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Pyrrolizidine alkaloids quantitatively regulate both scent organ morphogenesis and pheromone biosynthesis in male *Creatonotos* moths (Lepidoptera: Arctiidae)

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Summary. Males of *Creatonotos gangis* and *C. transiens* possess coremata ('scent organs') of drastically varying sizes (Figs. 2, 3), which release R(−)-hydroxydanaidal (Fig. 1A) in varying amounts. Both the size of the organs and their pheromone content depend on the ingestion of pyrrolizidine alkaloids (PAs; Fig. 1B, C) by the larvae. There is a direct correlation between amounts of PAs ingested and the size of the organs (Fig. 4). It is the absolute amount of PAs ingested which determines the expression of corematal size, structurally different PAs have identical effects (Table 2); PAs are no essential dietary factors for the general development of the moths (Table 1), and the morphogenetic effect is restricted to the coremata. The findings are discussed in terms of developmental, ecological and functional aspects.

Introduction

Males of the arctiid moths *Creatonotos gangis* and *C. transiens* possess eversible abdominal androconial organs ('scent organs', 'coremata') which are peculiar with respect to their use and role in sexual behaviour. In contrast to most other Lepidoptera (cf. Boppré 1984a), the coremata of *Creatonotos* are not expanded only when close to a female in the final phases of courtship. Rather, they appear to bring about assembly behaviour by releasing an attractant for both sexes (see Schneider 1983; Boppré and Schneider 1986; Wunderer et al. 1986). Furthermore, both the growth of the coremata and the biosynthesis of the pheromone which is eventually emitted by these organs depend on pyrrolizidine alkaloids (PAs; e.g. Fig. 1B, C). PAs are secondary plant substances of diverse structure found in many plants, particularly in certain Asteraceae, Boraginaceae and Fabaceae (Robins 1982; cf. Boppré 1986). These substances, when ingested by *Creatonotos* larvae, regulate the development of the size of the coremata which, in turn, release hydroxydanaidal (Fig. 1A) synthesized from the plant-derived growth regulator. This phenomenon has been recognized by Schneider and Boppré (1981), Schneider et al. (1982) and Boppré and Schneider (1986), who investigated field-caught males, and fed larvae with a variety of plants which either lacked or contained PAs. We now report on a series of experiments in which larvae were fed known amounts of pure PAs and which therefore allow a quantitative description of the gross effects of the PAs heliotrine and monocrotaline on these insects.

Abbreviation: PAs pyrrolizidine alkaloids

Material and methods

Insect breeding. Our cultures of *Creatonotos gangis* (L.) and *C. transiens* (Walker) (Fig. 2A) originated from eggs of females collected at a light near Dolok Merangir, North Sumatra, Indonesia, where both species occur sympatrically. Living females and/or freshly laid eggs were air-mailed or brought to Germany and bred in our laboratories. Although the larvae accepted a variety of European plants as food (Boppré and Schneider 1986), insects used for the experiments reported here were raised

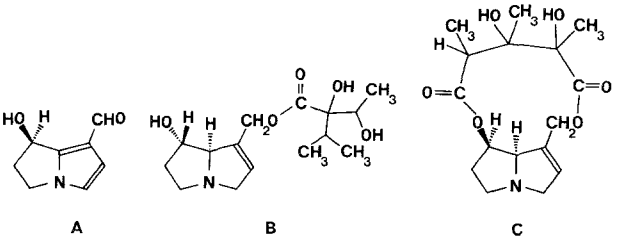

Fig. 1. Structures of R(−)-hydroxydanaidal (**A**), heliotrine (**B**), and monocrotaline (**C**)

on dandelion (*Taraxacum officinale* Web.; Asteraceae) and – in a few cases only – on wheat (*Triticum aestivum* L.; Poaceae). Our routine cultures are usually maintained on a semi-artificial diet (Bergomaz and Boppré 1986). With temperatures between 22 and 25° C, larval development took about 4–5 weeks, and moths emerged 7–11 days after pupation.

Larvae were kept in groups of 10–40, according to their size, in clear plastic Petri-dishes (90 mm in diameter, 16 mm high), and plastic boxes ($100 \times 100 \times 60$, $200 \times 200 \times 60$ mm) lined with moistened tissue paper, in rooms with a dark: light cycle of 12:12 hours. For quantitative feeding with PAs, larvae were kept singly in Petri-dishes for the rest of their development. Each series of experiments was done with one brood, i.e. under identical environmental conditions.

Quantitative feeding with PAs. For elucidating the effects of PA-ingestion by larvae for coremata development and pheromone biosynthesis in adult moths, during various stages of larval development (mainly in the final, i.e. the 7th instar) pure PAs (commercial heliotrine, Fig. 1B; monocrotaline (courtesy of J. Meinwald), Fig. 1C) were added quantitatively onto *Taraxacum* leaves: 50 µl of PA-solutions of different concentrations (0.4–20 mg PA/ml methanol) were applied onto pieces (approx. 20×20 mm) of fresh leaves with a dispenser pipette (Eppendorf). For this procedure, the pieces of leaf were laid on a plastic surface; if PA-solution did not stay on the leaf (due to small injuries, for example), it could be recognized as a residue on the plastic and the leaf was discarded. After evaporation of the solvent, one (or two) such PA-containing piece was offered to a larva in its Petri-dish. After consumption of the entire piece, the larva was fed untreated *Taraxacum* until pupation. To ensure that all larvae obtained PAs in the same physiological state, PA-treated leaves were fed only as the first meal after a moult. Effects of PAs transferred via the eggs (Boppré unpubl., cf. Results) were excluded by using larvae of parents which had been raised without access to PAs.

Quantification of corematal size. For quantitative determination of the sizes of coremata in moths raised from larvae which had ingested different amounts of PAs, the organs were artificially inflated by introducing a plastic tube (outer diameter 2 mm, inner diameter, 1.5 mm) into the severed abdomen of freshly killed one day old males. Gentle application of air pressure via a syringe protruded and expanded the coremata (for details see Boppré and Schneider 1986), which were photographed individually at half life-size on 35 mm film. The hair-bearing tubes were then extirpated at their bases and weighed on a micro balance (Mettler H20T), after the collapsed tubes had been dabbed on tissue paper (which took off body fluid sometimes pressed into the coremata during artificial inflation). Since corematal tissues naturally contain a certain amount of moisture, the organs were weighed instantly after excision; otherwise, unequal drying would make the data not comparable. For comparative purposes, some of the coremata were weighed again, after they had dried at room temperature. Weighed organs were kept individually in 1.5 ml micro test tubes (Eppendorf 3810) and stored in a deep freezer at −20 °C, until they were subjected to chemical analyses. The photographic negatives of some series were used for morphometric studies: they were projected to 6.8× life size and then, tube length, tube diameter, and length of hair were measured by use of a range-finder. (Data thus obtained are, however, not very accurate for large organs because the distal parts of the corematal tubes usually are bended and thus not entirely in the focal level.) The hair number was enumerated in representative samples by the following method: excised coremata were embedded in paraffine and cross-cut with a razor blade; the number of hairs was then counted from macrophotographs of the cross sections.

Quantification of hydroxydanaidal amounts. To measure the hydroxydanaidal content of coremata of individual males, the organs were extracted with 0.2, 0.5 or 1.0 ml of methanol, depending on size, and subjected to thin-layer chromatography (TLC) and spectrophotometry.

For TLC, used to assess hydroxydanaidal semi-quantitatively, 0.005–0.05 animal equivalents (i.e. 5–10 µl) of corematal extracts were spotted on Polygram SIL G/UV 254 sheets (Machery-Nagel) and run with methanol/chloroform/ammonia (14.5/85/0.5). After spraying with 10% 4-(dimethylamino)-benzaldehyde in concentrated HCl, which was diluted 1:4 with acetone just before use, pink spots with a Rf-value of 0.57 appeared. Treatment with aqueous $NaBH_4$ (which reduces hydroxydanaidal) after spotting resulted in dark bluish spots with a Rf-value of 0.4.

Most data reported below were obtained with a colour reaction evaluated spectrophotometrically. We followed a technique of J.A. Edgar (unpubl.): Individual coremata (or half-coremata) were extracted with 0.5–1 ml methanol. A volume of 20 µl of this extract was put onto 0.5–1.0 mg sodium borohydride in a test tube. 200 µl distilled water was added and the solution kept for 5 min. Then, 2 ml ethanol and 2 ml Ehrlich's reagent (= 3 g p-dimethylaminobenzaldehyde + 60 ml EtOH + 40 ml 14% BF_3) were added. The test tube was warmed for 5 min in a waterbath (40 °C), then cooled down and absorption was measured at 565 nm with a photometer (Spektronic 21, Bausch & Lomb). As a reference, we used hydroxydanaidal extracted with methanol from coremata of males, qualitatively fed with *Crotalaria* seed powder in agar (Boppré, unpubl.). The extract was then chromatographed on silica plates.

When the feeding experiments were carried out in 1981 and 1982 we were unable to quantify the absolute amounts of hydroxydanaidal of individual males. The organs had to be stored until 1985 before the quantitative chemical analyses could be done.

Results

Experiences from pilot experiments

In our initial cultures the coremata of males, the larvae of which had no access to PAs, showed up as two stalk-like projections giving rise to very few hairs (Fig. 2C–D). In contrast, feeding of PA-containing plants to larvae could result in males the coremata of which consisted of four tubes each of which being longer than the body and densely clothed with hairs (Fig. 2B; see also Schneider et al. 1982). This finding not only demonstrated that PAs are required for full corematal development, it also showed that there is a basic expression of coremata (the two projections) and that PAs not only cause an increase of size but also a differentiation of appearance of the organs. When evaluating the first feeding experiments, surprisingly, we found that in many control males coremata showed up as two stalks with more or less pronounced projections or even as four small tubes

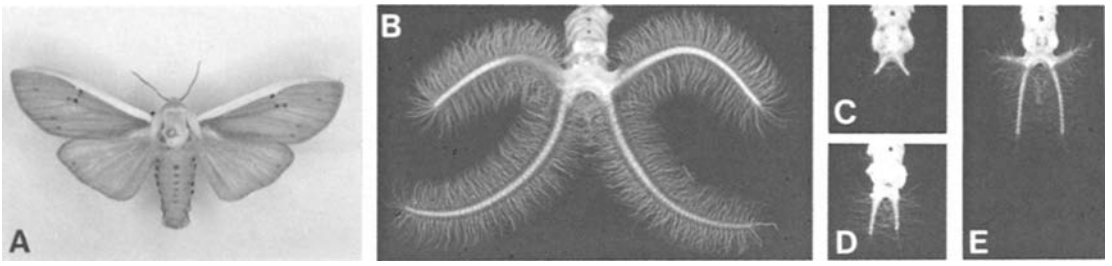

Fig. 2. Set male of *Creatonotos transiens* (**A**) and four artificially expanded coremata to show large (**B**) and small (**C–E**) expression of androconial organs and their size-relation to the insect. Natural size

Table 1. Mean values and standard deviations of weights of pupae (mg) of different feeding groups of *C. gangis* and *C. transiens*. Figures in brackets indicate sample sizes

Species	Sex	*Taraxacum* only (controls)	*Taraxacum* plus PA		PA fed
			100 µg	1000 µg	
gangis	m	328±34 (55)	342±30 (53)	346±27 (48)	heliotrine
			314±59 (45)	352±27 (54)	monocrotaline
	f	364±43 (86)	389±34 (40)	377±34 (41)	heliotrine
			369±56 (34)	380±35 (30)	monocrotaline
transiens	m	441±45 (54)	423±62 (38)	414±56 (44)	heliotrine
			376±48 (45)	435±29 (36)	monocrotaline
	f	526±58 (64)	487±64 (42)	498±66 (52)	heliotrine
			471±73 (39)	527±55 (40)	monocrotaline

(Fig. 2E, cf. Fig. 9). This turned out to be due to PAs transferred via the eggs (Boppré, unpublished): assuming proper developed coremata to be necessary for successful courtship, we had used PA-fed specimens for keeping our culture. In consequence of this experience, in the experiments reported here, only larvae of PA-lacking parents were used. However, note that the coremata of controls nevertheless varied considerably in size (cf. Fig. 9), and that the weights of the control coremata are less accurate than the values for larger organs, because in preparing and weighing the tiny organs there is a larger percentage of error.

PA-fed larvae were kept singly to avoid cannibalism which occasionally occurs in *Creatonotos* and would make quantitative measurements impossible.

Taraxacum leaves to which pure PAs had been applied were readily accepted by larvae of all instars of both species. Usually, a PA-treated piece of *Taraxacum* leaf was consumed within 30–60 min. Thus, the larvae are not repelled by PAs, even when applied in large amounts. This permits quantitative feeding.

Effects of PAs on general development

Ingestion of PAs had no effect on the duration of development. Also, we neither noticed any kind of malformation nor did we find any obvious difference in the general appearance between specimens from the control and experimental groups. By using the weights of the pupae as a parameter to assess general development, differences showed up between the sexes and species, respectively (females being heavier than males, *C. transiens* being heavier than *C. gangis*), but pupal weight never correlated with the amount of PA ingested by the larvae (for examples see Table 1).

Effects of PAs on corematal growth

As expected from previous results (see Introduction), males developing from larvae fed with different amounts of PAs possess coremata of strikingly different sizes. One can already appreciate by eye (Fig. 3) that the differences are due to a variety of parameters such as (i) length of tubes, (ii) diameter of tubes, (iii) length of hairs, and (iv) amount

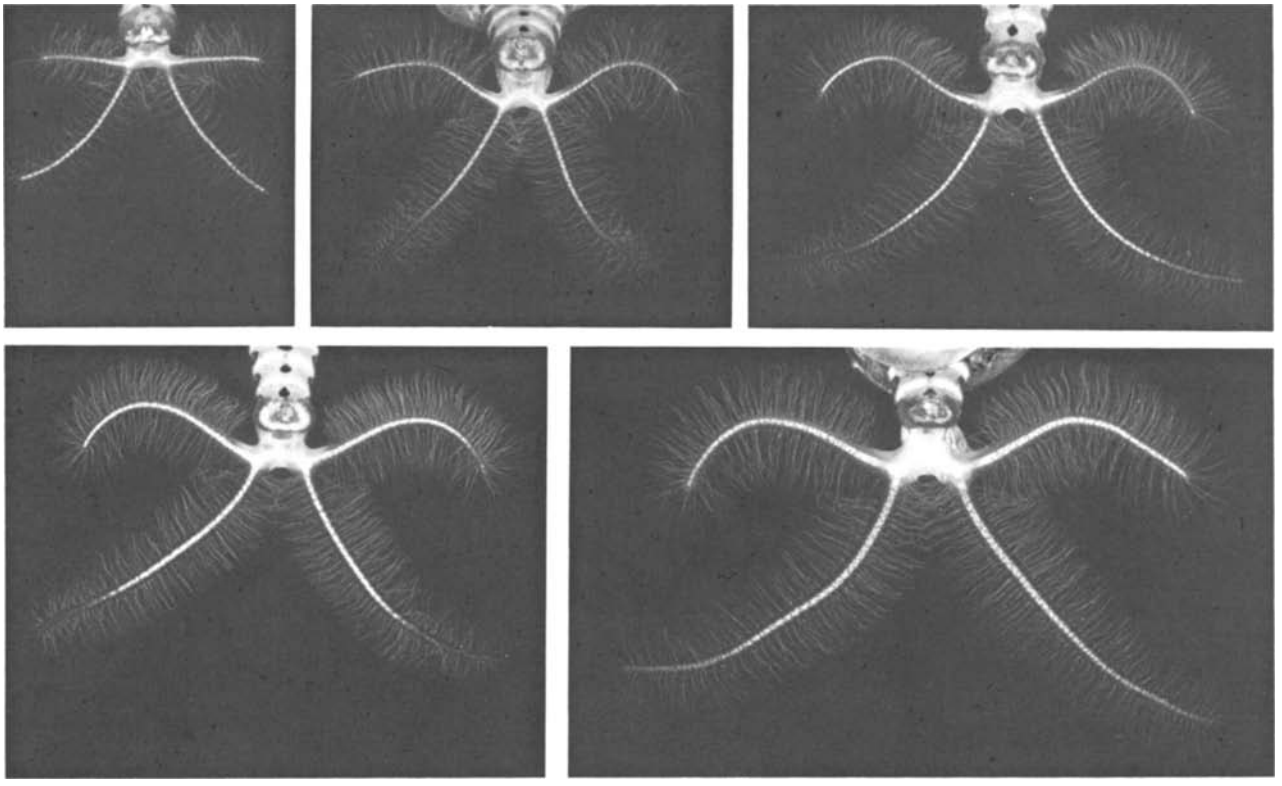

Fig. 3. Coremata of *Creatonotos transiens* representative of different feeding groups. Magn.: 1.5 ×

of hairs. Routinely, we quantified the sizes of the coremata simply by weighing the organs; data thus obtained represent the entire morphometry.

Figure 4A shows mean values and standard deviations of coremata weights of *C. gangis* plotted against the amount of heliotrine and monocrotaline, respectively, ingested by 7th instar larvae. It demonstrates that there is a positive correlation between the amount of PA eaten and the size (weight) of the coremata (Table 2A). However, corematal weights do not relate to pupal weights (Fig. 5). The same is true for *C. transiens* (Fig. 4B, Table 2B). For both species, there are neither qualitative nor quantitative differences in the effects of the two structurally quite different PAs heliotrine (Fig. 1B) and monocrotaline (Fig. 1C) (Table 2A, B). Thus, the following results are valid for both species and both PAs, unless otherwise stated. Feeding of 20 µg of PA in one meal at the beginning of the 7th instar results in coremata which are significantly larger than those of controls. With higher amounts of PAs fed to larvae, the coremata weight increases almost exponentially. It appears that coremata reach a maximum size if a larva has consumed 2 mg of PA. According to limited availability of pure PAs, we could only do feeding

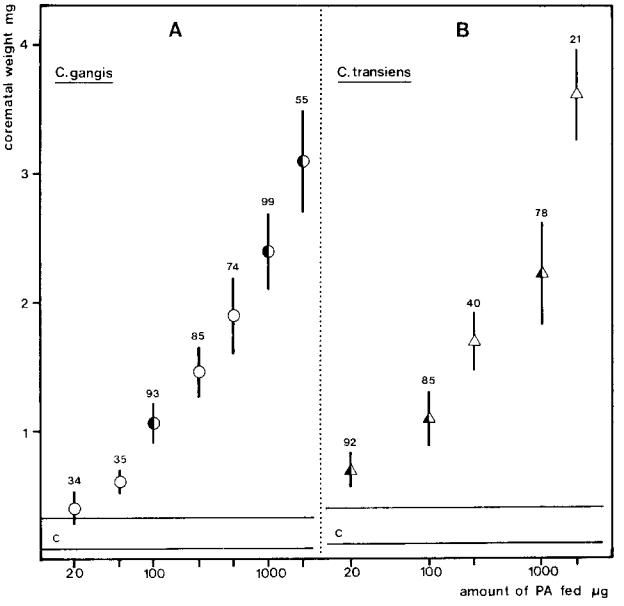

Fig. 4. Relation between size of coremata (expressed by their fresh weights) and amount of PA ingested by larvae of *Creatonotos gangis* (**A**) and *C. transiens* (**B**). Symbols represent mean values, bars standard deviations of coremata weights, numbers indicate sample sizes, and *c* range of controls. If symbols are blanc, the PA fed was heliotrine, if symbols are partly blackened, data of two insect groups, one fed with heliotrine, one with monocrotaline, were lumped together

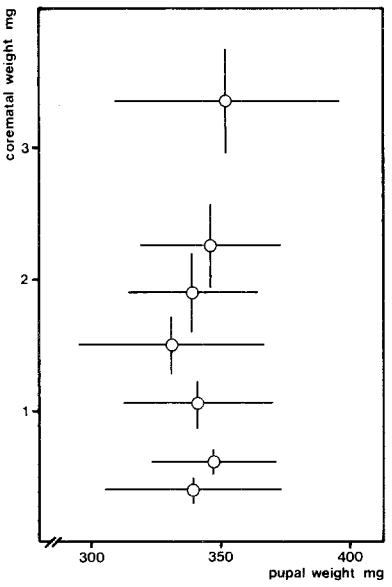

Fig. 5. Weights of coremata and pupae of different feeding groups of *Creatonotos gangis*. Mean values and standard deviations are plotted against each other

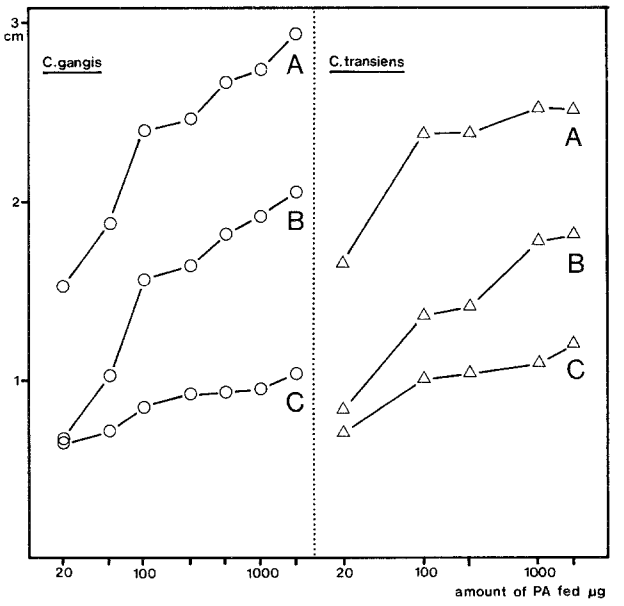

Fig. 6. Length of inner (**A**) and outer (**B**) corematal tubes and width of inner tube (including hairs) (**C** measured in the middle of the tubes) of samples of different feeding groups (PA = heliotrine) of *C. gangis* (left) and *C. transiens* (right)

Table 2. Mean values and standard deviations of weights of coremata (mg) of different feeding groups of *C. gangis* and *C. transiens*. Figures in brackets indicate sample sizes

A *Creatonotos gangis*

	100 µg	1000 µg	2000 µg
heliotrine	1.059 ± 0.18 (51)	2.247 ± 0.32 (47)	3.310 ± 0.30 (27)
monocrotaline	1.044 ± 0.22 (43)	2.510 ± 0.22 (52)	2.882 ± 0.40 (28)

B *Creatonotos transiens*

	20 µg	100 µg	1000 µg
heliotrine	0.599 ± 0.13 (38)	1.137 ± 0.20 (38)	2.214 ± 0.41 (42)
monocrotaline	0.624 ± 0.17 (35)	1.075 ± 0.18 (45)	2.249 ± 0.38 (36)

experiments utilizing higher PA-amounts with small numbers of insects. However, feeding some larvae with 3 or 4 mg did not result in apparently larger coremata, i.e. ingestion of 2 mg or so is sufficient to bring about maximal development.

Desiccation of dissected coremata at room temperature leads to a decrease of weight. However, this does not alter the relative correlation between PA amount fed and corematal size. The fresh weight of the coremata is about 2.1–2.4 × the dry weight, depending on the size of the organ.

In addition to corematal weights, some morphological characters were evaluated to demonstrate that the effect of PAs concerns all parameters making up the androconial organ. Figure 6 summarizes measurements of tube length and tube diameter (including hairs) of coremata of both species fed with different amounts of heliotrine. The numbers of corematal hairs counted in examples of both species and different feeding groups directly correlate to the corematal weights (Fig. 7). Naturally, the dose-response relationship is less prominent with individual morphological parameters than with the overall appearance of the organs and their weights.

All results discussed above come from insects which obtained PAs as their first meal after moulting to the 7th instar. If PAs are fed to other instars, or in several portions, no differences are obvious (Fig. 8), i.e., the insects apparently are able to ac-

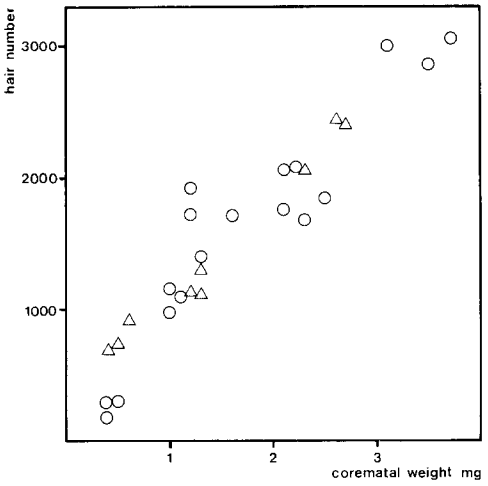

Fig. 7. Correlation between weight of 27 individual coremata and their number of hairs; *C. gangis* (○), *C. transiens* (△)

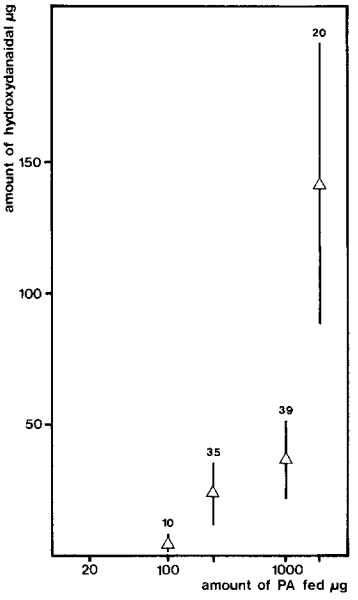

Fig. 10. Relation between amount of PA (heliotrine) ingested and hydroxydanaidal content of coremata of *C. transiens*. Cf. Discussion

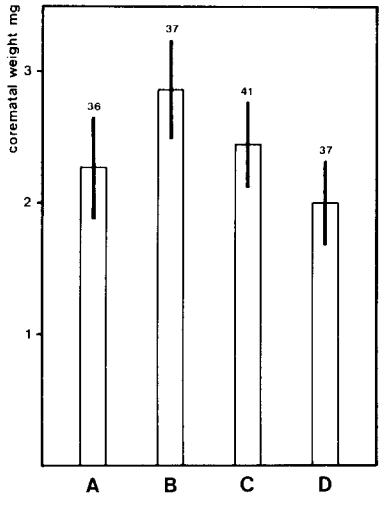

Fig. 8A–D. Weights of coremata of different feeding groups of *Creatonotos transiens*. Numbers indicate sample sizes, bars standard deviations. The same amount of heliotrine (1 mg) was fed in different instars and portions, respectively: at once in 7th instar (**A**), in three portions (333 µg each) in the 7th instar (**B**) and in the 5th, 6th and 7th instar (**C**), and at once in the 5th instar (**D**)

cumulate PAs and the concentration reached at the time of pupation is the decisive factor.

Effects of PAs on corematal development

As mentioned above, PAs not only cause increase of the size of coremata but also modify their general appearance, which is very obvious in tiny organs (Fig. 9). Comparing intermediate states of coremata expression obtained by feeding different amounts of PAs to larvae, indicate that the growth of the organs might follow certain 'steps', the amount of PAs ingested by the larvae determining which level will be reached. First, the central tubes become longer but do not increase in diameter. Then the lateral tubes growth out, until they reach about 3/4 of the length of the central tubes. More PA causes all four tubes to increase size steadily until reaching their maximal lengths, while even more PAs lead to thicker tubes and more hairs (cf. Fig. 3).

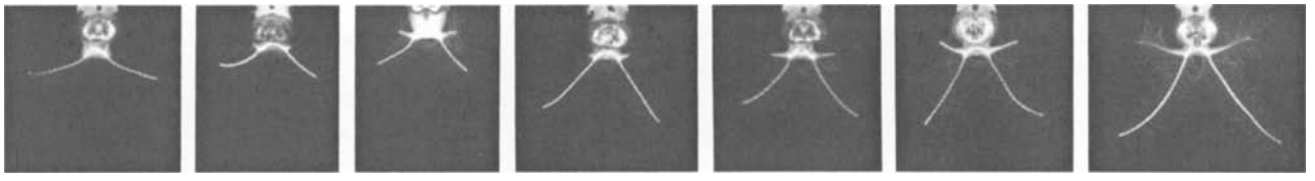

Fig. 9. Coremata of *Creatonotos gangis* illustrating variation of organs of control males and 'stepwise' growth regulation. See text. Natural size

Effects of PAs on hydroxydanaidal amounts

Thin-layer chromatography of extracts of freshly excised coremata indicated a direct correlation between corematal size and content of hydroxydanaidal. Quantification of hydroxydanaidal amounts by colour-reaction tests support this trend, however, the data vary strongly (Fig. 10) (see Discussion).

Discussion

The quantitative data provided above clearly prove that the ontogenetic development of the androconial organs in *Creatonotos gangis* and *C. transiens* does depend on the amount of certain secondary plant substances, namely pyrrolizidine alkaloids, ingested by the larvae. There are other cases where secondary plant compounds affect insect growth (see e.g. Beck and Reese 1975). However, in contrast to these cases, in *Creatonotos* the morphogenetic effect is restricted to a peculiar organ. Also, there are many other examples of male structural polymorphism or variation in insects, but in all these cases the factors causing the variance in expression are unknown.

Apart from the organ-specificity of the effect of PAs in *Creatonotos* we also demonstrate that (i) PAs are not essential dietary factors for the elementary metabolism or the general development of the moths, (ii) the growth of the coremata is dose-dependent, (iii) there is no strict specificity of the morphogenetic effect according to the structure of PAs, and (iv) the development of the coremata relates to the absolute amount of PAs ingested by the larvae, independent of the concentration and the stage of ingestion.

Creatonotos also provides us with the novel observation that the amount of pheromone synthesized is related to the size of the pheromone disseminating organ. There are other species where pheromone production – as in *Creatonotos* – depends on PAs ingested either by adults or by larvae: while male danaine butterflies gather PAs as adults, the arctiid *Utetheisa* moths ingest PAs as larvae – in both groups PAs are utilized as pheromone precursors (Schneider et al. 1975; Conner et al. 1981; see Boppré 1984a for further refs.). In these cases, PAs do not affect organogenesis. With respect to the pheromone amounts produced, in Danainae and in *Creatonotos* the maximal amounts found are in the range of 500 µg but there is great variation, and specimens kept without access to PAs have no dihydropyrrolizine pheromone components (Boppré et al. 1978; Schneider et al. 1982); in *Utetheisa*, comparatively small hydroxydanaidal amounts of 1.4 µg/male (average value) were reported (Conner et al. 1981).

So far, we neither know the mechanism of the regulation of corematal size development, nor do we know the biosynthetic pathway of the conversion of PAs into hydroxydanaidal, and it would be idle to speculate much about it. Further experimental studies, under way in our laboratories, should shed light on these questions. However, in addition to describing the phenomena, the data presented above do allow for some interpretation.

Developmental aspects

From our results we can conclude that each male individual has the potency to build large coremata, i.e., apparently there is no marked genetically inherited variation controlling the expression of this male character. There is, however, some non-genetically inherited variation because PAs are – if present in the parents – incorporated into the eggs and so affect (although not drastically) corematal size in the next generation (Boppré, unpublished).

The development of the tissue of the coremata anlagen requires the presence of a chemical which, in the natural situation, is obtained with the food, although it has no nutritional value. We have clearly demonstrated that feeding PAs to the larvae affects corematal development; however, one may not be certain that the unchanged PAs are the regulating substances, rather fragments and/or derivatives of PAs might be the crucial principles. Also, it is not known if PAs/PA derivatives act directly on the anlagen or if they influence other molecules which then trigger organogenesis. In this context, further studies will have to consider that (i) PAs are rather large molecules which probably cannot move freely in insect bodies and (ii) PAs possess properties which are noxious for tissues.

In any case, we can state on the one hand that corematal development is not a further case of allometric growth (because there is no relation between corematal size and other body parts); PAs can neither be seen as typical inductor substances (because of the dose-dependency of growth) nor as general regulator substances (because of the tissue specificity). PAs cause a modification in the literal meaning of the term, however, in detail it is not comparable with other cases of modification, and corematal development in *Creatonotos* appears to exhibit a novel growth-regulation phenomenon. It seems likely that a particular genetic programme is (directly or indirectly) activated by the PA in a graded way. With respect to the time of determi-

nation of final corematal size, transplantation experiments should give information; the PA-level could be read in the pupal stage only, or continuously during larval development. Since the absolute amount of PAs obtained is decisive and the time of ingestion of PAs by larvae is uncritical for the corematal expression, both ways would be possible.

Corematal development is the expression of adult male characters from a latent imaginal disc. With all probability, this development is controlled in *Creatonotos* – as in other Lepidoptera – by an interplay of juvenile hormone and ecdysteroids (see Riddiford 1980 for the sphingid moth *Manduca*). The anlage which gives rise to the coremata must be under the quantitative command of the PA or its executor. It is here where we see a chance to uncover experimentally the growth determination – the gene expression – mechanism(s) of this peculiar system.

Ecological aspects

Our results were obtained from insects fed with substitute foodplants and provided with PAs in crystaline form, which is a very artificial situation. However, as shown by what follows, we can be certain that the information gathered in the laboratory not only provides some knowledge on development phenomena but is of relevance in ecological terms. Because too little is known about the biology of *Creatonotos* (see Boppré and Schneider 1986), discussing our findings in an ecological context leaves us with open questions. However, some conclusions can be drawn.

The fact that PAs with structures as different as those of heliotrine and monocrotaline (Fig. 1 B, C), were found to affect both corematal growth as well as pheromone biosynthesis in an identical way, reveals a relatively low specificity of PA-utilization in *Creatonotos* (see also Bell et al. 1984). Nevertheless, it should be noted that not all compounds matching the (chemical) definition of PAs are equally suited for these insects (Boppré, unpublished; cf. Boppré 1986).

Creatonotos larvae can utilize PAs from a variety of plant species, and thus do not depend on the availability of a particular PA-plant. Also, they do not need to ingest PAs continuously but are able to accumulate PAs and cope with high PA-quantities at once. If PAs have a significant influence on the reproductive success of *Creatonotos* (see below), the insects' ability to sequester PAs of various molecular structure can be seen as an advantageous trait.

Recognizing the effects of PAs on the morphology and chemical composition of the andoconial organs one would suspect that, in the field, *Creatonotos* larvae would feed on PA-containing plants exclusively and that all males would have large coremata with a high pheromone content. However, the larvae are polyphagous and do not feed exclusively on PA-plants – as, e.g., *Utetheisa* do –, and in field-caught males both corematal size and hydroxydanaidal amounts vary drastically (Boppré and Schneider 1986). Thus, the observed male variation is an individual matter, dependent on whether or not and how much PA is gathered. Nevertheless, PA-uptake is not apparently a chance phenomenon: larvae of *Creatonotos* like feeding on glass-fibre if it had been soaked with PAs, i.e. the larvae detect PAs, and pure PAs are feeding stimulants (Boppré unpublished). *Creatonotos* larvae are thus pharmacophagous (Boppré 1984b).

Comparing the corematal weights of field-caught males (Boppré and Schneider 1986) with our dose-response curves (Figs. 3 and 8) suggests that the larvae of males with coremata of average size probably had gathered about to 2 mg PA. The amounts of PAs offered to individual larvae in our experiments would thus be in an 'ecological range', but the occurrence of larger organs indicates that in the field larvae consume even higher amounts. Also, we did not find hydroxydanaidal amounts as high as reported for organs of field-caught males (400 µg, Schneider et al. 1982; cf. below). However, since we do not know which type(s) of PAs the caterpillars actually obtained in the field, the data of field-caught and laboratory-reared might not be comparable: perhaps, the PAs tested by us are not 'optimal' for regulating organogenesis, i.e. other PAs might have a different (stronger or weaker) dose-response relationship. This argument not only accounts for the organogenetic effect but also for pheromone biosynthesis. Furthermore, a given PA might affect coremata development in a different way than pheromone biosynthesis. Only quantitative feeding experiments with a variety of structurally different PAs (which are not available presently in sufficient amounts) will shed light on this aspect which is of major importance in an eco-ethological context.

The data provided above on pheromone amounts found in the different feeding groups show some relation to corematal size. However, they vary unexpectedly greatly within a feeding group. This is surprising since the data of the coremata weights indicate that there is relatively little error in our method of feeding larvae quantitative-

ly with PAs. Very likely hydroxydanaidal had evaporated and/or decomposed while the coremata had been stored (for three years) prior to chemical analyses. We have to await further experimental results to see whether or not the observed large variation is an artefact and now do not interprete the values found.

Functional aspects

Multiple adaptations and physiological 'cost' attend corematal development and pheromone production in *Creatonotos*, and one must ask what significance PAs have for reproduction. Presently, the details of courtship behaviour, the significance of male pheromones, the role of female pheromones, and in particular, the consequence(s) of the pheromone amount of the individual male, are all uncertain. In confinement PAs are not essential for reproductive success of male *Creatonotos*, which is in contrast to other Lepidoptera utilizing PAs for male pheromone biosynthesis (cf. Boppré 1986). The present knowledge and the challenging eco-ethological problem behind the relationship of *Creatonotos* to plants containing pyrrolizidine alkaloids is discussed extensively by Boppré and Schneider (1986; see also Wunderer et al. 1986).

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