Macromolecular synthesis in wing discs of *Spodoptera mauritia* Boisd: Effects of a juvenile hormone analogue

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Last instar larvae of *S. mauritia* treated topically on day 0, day 1, day 2 and day 3 with a daily dose of 25 μg juvenile hormone analogue (JHA) moulded into supernumerary larvae. The imaginal discs of the supernumerary larvae especially those of mouthparts and thoracic appendages showed pupal characteristics. However the wing discs, which showed only partial differentiation, were uneverted and highly tanned. In an effort to provide an explanation to this anomaly the RNA, DNA and protein profile in the wing discs of supernumerary larvae were studied. Quantitative analysis of DNA, RNA and protein showed a considerable increase in the amount of DNA and protein and a decline in RNA level. SDS-PAGE analysis of wing disc proteins of JHA treated larvae showed a reduction in the expression of many major proteins that were predominant in the wing discs of control larvae. The results suggest that JHA induced inactivation of genes involved in the synthesis of proteins needed for evagination process may be responsible for the formation of uneverted, partially differentiated pupal wing discs in supernumerary larvae.

In lepidopteran insects the imaginal discs of antennae, proboscis, labial palps and legs develop in intimate association with corresponding larval appendages. The wing imaginal discs on the other hand are not associated with any larval structures, instead they form discrete epidermal placodes that evaginate and become pupal and adult wings. Hence imaginal discs of wings have been ideal tissues for studying the mode of action of insect hormones. Juvenile hormone (JH) treatments of final instar larvae of many lepidopterans often result in the production of supernumerary larvae. Treatments of last instar larvae of *Spodoptera mauritia* Boisd. (Lepidoptera : Noctuidae) with JH analogue (JHA), hydroprene induces similar morphogenetic effects. Instead of pupating the JHA treated larvae moult into supernumerary larvae having pupal characteristics in the imaginal discs of mouthparts and thoracic appendages. The wing discs, however show only partial differentiation, remain uneverted but highly tanned. Why this happens remains undetermined. In an effort to provide an explanation to these observations DNA, RNA and protein profile in the wing discs of supernumerary larvae of *S. mauritia* obtained after treatments of JHA, hydroprene have been analysed.

Sixth instar larvae (last larval instar) of *S. mauritia* were taken from the laboratory stock culture reared and maintained as described earlier. The age of the larvae was designated as day *n* where day 0 indicates the day of ecdysis to this stage. The JHA, hydroprene (obtained as a gift from Dr. G. B. Staal, Zoecon Corporation, California) was dissolved and diluted in acetone to obtain 25 μg JHA/5μl. Sixth instar larvae were treated topically on day 0, day 1, day 2 and day 3 with a daily dose of 25 μg JHA on the abdominal tergites using a Hamilton microsyringe. Larvae kept as controls were treated in a similar manner with 5 μl acetone. Wing discs were dissected on day 5.

Preparation of tissues and estimation of DNA, RNA and protein — Wing discs of sixth instar larvae were easily identified by the presence of a large tracheole mass on their bases. Wing discs were dissected in insect saline under a dissection microscope. Adhering fat bodies and other tissues were carefully removed. Forty wing discs having an average total weight of 25 mg were utilized for the preparation of each sample. Acid soluble components and pigments in the disc tissue were washed off as per the schedule of Mittmayer et al. The residue was incubated twice with 0.5 ml hot (80°C) perchloric acid (0.5M) for 30 min. These extracts were centrifuged at 1000 rpm. The supernatants were used for nucleic acid analysis. The pellet of disc tissue was dissolved in cold (4°C) 0.1N NaOH and used for protein analysis. DNA was esti-
mated by the diphenylamine method and RNA by orcinol reaction. Amount of protein was estimated by the method of Lowry et al. against a bovine serum albumin standard (Sigma Chemical Co., St. Louis, U.S.A.). The colour intensities were read in a Shimadzu UV mini spectrophotometer.

Electrophoresis—The wing discs of treated and control larvae were dissected and washed thoroughly in insect saline. The tissues were homogenized in distilled water using a glass homogenizer. The homogenate was then centrifuged at 2000 rpm for 10 min and the resulting aqueous supernatant was kept at 0°C until use. SDS-PAGE in denaturing conditions was run on 10% polyacrylamide gel using vertical slab gel electrophoresis apparatus (Bangalore Genei Pvt. Ltd., Bangalore) according to the procedure of Laemmli.

Gels were stained to visualize separated protein bands by soaking in Coomassie Brilliant Blue R-250.

In the last instar larvae of S. mauritia, the paired fore and hind wings are located on the dorsolateral regions of meso and metathoracic segments, respectively. During early stages of sixth instar larval development they appear as very small pouches of tissues. The discs became more visible from day 3 onwards. On day 3 and day 4 they develop into more or less membranous round structures. The wing discs of mature larvae began their metamorphosis by evertine and unfolding to produce the shape of the adult structure into which they will differentiate. The process called evagination of wing discs occurs during the transformation of larva from wandering stage to prepupal stage. The evaginated wing discs of day 5 larvae are thin, transparent and membranous. In addition the everted forewing discs are very large and much folded. Due to the shortening of the length of the larva on day 5, the fore and hind wing discs get close to each other. Any indication of tanning process could not be observed in the wing discs during sixth instar larval development. There have been numerous in vitro studies on the evagination of wing discs, which reveal that this process is an intrinsic response of disc to a suitable titre of ecdysteroids.

The JHA treated larvae moulted into imperfect supernumerary larvae on day 5. The supernumerary larva resembled normal last instar larvae in appearance but possessed pupal characteristics in the head and thoracic appendages. Thus the imaginal discs of these structures exhibit normal pupal differentiation. However, the paired wing discs remained unevorted. The wing discs were partially tanned and sclerotised. The present results demonstrate that the various imaginal discs respond differently to exogenous application of JHA. None of the supernumerary larvae pupated and died after surviving for 48 hr.

In S. mauritia as in other lepidopterans there are two peaks in haemolymph ecdysteroid titres, one at the transition from feeding stage to the post-feeding prepupa and the other in association with pupal cuticle formation. The first small peak of ecdysteroids has been implicated in the change in epidermal commitment (hence called commitment peak of ecdysteroids) from larval to pupal type and the second major increase promotes pupal cuticle secretion and successful larval-pupal transformation.

Quantitative analysis of DNA, RNA and protein contents in the wing discs of supernumerary larvae formed after JHA treatment showed a considerable increase in the amount of DNA and protein and a significant decline in RNA level (Fig. 1). In lepidopterans low levels of endogenous ecdysteroids have a stimulatory effect on DNA synthesis while higher levels blocks it. Studies on the haemolymph ecdysteroid titre in S. mauritia have shown that the commitment peak of ecdysteroids did not appear in JHA treated larvae, the large premoult peak preceding the larval-pupal moult was also missing and a much smaller premoult peak appeared on day 4 in the treated larvae. The longer exposure of discs to low level of ecdysteroids may presumably be the reason for the increase in the concentration of DNA in the wing discs of JHA treated insects. Even though

![Fig. 1—DNA, RNA and protein content in the wing discs of supernumerary larvae in response to repetitive treatment of 25 μg JHA.](image-url)
the RNA level was low, the protein level was quite high in the wing discs of JHA treated larvae of *S. mauritiana*. The apparent contradiction cannot be clearly explained. However one cannot rule out the possibility that certain proteins needed for tanning and sclerotisation accumulate from haemolymph as has been observed in other holometabolous insects\(^{-23}\). Absence of proper differentiation of wing discs is probably due to the lack of intrinsic RNA and protein synthesis in the wing discs.

Electrophoretic analysis of wing disc proteins of day 5 larvae kept as controls showed the presence of 16 protein bands (Fig. 2). Analysis of protein pattern in the wing disc of JHA treated supernumerary larvae showed a reduction in the expression of many major bands that were predominant in the wing discs of control larvae. The observed low amount of major peptides or the absence of a few peptides indicate that high titre of JH may have an inhibitory effect on the wing disc protein synthesis. Further the appearance of a specific 39.81 kDa protein in the wing disc of JHA treated pupae strongly suggest that the hormone analogue may direct the synthesis of abnormal protein in wing discs. More likely the action of JHA might be indirect. As mentioned earlier JHA induced ecdysteroid deficiency might be responsible for the low concentration or disappearance of many peptides. The processes of evagination and cuticle deposition in the wing discs were induced by relatively high titre of ecdysteroids\(^{-24-26}\). Thus it seems that JHA induced inactivation of genes involved in the synthesis of proteins needed for evagination process may be responsible for the formation of unevverted, partially differentiated pupal wing discs in supernumerary larvae.

References


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