of JH. A. Acetone treated control sternite. B. .002 µg JH/animal. C. .016 µg JH/animal. D. .05 µg JH/animal. E. .16 µg JH/animal. F. Pupal cuticle from an acetone treated control. ab: abnormal bristle; ac: aberrant cuticle.

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References: see next page.

References: 1) Bryant, P.J. and J. Sang 1968, Nature 220:393-394; 2) Ashburner, M. 1970, Nature 227:187-189; 3) Madhavan, K. 1973, J. Insect Phys. 19:449-453; 4) Postlethwait, J.H. (In prep.); 5) Slama, K. 1971, Am. Rev. Biochem.; 6) Williams, C.M. 1970, In: Chemical Ecology. Academic Press; 7) Garcia-Bellido, A. and J. Merriam 1971, Develop. Biol. 26:264-276; 8) Guerra, M., J. Postlethwait and H. Schneiderman 1973, Develop. Biol. 32:361-372; 9) Robertson, C. 1936, J. Morph. 59:351-399.

Vogel, E. Zentrallaboratorium für Mutagenitätsprüfung, Freiburg i. Br., Germany. Strain variations in response to certain indirect mutagens in D. melanogaster.

Strain differences in sensitivity to insecticides such as DDT, parathion and others, as well as cross-resistance are well-known in Drosophila. Among the factors considered to cause such effects is variation in enzyme activity of mixed-function oxidases localized in the microsomes (e.g. R.L. Metcalf, Ann. Rev. Entomol. 12:229, 1967). Since metabolic activation of indirect carcinogens such as aryldialkyltriazenes and azoxyalkanes is also performed by mixed-function oxidases (R. Preussmann et al., Ann. Acad. Sci. 163:697-716, 1960), the question that presented itself was whether similar effects might occur with respect to chemical mutagens.

The strains selected to study this question were our wild strain Berlin K and a resistant one, Hikone R. Dosage-mortality effects and the induction of X-chromosome recessive lethals were analyzed by treating adult males of the two strains. 1-2 day old males were exposed to test solutions of 1.0 mM/l 2,4,6-trichloro-phenyldimethyltriazene or 1.3 mM/l azoxymethane for three days and recessive lethals tested for. To recover stage-dependent sensitivity differences, three broods of three days duration each were set up (Table 1).

Table 1. Frequencies of X-chromosome recessive lethals induced by 2,4,6-trichloro-phenyldimethyltriazene (a) and azoxymethane (b).

Expt.	Strain	Brood I leth./chrom.	%	Brood II l./chr.	%	Brood III l./chr.	%	I-III (II) l./chr.	%
a	Berlin K	131/553	23.7	102/364	28.0	sterile		233/917	25.4 ± 1.4
	Hikone R	29/617	4.7	39/603	6.5	20/753	2.7	88/1973	4.5 ± 0.5
b	Berlin K	6/617	0.97	27/582	4.6	2/202	0.99	35/1401	2.5 ± 0.4
	Hikone R	5/608	0.82	49/603	8.1	sterile		54/1211	4.5 ± 0.6

The experiments revealed pronounced differences in mutation frequencies between both strains. Recessive lethals were induced to a much greater extent in Berlin K males by the triazene, while more lethals were produced by azoxymethane in Hikone R males. Analyses of the data from the different brood pattern experiments (I - III) using the χ^2 test revealed highly significant differences between the samples.

With the compounds so far tested, there was a positive correlation between toxicity and genetic activity for triazenes and azoxyalkanes. Triazenes were more toxic to Berlin K males, while Hikone R males showed higher sensitivity to azoxymethane (as well as the structural isomer of azoxyethane-diethylnitrosamine).

The data are interpreted to be due to genotype-dependent differences in activation of these indirect mutagens resulting in differing concentrations of mutagenic products in various parts of the body including the gonads. This assumption is supported by:

(1) the positive correlation between mutation frequency and the observed sterilizing effects.

(2) the inhibitory action of proper enzyme inhibitors on mutation induction by indirect mutagens (Vogel, unpublished), and

(3) our finding that the mutation frequency in Berlin K - Hikone R hybrids (Berlin K ♀♀ x Hikone R ♂♂) treated with the triazenes is almost exactly half that in the wild strain.

Whatever the correct explanation of the result is, the data show that group-specific cross-resistance to certain chemical mutagens seems to exist in Drosophila.