Postlethwait, J.H. University of Oregon, Eugene. Effects of juvenile endocrine organs and juvenile hormone on the metamorphosis of Drosophila.

Metamorphosis of Drosophila is blocked by substances with juvenile hormone (JH) activity (1-4). The experiments reported here compare the JH syndrome to the effects of juvenile endocrine organs implanted into ready to pupate hosts.

The anterior or posterior thirds of first instar Drosophila larvae were implanted into ready to pupate third instar hosts. In other experiments first instar brain-ring gland complexes or late third instar imaginal discs were implanted into mature larval hosts. After metamorphosis the host was dissected and the position of the implant recorded. The cuticle of the host and the implant were then mounted in Gurr's water mounting medium between two cover glasses. D. virilis and D. melanogaster were used as donors and hosts. The data for both were similar, but were more extensive for D. virilis, and so these appear in Table 1.

Table 1. The effect of implanted parts of first instar upon metamorphosing Drosophila virilis hosts.

Implant	Age of implant	Age of host	Number of recovered hosts	Number of hosts with aberrant cuticle above implant
Anterior third	First instar	Mature third instar	84	17 (20%)
Posterior third	First instar	Mature third instar	87	1 (1%)
Anterior third minus brain-ring gland complex	First instar	Mature third instar	99	5 (5%)
Brain-ring gland complex	First instar	Mature third instar	74	14 (19%)
Imaginal disc	Third instar	Mature third instar	48	0 (0%)

When abdomens of adult hosts were examined abnormalities were evident in 17 (20 percent) of the 84 abdomens that had been implanted with the anterior third of first instar donors. Only 1 abnormality was encountered among 87 abdomens that had received the posterior third of first instar larvae. The abnormalities were localized in the cuticle immediately overlying the implant; they were encountered only when the implant was superficial, never when it was deeply imbedded in the abdomen. As illustrated in Figure 1B, the abnormalities consisted of one or more localized zones closely resembling pupal cuticle (Figure 1D) and differing from adult cuticle (Figure 1A) in terms of the absence of pigment or hairs. Bristles distinctive of adult cuticle were either absent or of aberrant size and shape. Vogt (5) apparently observed similar inhibition of adult differentiation after the implantation of adult corpora allata into mature third instar hosts of D. hydei.

The ring gland of first instar larvae proved to be too small to be dissected and transplanted as such. Therefore, the brain-ring gland complex was implanted into mature larval hosts (Table 1). When the latter emerged as adults, 14 (19 percent) of 74 individuals showed typical integumentary defects. Here again, the local inhibition of metamorphosis was conditional upon the close proximity of implant and overlying cuticle. Control animals received the anterior third from which the brain-ring gland complex had been removed. Only 5 percent of these animals showed the typical defect. This could be due to the retention of the ring gland by some of the anteriors since the ring gland grasps the pharynx rather tenaciously. As a final control imaginal discs from mature larvae were implanted into hosts of the same age, and none of these hosts showed any abnormalities.

These studies, coupled with Vogt's results, show that the ring gland of adult Drosophila and the brain-ring gland complex of first instar Drosophila are capable of inhibiting metamorphosis of the abdomen.

In order to test the effect of exogenously supplied juvenile hormone, 0.005 to 5 μg of C18-Cecropia hormone was applied to white puparia of D. melanogaster or D. virilis in 0.3 μl acetone. 0.3 μ l acetone served as control solution. Seven days after treatment eclosed and uneclosed animals were dissected and their cuticular parts prepared for microscopic observation.

Individuals receiving in excess of 0.05 μg formed defective pharate adults which failed to emerge. As illustrated in Figure 1C, the abdominal tergites of individuals receiving low doses of JH showed patches of cuticle identical to those produced by the implantation of brain-ring gland complexes. Individuals treated with the highest doses showed underneath the pupal cuticle an entire "adult" abdomen covered by an aberrant cuticle showing an almost complete suppression of pigmentation and of bristles and hairs. The cuticle in many cases was indistinguishable from pupal cuticle. Although the aberrant cuticle could not be distinguished from pupal cuticle it must be noted that the normal pupal cuticle lacks any projections or irregularities which in unstained whole amounts permit its positive and unambiguous identification. But we can conclude that exogenously supplied synthetic JH causes a general syndrome identical to that produced locally by an implanted active young larval brain-ring gland complex, and in both cases the aberrant cuticle is indistinguishable from pupal cuticle.

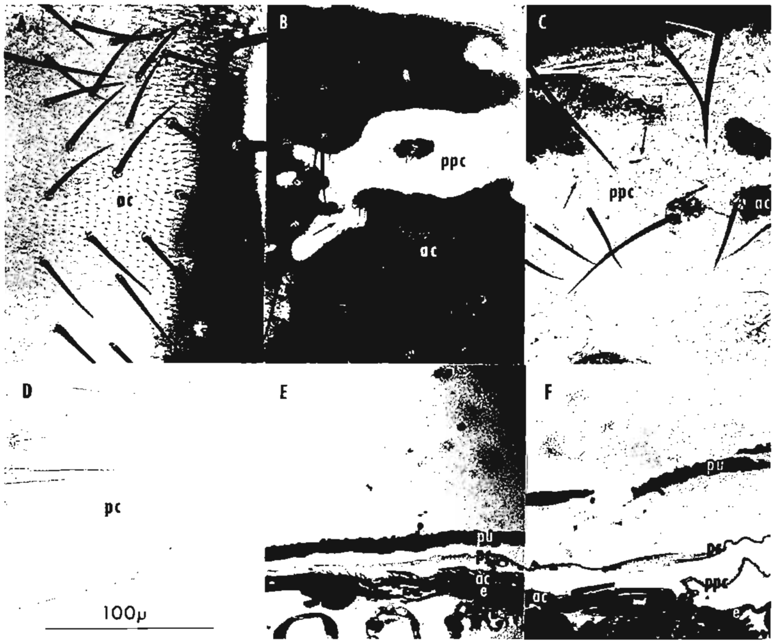

Figure 1. A. Adult cuticle from an acetone treated control.
B. Aberrant adult cuticle lying above the implanted anterior third of a first instar larva.
C. Aberrant adult cuticle from a JH treated pharate adult.
D. Pupal cuticle from an acetone treated control.
E. Histological section of an acetone treated control.
F. Histological section of a JH treated pharate adult.
ac, adult cuticle;
e, epidermis
pc, pupal cuticle
ppc, aberrant adult cuticle
pu, puparium
arrows, abnormal bristles

Experimental animals were also examined histologically by fixation in Bouin's and histological sections were prepared and stained in azocarmine-azan and hematoxylin. All individuals showed three cuticular layers. In the case of the acetone-treated controls, these layers consisted of an external red-staining puparium about 8 μ thick, an intermediate approximately 1 μ thick blue-staining pupal cuticle, and an innermost red-staining adult cuticle about 3 μ thick covered with bristles and hairs (Figure 1E). In the JH-treated individuals (Figure 1F) the two outer cuticles were as in the controls except that the intermediate layer often stained pink rather than blue. Interest of course centered on the innermost cuticle of the experimental flies. This cuticle showed zones which in terms of thickness, staining properties, and surface architecture were indistinguishable from pupal cuticle. Emphasis should be placed upon the color of the aberrant zones, which was blue, a pupal characteristic, rather than red, an adult characteristic.

Although the aberrant cuticle formed under the influence of either JH or juvenile endocrine organs cannot be distinguished from pupal cuticle by the methods here employed, one cannot conclude that the aberrant cuticle is in fact pupal cuticle. Bhaskaran (6) has shown that in Sarcophaga JH causes the production in the abdomen of an abnormal adult cuticle without hairs and bristles in mild cases, and complete suppression of histoblast spreading with no abdominal adult cuticle being formed in severe cases.

References: (1) Dearden, M. 1964, J. Insect Phys. 10:195-210; (2) Bryant, P. and J.

Sang 1968, Nature 220:393-394; (3) Ashburner, M. 1970, Nature 227:187-189; (4) Madhavan, K. 1973, J. Insect Phys. 19:441-453; (5) Vogt, M. 1946, Nature 157:512; (6) Bhaskaran, G. 1972, J. Exp. Zool. 182:127-134.

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Ganetzky, B. and J. Figenshow. University of Washington, Seattle, Washington. An influence of the compound-generating exchange on ring recoverability in tandem-metacentric compound-X chromosomes.

Lindsley and Sandler (1965) noted that a difference in the pericentric heterochromatic content of two different tandem metacentric chromosomes resulted in striking differences in the transmission of tandem rings derived from these. Since the exchange that leads to the generation of a tandem metacentric can occur in different places in the heterochromatin, it is apparent that the location of this exchange can affect the subsequent behavior of the compounds generated.

We have constructed a number of different tandem metacentrics whose variability in behavior is consistent with this idea. The compounds were recovered from females of the constitution: X·Y^L, In(1)sc^4, EN^R y sc^4 sn g/Df(1)v^{74}, X·Y^L, In(1)sc^4, EN^R y sc^4 g/Df(1)g^1 y f B, or X·Y^L, In(1)sc^4, EN^R y sc^4 sn g/y cv v f, after irradiation with 2000 R. The compounds recovered showed striking differences, both within and among different constructions, in respect to the ring progeny generated. The results of the constructions are summarized in the following table:

Genetic constitution	No. C(1)TM recovered	No. stable (no ring progeny)	No. unstable (20-25% ring progeny)
X·Y^L, In(1)sc^4, EN^R y sc^4 sn g / Df(1)v^{74}	13	12	1
X·Y^L, In(1)sc^4, EN^R y sc^4 sn g / Df(1)g^1 y f B	15	8	7
X·Y^L, In(1)sc^4, EN^R y sc^4 sn g / y cv v f	9	4	5

An example of the behavior in crosses of C(1)TM/y^+Y x Y^SX·Y^L, y B/O of stable and unstable compounds is:

	Pat♂♂	TM♀♀	Homo.♀♀ (v)	Ring♀♀	Ring♂♂	Exc.♂♂	Exc.♂♂ / Reg.♂♂	TM♀♀ / Reg.♂♂
Unstable	3753	1055	60	1118	1674	200	.05	.28-.30
Stable	1554	613	-	0	0	29	.02	.39

It should be pointed out that in the cases where the tandem metacentric chromosomes are stable they cannot be distinguished genetically or cytologically from reversed metacentrics. However in every case examined where the chromosome was unstable the breakdown product was a ring confirming the compound's identity as a tandem metacentric. Whether stable or unstable, the compounds were heterozygous for the expected markers, consistent with their being tandem metacentrics. Analysis of the data from the stable compounds suggests that rings are formed but they behave as dominant lethals.

Several points of interest emerge from the data presented above. First, since the behavior exhibited by tandem metacentrics can vary quite drastically even when the chromosomes used in their construction are identical, it seems that the initial exchange leading to the