

emergence with *D. sukutii* males and subsequently injected with an extract from their own species produced a significantly greater number of hybrid progeny than did controls (table 2). The small number of hybrids produced by even treated females was not unexpected considering the scarcity of sperm transferred during interspecific copulation, and the unusual route by which stimulant was administered. Rather unexpected was the fact that vigorous hybrids of both sexes were produced in almost equal frequency. Moreover, the female hybrids were fertile when backcrossed to the parent species, although the male hybrids were sterile.

These results indicate that the genetic incompatibility between the 2 species is very small, and that the species-specificity of the paragonial substances plays a certain role as a hybridization barrier, though the pre-mating barriers are obviously of primary importance. I would like to propose the term 'paragonial sterility' for such gametic isolation. With paragonial sterility, a female first inseminated by a heterospecific male, and then by a conspecific male will not suffer the reduction of fertility found with 'insemination reaction', another gametic isolating mechanism

known in *Drosophila*¹⁰. Perhaps such paragonial sterility, having been favored by natural selection, will be discovered in other species, especially those whose pre-mating isolation is incomplete.

- 1 Acknowledgment. I wish to thank Dr B. L. S. Pierce for reading the manuscript.
- 2 Leopold, R. A., A. Rev. Ent. 21 (1976) 199.
- 3 Leahy, S. R. M. G., and Craig, Jr, G. B., Evolution 21 (1967) 41.
- 4 Chen, P. S., Experientia 32 (1976) 549.
- 5 Chen, P. S., Fales, H. M., Levenbook, L., Sokoloski, E. A., and Yeh, H. J. C., Biochemistry 16 (1977) 4080.
- 6 Bock, I. R., and Wheeler, M. R., Univ. Texas Publ. 7213 (1972) 1.
- 7 Garcia-Bellido, V. A., Z. Naturforsch. 19b (1964) 491.
- 8 Baumann, H., J. Insect Physiol. 20 (1974) 2347.
- 9 Baumann, H., J. Insect Physiol. 20 (1974) 2181.
- 10 Patterson, J. T., Proc. natl Acad. Sci. USA 32 (1946) 202.

0014-4754/83/020190-03\$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1983

Differential aminotransferase activity in normal, allatectomized, brain-cauterized and juvenoid treated male and female bugs (*Lohita grandis*)¹

Sanjay Mandal²

Entomology Laboratory, Zoology Department, The University of Burdwan, Burdwan-713104, West-Bengal (India), February 24, 1982

Summary. L-Alanine:2-oxoglutarate aminotransferase (GPT, 2.6.1) and L-aspartate:2-oxoglutarate aminotransferase (GOT, 2.6.1) activity were different in different tissues of each sex and also in the 2 sexes. In all tissues of both sexes GPT activity was found always to be higher than GOT activity. Allatectomy leads to a decline in the activity of both enzymes in all tissues of males and females. Juvenoid treatment of the allatectomized insects reverses the effect of allatectomy. After both allatectomy and brain-cauterization and extremely high decline of the activity of both enzymes in comparison to cases in which only allatectomy had been carried out.

The literature on the effects of juvenoids, allatectomy and ablation of median neurosecretory cells at the level of the whole organism and at the biochemical level in different insect species has grown exponentially over the past few decades, but there have been few demonstrations of direct or indirect relationships between the application of a particular juvenoid, or allatectomy or brain-cauterization, and synthesis or activity of a particular enzyme^{3,4}. The 1st indication of differential cellular enzyme activity in normal and sterile boll weevils was given by Chang et al.^{5,6}, and the occurrence of different transaminase activities in different insect tissues was reported by Bheemeswar and Sreenivasaya⁷ in *Bombyx mori*, Chen and Diem⁸ in *Drosophila melanogaster*, and Mandal et al.⁹ in *Schizodactylus monstrosus*. A decline in the activity of transaminase resulting from allatectomy in adult insects was first reported by Wang and Dixon¹⁰. The application of juvenile hormone inducing the activity of acid phosphatase in different tissues was reported by Beel and Feir¹¹. Such fragmentary findings demonstrate that corpora allata play an important function on the cellular enzyme level, apart from their main gonadotropic role in adult insects. Therefore it is of academic interest to know actually what role the different endocrine centres play in affecting the cellular enzyme level, and whether there are any sexual differences in their function. The present investigation was undertaken to find out the effects of brain, corpora allata and synthetic juvenoids on the

transaminase activity in adult male and female *Lohita grandis* Gray (Pyrrhocoridae: Heteroptera: Hemiptera). The insects were reared in the laboratory following the procedure described elsewhere¹² and emerging adult (4 h after emergence) males and females were used in the experiments. The necessary organ tissues for the experiments were collected by dissecting the insects under Ringer's solution mixed with phenyl thiourea. The hemolymph was collected separately in ice-cold centrifuge tubes previously coated with phenyl thiourea to inhibit the tyrosinase activity. Allatectomy was performed following the method outlined by Stay and Tobe¹³, and brain-cauterization was performed using the technique of Girardie¹⁴. The juvenoid used here was the highly active compound of hemipteran bugs which was synthesized and bioassayed by Filho et al.¹⁵ and Pinchin et al.^{16,17}. This juvenoid compound was a derivative of N-geranylaniline and its chemical structure is N-(2,5-dichlorophenyl)-3,7-dimethyl-2,6-octadienylamine. It was injected at a dose of 20 µg/insect in 10 µl olive oil as solvent. Control insects received only the same quantity of olive oil. Insects were sacrificed after 24 and 48 h after each treatment. The enzyme was estimated following the methods described by Reitmann and Fränkel¹⁸ and the protein content was determined by the method of Lowry et al.¹⁹ using bovine serum albumin as standard. In each treatment 60 females and 40 males were used and all surgically treated animals were kept at

25°C ± 0.5°C and 80% relative humidity. The data were processed statistically using Student's t-test and an analysis of variance.

The results obtained here show that in normal insects, i.e. in the control insects, GPT activity was always higher than GOT activity in all tissues and in both sexes (tables 1 and 2). The quantity of these 2 cellular enzymes in the different tissues from both sexes of the normal insect also shows differential results, e.g. hemolymph from male insects shows a 2–3 times higher activity of both GOT and GPT than hemolymph from female insects, whereas fat bodies and gonads from female insects show a greater activity of both GOT and GPT than in male insects (tables 1 and 2). Similarly, the different tissues from a single sex also show differential results in the GOT and GPT activity. Allatectomy leads to a general decline of both the GOT and GPT activity ($p < 0.05$), except in the testis where allatectomy did not produce any significant change. There was sometimes a greater decrease when the enzyme assay was performed a longer time after allatectomy than when it was carried out after a short time. This was most significant in the case of the ovary ($p < 0.01$). One interesting result obtained after allatectomy is that both GOT and GPT were affected simultaneously in the same manner (tables 1 and 2). A treatment of the allatectomized insect with juvenoid reverses the effect of allatectomy. Both the GOT and GPT activity were induced significantly except in the testis where the changes were not so apparent. After both allatectomy

and brain-cauterization both enzymes decrease to an abnormally low level (lower than from any other operation) which was significantly different from that produced by any other treatment performed here ($p < 0.01$) and ($p < 0.05$). It was also interesting to note that after these 2 operations both GOT and GPT sometimes showed no activity.

The role of L-aspartate:2-oxoglutarate aminotransferase (GOT) and L-alanine:2-oxoglutarate aminotransferase (GPT) in insects, particularly in plant sap feeders, during the post-embryonic developmental and adult period has been thoroughly studied by several workers^{7,8,10}. The differential activity of these 2 cellular enzymes obtained here in the 2 sexes and also in the different tissues indicates that the importance of these 2 enzymes is sex and tissue specific. After allatectomy both enzymes show similar and coordinated patterns of change in all the tissues of both sexes (except in testis) which indicates that the role of the corpora allata were more or less similar in both sexes. But if we consider the amount of the enzyme changes in each tissue of both sexes then the results clearly indicate that the potentiality of responsiveness to the corpus allatum secretion of the different tissues from each sex was different²⁰. The insignificant changes of GOT and GPT in the testis after allatectomy might be due to the fact that the biochemical events occurring in the testis are not controlled by the corpus allatum secretion whereas the rapid inhibition of the activity of these 2 enzymes in the ovary after allatectomy

Table 1. The fluctuation of L-aspartate: 2-oxoglutarate aminotransferase (GOT) activity in adult males and females of *Lohita grandis* after different treatments. Activity expressed in terms of micromoles of oxaloacetate formed/min/mg protein

Source	Sex	Control***	Allatectomized		Allatectomized + JHa-treated		Allatectomized + brain-cauterized	
			24 h	48 h	24 h	48 h	24 h	48 h
Hemolymph	♂	6.00 ± 0.05	4.85* ± 0.05	4.00 ± 0.16	5.98 ± 0.03	7.25* ± 0.20	4.12 ± 0.07	3.00** ± 0.12
	♀	3.25 ± 0.20	1.25* ± 0.22	0.92 ± 0.08	4.21 ± 0.16	5.00** ± 0.28	1.15 ± 0.02	0.02** ± 0.04
	♀	9.70 ± 0.15	7.00* ± 0.03	6.35 ± 0.22	9.00 ± 1.20	10.25* ± 0.23	4.64** ± 0.25	4.05 ± 0.07
Fat-body	♂	16.34 ± 0.92	9.25** ± 0.35	6.30 ± 0.65	15.75 ± 0.12	18.26** ± 1.20	8.28** ± 0.02	8.00 ± 1.25
	♀	4.80 ± 0.15	4.00 ± 0.02	4.25 ± 0.88	4.71 ± 0.06	5.24 ± 0.77	2.76 ± 0.24	2.00** ± 1.26
	Ovary	11.25 ± 0.25	5.00* ± 0.07	4.25** ± 0.26	11.24 ± 0.07	11.95** ± 0.02	4.88 ± 0.36	4.00** ± 0.05

*Significant in comparison with control only, $p < 0.05$; $n = 12$; **significant in comparison with each treatment and with control, $p < 0.05$; $n = 12$; ***as there were no significant differences found between the controls of each treatment, only the mean values are given here.

Table 2. The changes of L-alanine:2-oxoglutarate amino-transferase (GPT) activity in adult males and females of *Lohita grandis* after different treatments. Activity expressed in terms of micromoles of pyruvate formed/min/mg protein

Source	Sex	Control***	Allatectomized		Allatectomized + JHa-treated		Allatectomized + brain-cauterized	
			24 h	48 h	24 h	48 h	24 h	48 h
Hemolymph	♂	11.30 ± 0.75	9.00 ± 0.12	8.56* ± 0.06	11.56 ± 0.02	13.00** ± 0.13	6.34 ± 0.03	5.00** ± 0.42
	♀	5.92 ± 0.84	3.21 ± 0.76	3.00* ± 0.50	7.30* ± 0.55	7.90 ± 0.13	2.00 ± 0.61	1.85** ± 0.09
	♀	14.96 ± 0.33	12.00* ± 0.32	9.25** ± 0.25	14.21 ± 1.24	18.09* ± 0.98	7.22** ± 1.25	6.16** ± 0.90
Fat-body	♂	28.05 ± 1.20	16.30* ± 0.05	15.88** ± 1.29	28.92 ± 1.50	35.00** ± 2.23	14.00* ± 2.29	11.88** ± 1.05
	♀	6.12 ± 0.50	6.00 ± 0.72	5.56 ± 0.16	6.11 ± 0.05	6.59 ± 1.28	4.76 ± 0.10	4.08** ± 0.35
	Ovary	20.05 ± 0.92	18.17 ± 0.06	15.00* ± 0.93	20.92 ± 1.06	20.00** ± 0.88	7.39** ± 0.16	7.98 ± 1.95

*Significant in comparison with control only, $p < 0.05$; $n = 12$; **significant in comparison with each treatment and with control, $p < 0.05$; $n = 12$; ***as there were no significant differences found between the controls of each treatment, only the mean values are given here.

clearly reveals the corpora allata and ovary interaction. But the question why the level of the 2 enzymes showed a maximum decrease after both corpora allata and brain operation remains unanswered.

The role of juvenile hormone on the GOT and GPT activity has been proved by injecting the juvenile hormone analogue into allatectomized insects, which reverses the effect of allatectomy. But the results after the application of juvenoid to allatectomized insects for the different tissues in both sexes are different, and this signifies that the sensitivity of the different tissues and sexes to the juvenile hormone analogue used here were different. The results obtained here show that the tissues most sensitive to juvenile hormone analogue action and those with the greatest potential for response, are the fat body and the ovary, and to some extent the hemolymph, too. It was also shown that the females were more sensitive to the juvenoid than the males. The main cause for this type of differential response to juvenoid and other hormone action will be clear when the molecular mode of hormone action on insects is discovered.

- 1 Part of the Ph.D. thesis, Department of Zoology, University of Burdwan, Burdwan 1982.
- 2 Acknowledgments. Author is grateful to Prof. A.M. De Oliveira Filho (Universidade Federal do Rio de Janeiro e Instituto de Pesquisas da Marinha, Brazil) for the kind gift of the juvenoid sample and for providing the bioassayed data and literature concerning this juvenoid in various hemipteran insects. This work was supported by the Council of Scientific and Industrial Research (New Delhi).
- 3 Mandal, S., Roy, S., and Choudhuri, D.K., *Acta physiol. hung.* 58 (1981a) 53.
- 4 Mandal, S., Roy, S., and Choudhuri, D.K., *Curr. Sci.* 50 (1981b) 871.
- 5 Chang, Y.H., Haynes, J.W., Frazier, J.L., and Heitz, J.R., *Comp. Biochem. Physiol.* 62B (1979) 51.
- 6 Chang, Y.H., Frazier, J.L., and Heitz, J.R., *Comp. Biochem. Physiol.* 62B (1979) 45.
- 7 Bheemeswar, B., and Sreenivasaya, M., *Curr. Sci.* 21 (1952) 235.
- 8 Chen, P.S., and Bachmann-Diem, C., *J. Insect Physiol.* 10 (1964) 819.
- 9 Mandal, S., Ghosh, B., and Choudhuri, D.K., *Insect Sci. Appl.* (1982) in press.
- 10 Wang, S., and Dixon, S.E., *Can. J. Zool.* 38 (1960) 275.
- 11 Beel, C., and Feir, D., *J. Insect Physiol.* 23 (1977) 761.
- 12 Mandal, S., Ghosh, B., and Choudhuri, D.K., *Curr. Sci.* 51 (1982) 51.
- 13 Stay, B., and Tobe, S.S., *Gen. comp. Endocr.* 33 (1977) 531.
- 14 Girardie, A., *Bull. Soc. zool. Fr.* 91 (1966) 423.
- 15 De Oliveira Filho, A.M., Pinchin, R., Figueiredo, M.J., Muller, C.A., Goncalves, J.R.A., and Gilbert, B., *Rev. Brazil Biol.* 41 (1981) 197.
- 16 Pinchin, R., De Oliveira Filho, A.M., Figueiredo, M.J., Muller, C.A., Gilbert, B., Szumlewicz, A.P., and Bengon, W.W., *J. econ. Ent.* 71 (1978a) 950.
- 17 Pinchin, R., Zocher, D.H.T., Carvalho, M.P.M., and Pinto, M.C.R., *Brazil Rev. latinoam. Quim.* 9 (1978b) 195.
- 18 Reitmann, S., and Fränkel, S., *Am. J. clin. Path.* 28 (1957) 56.
- 19 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., *J. biol. Chem.* 193 (1951) 265.
- 20 Gilbert, L.I., and King, D.S., in: *The physiology of insects*, vol. 1, p.292. Ed. M. Rockstein. Academic Press, New York and London 1973.

0014-4754/83/020192-03\$1.50 + 0.20/0

©Birkhäuser Verlag Basel, 1983

Evidence for the neurohormonal basis of commitment to pupal diapause in larvae of *Sarcophaga argyrostoma*¹

J.M. Giebułtowicz and D.S. Saunders

Department of Invertebrate Physiology, The Zoological Institute, University of Warsaw, Zwirki i Wigury 93, PL-02-089 Warsaw (Poland), and Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT (Great Britain), May 19, 1981

Summary. Implantation of brain-ring gland complexes from short-night larvae into long-night larvae reversed the diapause 'programme' of the recipients after metamorphosis to the pupal stage. The converse experiment did not induce diapause. These results demonstrate that the larval endocrine centers are photoperiodically programmed for diapause or non-diapause development long in advance of the diapause stage.

One of the more important unresolved problems in insect developmental biology is how environmental factors (specifically photoperiod) induce seasonally appropriate commitments to diapause or non-diapause development. In the flesh fly, *Sarcophaga argyrostoma*, for example, larvae raised in short daylengths (long nights) enter an overwintering pupal diapause, whereas those raised in long days (short nights) adopt an alternative summer pathway with uninterrupted pupal development and successive generations of flies²⁻⁴.

The minimal requirements for such a response are a photoreceptor, a 'clock' to measure daylength (or night-length) and to integrate such information, and an effector system to control the onset of the diapause state. Investigations into the nature of the photoperiodic clock are still restricted to largely formal analyses: these show that night-length is the component of the environment that is measured, and that the clock is part of the insect's circadian system⁵⁻⁸. Next to nothing is known about the concrete physiology of time measurement, and this situation is

unlikely to change until more is known about the physiology of circadian pacemakers. On the other hand, there is some concrete knowledge of the hormonal basis of the diapause state. Several investigators have shown that pupal diapause in *Sarcophaga* spp. may be terminated by an administration of exogenous ecdysteroids⁹⁻¹¹, and it is thought that pupal diapause in these flies is a result of the inactivation of the cerebral neurosecretory cells, a consequent hiatus in the secretion of the prothoracotropic hormone (PTTH), and a halt to ecdysone production by the ring gland¹². Diapause occurs at a precise morphogenetic stage⁹ in a 'trough' between 2 periods of ecdysteroid release, the former correlated with puparium formation and pupation, the second initiating adult differentiation¹³. In diapause pupae, therefore, the second, adult-initiating pulse of ecdysterone does not occur and development ceases¹⁴.

The most obvious gap in our understanding is how the clock, having discriminated between a short and a long night, then controls the secretion or retention of PTTH.