

Insects Pharmacophagously Utilizing Defensive Plant Chemicals (Pyrrolizidine Alkaloids)*

Michael Boppré
Universität Regensburg,
Zoologie-SFB4/B6,
D-8400 Regensburg

Insects of several orders (mainly Lepidoptera) gather alkaloids independent of feeding behaviour. By storing these secondary plant metabolites, the insects gain protection from predators, and in various species the plant chemicals are used as precursors for the biosynthesis of male pheromones; in *Cretonotos* they even regulate specifically the development of the androconial organs. The variety of aspects involved in this link between chemical defence and sexual communication, as well as its consequences, are discussed.

* Dedicated – with deep gratitude – to Professor Dr. Dietrich Schneider

Insects and Plants

Although most flowering plants depend on insects for pollination, they have also much to fear from insects as herbivores. Thus, on the one hand, plants have evolved features like alluring colours and fragrances to attract insects for fertilization, and even pay for this service by sacrificing pollen and providing nectar. On the other hand, they have armed themselves to do battle with the phytophagous insect horde. This they have done by developing a great variety of protective devices, mainly chemical. In turn, insects have taken up the gauntlet and developed adaptations enabling them to cope with the plants' defensive arsenal.

Insect-plant relationships and "co-evolution" are not only most challenging subjects for biological research, but are also important for practical purposes because man is affected in his needs for agriculture and forestry. This paper deals with only a small section of the wide field of *chemical ecology*: for insects plants can be much more than merely nutrient sources, and insects may even come to be dependent on plant defensive chemicals for their reproductive success. In particular, the variety of aspects involved, and the consequences of a link between chemical defence and sexual communication are discussed.

Protective Allelochemicals

Many if not most *secondary plant substances* (i.e., those chemicals not belonging to the essential molecular outfit of each plant cell) are believed to serve as agents to protect plants from being attacked, and they can therefore be classified as *allelochemicals*, i.e., as non-nutritional chemicals affecting members of other species (see, e.g., [1–7] for general accounts).

Various insects cope with noxious plants by excreting the allelochemicals or by metabolizing them into harmless derivatives; such species, at least, take advantage of a protected niche (e.g., [8]) – grazers usually avoid such plants. Some insects use allelochemicals for food detection, and many elaborate their relation to toxic plants by accumulating and storing harmful plant chemicals to gain ("second-hand") protection from insectivorous vertebrates (mainly birds, reptiles, amphibians, and small mammals; e.g., [9–12]). Chemical protection is nevertheless not absolute and depends on both dose and predator.

Insects equipped with self-made or with plant-derived defensive/protective chemicals usually ad-

vertise their unpalatability by *aposematic* signals (e.g., conspicuous “warning” coloration), which facilitate learning by potential predators. Such communication is advantageous to both predator and potential prey. In addition, numerous relatively palatable insects have also evolved aposematic characters and resemble unpalatable species in their outward appearance; as a result, they gain protection because predators fail to discriminate rapidly between the distasteful (the *models*) and tasty look-alikes (the *Batesian mimics*).

A well-studied case is *Danaus plexippus*, the American Monarch butterfly (subfamily Danainae). The larvae of this species feed on milkweed plants (Asclepiadaceae); from these they accumulate cardenolides (cardiac glycosides), which they retain through the pupal stage; cardenolides are thus present in the imago [14]. Classic feeding experiments by Brower et al. [15, 16, 12] proved the almost 100-year-old hypothesis of Slater [17], that plant-derived chemicals can be the basis for distastefulness in insects, and thus the reason for the appearance of mimicry. However, not all specimens of *Danaus plexippus* are chemically protected: within this species there is a *palatability spectrum*, individuals ranging from palatable to completely unpalatable (but cf. below); this variation relates to the larval host plants, which contain cardenolides in varying amounts and of different types. The same is true for various other danaines (see [12, 18] for details and refs.).

Butterflies and Dead Plants

Entomologists in Asia and South America have noticed that *withering* and *dead* plants of several taxa, including *Heliotropium* (Boraginaceae), attract particularly male danaine butterflies of several genera. Collectors have used dried material of such plants as baits (refs. in [19, 20]). The significance of this peculiar butterfly behaviour was a puzzle, and the phenomenon was not considered when studies on chemical communication of danaines began – but eventually it turned out to be a key for understanding danaine reproductive biology: male Danainae possess abdominal scent organs (Fig. 1) which are everted in the final stages of their visually initiated courtship behaviour and release pheromones necessary to gain acceptance by a female [21–23]. In extracts of these androconial organs, Meinwald and co-workers found danaidone (Fig. 2D), the structure of which resembles pyrrolizidine alkaloids (Fig. 2A, B) previously

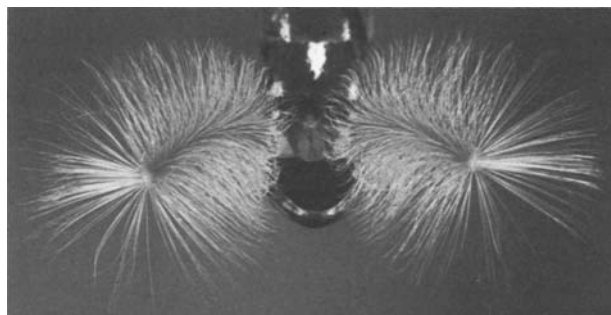


Fig. 1. Expanded male hairpencils of *Euploea* sp. (Danainae)

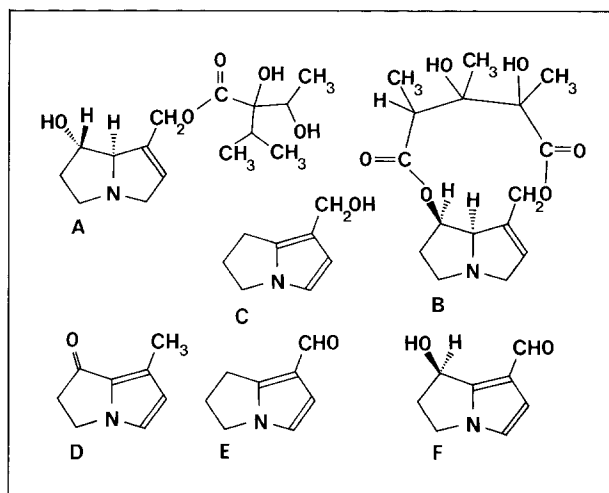


Fig. 2. Structures of the pyrrolizidine alkaloids heliotrine (A) and monocrotaline (B), of retronecinepyrrole (C), a possible intermediate in the biosynthesis of the dihydropyrrolizine pheromones danaidone (D), danaidal (E), and hydroxydanaidal (F)

known only from certain plants. While danaidone and other dihydropyrrolizines (Fig. 2D–F) were subsequently found in greatly varying amounts (up to 500 μg) in hairpencils of field-caught males of almost all Danainae studied, in indoor-raised males these common major components of species-specific odour bouquets were entirely missing (refs. in [18]). Eventually, finding male *Danaus chrysippus* “feeding” (cf. below) on dead *Heliotropium* plants (cf. Fig. 3A, B) led to laboratory experiments which established that the adult butterflies gather pyrrolizidine alkaloids from dead plant tissues and utilize them for biosynthesis of their major pheromone components [24–26]. This was the first demonstration of the dependency of male Danainae for courtship success upon plants which are not their food plants, and of the fact that some butterflies have to actively gather pheromone precursors as adults. (Further peculiarities of danaine *pheromone biology* are reviewed in [18, 27–29].)

Pyrrolizidine Alkaloids (PAs)

Because other insects turned out to have similar relationships to plants containing pyrrolizidine alkaloids (PAs), these allelochemicals require a brief introduction. Chemists define PAs (e.g., Fig. 2 A, B) as esteralkaloids, the aminoalcohols of which (the necines) originate from the bicyclic pyrrolizine skeleton and possess mainly one or two alcoholic functions. The necic acids are aliphatic, mono- or dicarboxylic, often branched and/or functionalized and contain 5–10 carbon atoms. Because of the tertiary aminic function, many PAs oxidise readily to N-oxides and often occur as such. (For general accounts on PAs see [30, 31].)

In the plant kingdom, PAs are widespread; they have been found in species of 61 genera belonging to 13 families [31, 32], but the structures of the PAs in the diverse plant species differ considerably. In general, PA plants possess not one but several PAs, and amounts of PAs may vary greatly between and even within individual plants of the same species, depending on their physiological state; in some cases PAs appear to be temporary products. The highest concentrations have been found in seeds which may contain up to 5% PAs of their dry weight (see [30] for details). Herbivorous mammals usually avoid PA plants, which indicates the protective function of PAs for plants [3, 30].

PAs are frequently consumed by humans, e.g., in medicinal herbal teas or unintentionally with food, and account for a global health problem: vertebrate metabolism converts many PAs into toxic necine pyrroles (Fig. 2C), which are, for instance, hepatotoxic, mutagenic, and carcinogenic (reviews: [33–36]). This importance of PAs for hu-

man and veterinary medicine has caused these compounds to be pharmaceutically well studied. In summary, PAs comprise a great structural diversity. In introducing the following paragraphs it must be stressed that although insects do not respond to all PA-containing plants (see below), those PAs that insects can utilize are of several types. Since the specificity of the insects' relations to PAs is not fully understood the general term PAs is used here, but does not imply lack of specificity.

Adult Insects and PAs

Male danaine butterflies can obtain PAs not only from the *Heliotropium* plants already mentioned. Withered and decomposing foliage, stems, roots, and particularly seeds of several species attract the butterflies and provide them with PAs (cf. Fig. 3). These PA plants include also *Tournefortia* (Boraginaceae), *Senecio*, *Eupatorium* (Asteraceae), *Crotalaria* (Fabaceae), and others; they contain mainly macrocyclic diesters and monocarboxylic esters with retronecine and heliotridine moieties. Experimentally, not only baits made of withered parts of these plants, but also PAs extracted and purified from them (e.g., axillaridine) lure the insects and stimulate ingestion.

Many species belonging to several genera of ithomiine butterflies (the sister-group of Danainae), and certain arctiid (e.g., *Halisidota*, *Rhodogastris*, *Digama*) and ctenuchiid (e.g., *Euceron*, *Euchromia*) moths exhibit the same behaviour of gathering PAs. To date, species of 32 genera of Lepidoptera have been reported as visiting PA sources ([19, 20] and refs. therein, [37]); however, for many species



Fig. 3. Male danaine butterflies congregating at a withered part of *Heliotropium* (A, *Amauris niavius*) and at dry *Heliotropium* in a gauze bag put out as bait (B, *Danaus chrysippus*, *Tirumala petiverana*, *Amauris ochlea*). *Rhodogastris* applying fluid via its proboscis onto a dry root of *Heliotropium* to dissolve PAs (C)

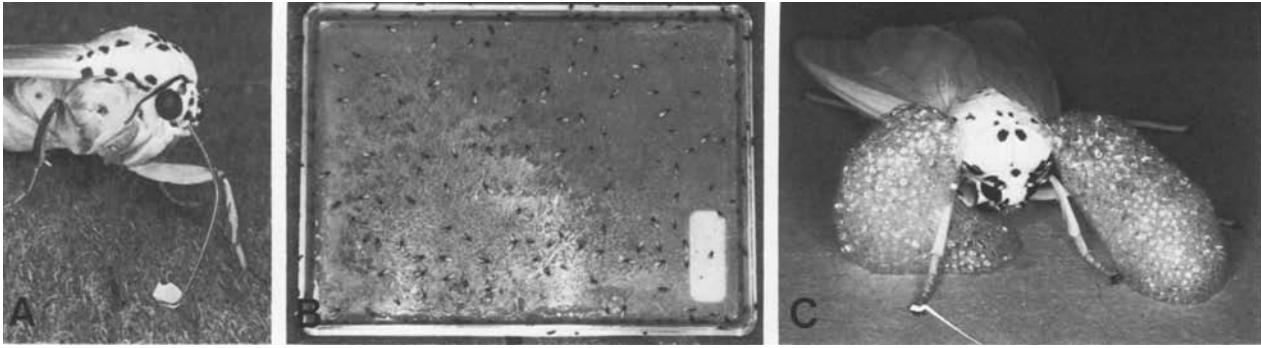


Fig. 4. *Rhodogastria* dissolving a crystal of monocrotaline (A), Kenyan chloropid flies attracted to a dish containing axillaridine (B), *Rhodogastria bubo* exuding froth after mechanical stimulation (C)

listed by Pliske [19], their relation to PA plants requires substantiation because only few specimens were baited. Depending on the species, there can be sex-bias; attraction of Danainae and Ithomiinae is strongly male-biased, in the Arctiidae and Ctenuchidae it is either male-, female- or not sex-biased. All these Lepidoptera approach PA sources upwind and are thus apparently guided by olfaction (see below). Once on the plant, they walk, probing its surface with extended mouthparts, and eventually they settle and apply a fluid onto dry tissue via their proboscides (Fig. 3C). The insects then reimbibe this fluid together with the dissolved PAs.

Gathering PAs is not a phenomenon restricted to Lepidoptera (cf. [19]). Studies in Africa, for instance, have revealed that apart from butterflies and moths, male flea beetles (Chrysomelidae: Alticinae: *Gabonia*; [38, 39]), both sexes of certain grass flies (Diptera: Chloropidae; Fig. 4B; [37]), and of grasshoppers (Orthoptera: Pyrgomorphidae: *Zonocerus*; [40]) (larvae and adults) are attracted to PA baits.

In summary, visiting plants (which are not food plants) to gather PAs is quite a widespread phenomenon. Although we now know of many such “PA insects”, our knowledge may amount to no more than the tip of the iceberg, because only limited habitats have been checked for insect-PA relations.

The fact that dry rather than green plants are attractive for adult PA-searching insects is not because fresh plants lack PAs, but probably relates to the lack of volatile stimuli eliciting attraction in insects (see below). Adult Lepidoptera with their sucking mouthparts also face the problem of the mechanical accessibility of the allelochemicals in fresh plants. In Danainae it has been observed how this can be overcome: in East Africa, flea beetles (*Longitarsus*) were found chewing small holes in

fresh *Heliotropium* plants. The withering edges of these holes attracted danaine butterflies, which then exhibited a peculiar behaviour: with their legs they scratched radially from these holes and damaged the tissue significantly, thus gaining access to the plant’s juices, which they imbibed (Fig. 5) [41].

The nectar of some – but not all – PA plants contains PAs [42–45] and can be another natural PA source. Since butterflies which do not utilize PAs seem to avoid PA-rich nectar (as in *Gynura*; [45]), one may ask if the PA insects are attracted by nectar or by PAs, and whether non-PA insects are even repelled (see below).

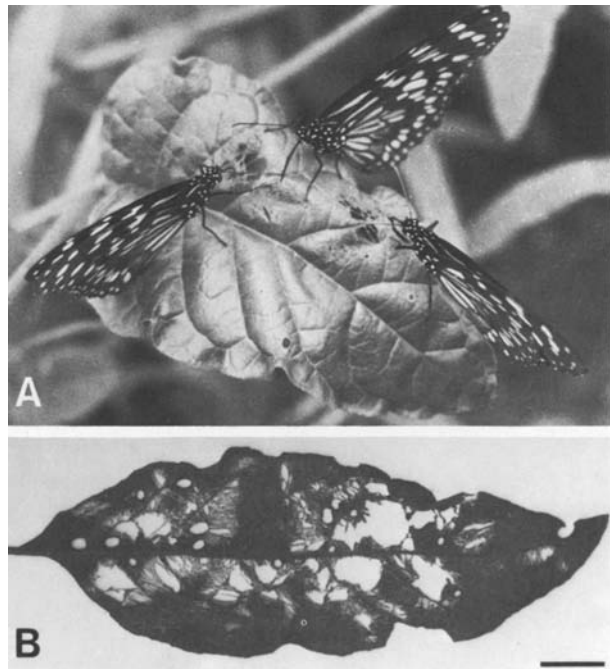


Fig. 5. Male *Tirumala petiverana* scratching a leaf of *Heliotropium* and imbibing PAs (A), and a damaged leaf showing beetle holes and scratching tracks of butterflies (B) (bar 1 cm)

Pharmacophagy

Secondary plant substances are usually obtained together with food, and the uptake of non-nutritional chemicals from host plants is basically accidental. If a given insect feeds on a certain plant containing peculiar secondary compounds, one cannot conclude that it is necessarily able to detect these substances. Insects are often unable to perceive allelochemicals, even if they utilize them [46]. However, with respect to PA insects, there is not only no doubt that it is PAs which the insects are after, but also that it is PAs and not some other plant substance(s) which guide them to PA sources: the insects react to pure PAs in the same fashion as to PA plants, and PAs alone chemically mediate the entire behavioural sequence of locating and taking up PAs (upwind flight, applying and re-imbibing solvent) (Fig. 4A, B; [19, 20, 37, 47, 48]). As shown electrophysiologically, the head space of PA crystals elicits sensory responses in specialized antennal sensilla (e.g., of *Rhodogastria*; [49]). However, because PA molecules are quite large and heavy, the question arises of whether the entire PA molecule can be odorous for insects; there is some evidence that breakdown and/or reaction products of PAs account for the crucial sensory stimulation in insects, and perhaps the different insects react to different “PA odors” [19, 47, 48]. This would also explain why the attractive power of decaying and dead plants varies and eventually fades. In any case, airborne stimuli due to PAs somehow make possible the location of PA sources where gustatory stimuli seem to mediate ingestion [19, 37, 47].

Visual cues are not necessarily involved in locating PA sources, either in danainae or in other species, since PA-containing dishes, providing no natural visual stimulus, are effective baits. In the field, danainae in search of PAs are often observed to fly to conspecifics, and aggregations thus build up. Perhaps, in butterflies, visual stimuli of conspecifics facilitate orientation, but a more probable explanation of the tendency to aggregate is that the individual takes advantage of participating in the fluid applied onto the plant by others; probably for the same reason, *Rhodogastria* moths also often congregate, and *Gabonia* beetles are particularly attracted to spots moistened by butterflies or moths [38].

As demonstrated, many of the insects which sequester PAs gather these allelochemicals *independently* of true feeding behaviour (i.e. ingestion of nutrients), responding to PAs alone. Thus, peculiar adaptations at the sensory, behavioural and physio-

logical level are required for PA-gathering, making it a distinct type of insect-plant association, termed *pharmacophagy* and defined as follows: “Insects are pharmacophagous if they search for certain plant substances directly, take them up, and utilize them for a specific purpose other than primary metabolism or (merely) food-plant recognition” [46].

Larvae and PAs

PA-containing plants are also normal food plants for numerous insects of several orders, particularly for their larvae. This represents a less advanced relation to PA plants than that exhibited by adult insects gathering PAs pharmacophagously (see above), and it cannot be predicted if they all utilize the PAs ingested with their food: each case requires experimental investigation. The larvae of certain species probably do not sequester PAs but convert and/or excrete them, yet some species are known to utilize PAs for specific purposes (see, e.g., [9, 50, 51], below). Nevertheless, not all are pharmacophagous, but *Creatonotos* (Lep.: Arctiidae) provides an example of larval pharmacophagy with respect to PAs: the caterpillars feed eagerly on glass fiber (and other materials) if it has been contaminated with pure PAs [37] (see below).

Significance of PAs for Insects

For most species which gather PAs, the significance of PA sequestration is either unknown or is a matter of analogy and speculation. After it had been established that PAs serve as pheromone precursors in *Danaus*, such a function was found in other species, the use of PAs for defence was recognized and, eventually, we learned about a morphogenetic effect of PAs. Thus, to date, three modes of PA utilization are known:

PAs as Pheromone Precursors

As stated above, male Danainae use PAs as precursors for the biosynthesis of dihydropyrrolizines (Fig. 2D–F), which they need in order to gain acceptance by a female. The biosynthetic pathway is not known but might include a toxic pyrrole (Fig. 2C), which has been found in hairpencils of *Euploea* [52]. With *Danaus chrysippus* it has been demonstrated that in addition to ample PA uptake, in order to produce physiological amounts of danaidone, the males first need to place their abdominal hairpencils in contact with their own alar

glands ([53], cf. [18]; Fig. 7, 3). Ithomiinae are still poorly investigated with respect to male pheromones, but the males of a few species are known to convert the acid (not the necine) moiety of certain pharmacophagously gathered PAs to lactone pheromones [54]. These are released from alar fringes and appear to serve as multi-species aggregation pheromones; they have also been said to mediate male-male recognition (refs. and discussion in [27]). Male arctiid moths of several species, e.g., *Utetheisa* [55, 56] and *Cretonotos* (see below), again utilize the necine ring and synthesize hydroxydanaidal (Fig. 2F), just as some Danainae do, although in these arctiids it is the larvae which take up PAs. In *Utetheisa*, hydroxydanaidal is an essential close-range courtship stimulus [56]; in *Cretonotos* its use is clearly different but not yet fully understood (see below). Note that in Danainae and in *Cretonotos* there is great individual variation in the amounts of pheromone produced – due to the amount of PAs ingested – and males can possess up to 0.5 mg ([53, 68], below). In *Utetheisa* the reported average hydroxydanaidal amount is 1.42 µg [56].

PAs as Defensive Agents

As mentioned, PAs seem to serve as protective chemicals for the plants, and it has been assumed that the insects might store PAs for their own chemical defence [57]. This hypothesis is supported by the fact that the majority of insects involved with PA-containing plants are aposematically coloured, many are mimicry models, and several are known to be avoided by potential predators (e.g. [58]).

A variety of species which either feed as larvae on PA plants or gather PAs as adults have been investigated chemically for this aspect and found not only to retain but also accumulate and store PAs in their bodies [9, 55, 57, 59–64, 37]; PAs are also incorporated into the eggs [59, 63, 51, 65, 66]. There is, not unexpectedly, considerable variation in the amounts of PAs stored by the species; in species ingesting PAs as larvae, the physiological state of the host plant is of influence, and, particularly in species of which the adults gather PAs, there is great individual variation due to the availability of PAs in the habitat and the success of the individual in gathering PAs. Maximal amounts of PAs reported are in the range of 3 mg per insect [63]. Part of the stored PAs occur as N-oxides; whether the insects take up N-oxides from plants or oxidise ingested free bases seems to depend on both plant and insect species.

Rhodogastris moths employ PAs in active defence: when disturbed, the moths emit from prothoracic glands a defensive froth (Fig. 4C) which contains N-oxides of PAs [48].

In addition to chemical studies, feeding experiments on predators were carried out with some Lepidoptera, and they demonstrated that insects are rejected due to stored PAs by predators such as spiders and thrushes [62–64]. Tests with a variety of animals (including mice, toads, frogs, lizards, titmice, ants, cockroaches, locusts, and various lepidopteran larvae) have also demonstrated that PA-contaminated food is rejected by taste [67]. Humans find PAs bitter, and when vertebrates taste PAs, signs of discomfort are often observed. PAs are thus definitely unpalatable to a very diverse range of animals and the mode of action of PAs as defensive chemicals is not their noxious long-term effects (see above), but stimulation of taste receptors responsible for rejection behaviour; PAs are predator deterrents. Since PAs do not cause instant harm, sensitivity to avoiding PA-containing food is relative, depending, for example, on the degree of hunger. It is also especially interesting that insects are generally deterred by PAs. (For refs. and extensive discussion of the defensive role of PAs see [12, 67].)

PAs as Growth Determinants

The larvae of *Cretonotos gangis* and *C. transiens* (Lep.: Arctiidae) are polyphagous but they can gather PAs pharmacophagously. If they ingest PAs, they sequester them to the adult stage, and the males synthesize hydroxydanaidal (up to 500 µg) from them [68]. Males collected in the field not only differ greatly with respect to the amounts of stored PAs and pheromone, but the size of the pheromone-disseminating organs also varies strikingly: these *coremata* appear as tiny stalk-like projections, or as four very large hair-covered tubes, or may be intermediate in size (Figs. 6A–C, 7, 7) [68–70]. Quantitative feeding experiments with pure PAs revealed that the size of the *coremata* relates directly to the amount of PAs ingested by the larvae [66]. Surprisingly, the morphogenetic effect of the secondary plant substances is restricted to the scent organs [66].

As reviewed above, only a few PA insects have been investigated in any detail, and of the others we only know of their association with PAs without having direct functional evidence. It is not

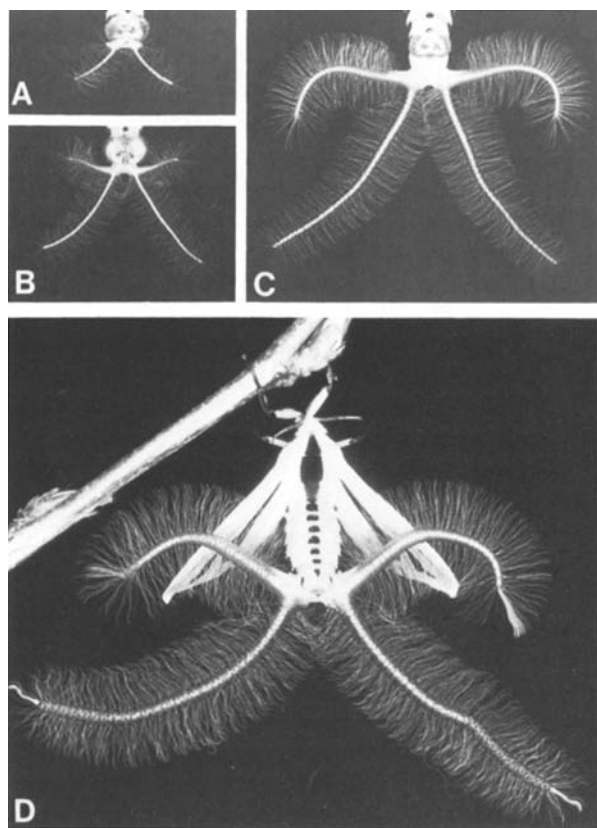


Fig. 6. Differently expressed androconial organs (A–C, natural size), depending on amount of PAs ingested by larvae of *Creatonotos gangis* and male displaying his coremata (D)

worth speculating here whether PAs in the variety of species might have other/additional functions to those discussed above (e.g., a nutritional one; [19, 71]). In summary, present knowledge strongly suggests that storage of PAs for defence is the original as well as a common feature of PA insects¹, and also that the use of PAs as male pheromone precursors is widespread. However, the insects in question are quite diverse, and although their relation to PAs is phenomenologically similar, generalizations on functional aspects should be approached with caution.

PA Utilization and Sexual Selection

The common appearance of PA utilization for both protection and pheromone biosynthesis shows a link between chemical defence and chemi-

¹ For *D. plexippus* and some other Danainae which erratically obtain cardenolides from larval food plants (see above), sequestration of cardenolides might be an additional, secondary defensive factor, perhaps merely a side-effect of using cardenolide-containing host plants which provide a food resource generally avoided by herbivores ([27, 61], see [14])

cal communication. This sheds fresh light on the question of the significance of male pheromones in the Lepidoptera, which is little understood particularly with respect to the information they signal to the female [27]. The function of dihydropyrrolizines in Danainae is particularly puzzling, since they are joint pheromone components in sympatric species and require costly efforts to be synthesized. On the grounds that *Utetheisa* moths store PAs for defense and use them as precursors for male pheromones, Eisner and Conner [62, 56] advanced the hypothesis that the pheromonal content of coremata could be an indicator of the defensive vigor of the male and be decisive for the female in the context of sexual selection. The authors assume this hypothesis to be applicable to danaine butterflies, but admit that their assumptions are speculative and that certain fundamental questions await answers.

K.S. Brown Jr. recently reported that male Ithomiinae spermatophores containing up to 23% of their dry weight as PAs transfer large amounts of PAs to the female (up to 1.7 mg; [63]); the same occurs in other species [65, 37]. PA transfer during copulation not only explains why PAs are found in females of species which gather PAs in male-biased fashion [63, 60, 61], but it indicates a female's interest in PA-rich males: PAs obtained during copulation probably protect the female and her offspring. Particularly in species gathering PAs pharmacophagously, apart from the benefit for the female of obtaining PAs, the information signaled to a female by a PA-derived male pheromone also reflects a male's general traits, even demonstrating probation of inherited traits. All these aspects must be considered in the context of the hypothesis of Eisner and Conner. It must be stressed, however, that in the different groups PA-derived male pheromones play different roles, and that not all PA insects use PAs as pheromone precursors. Still, the finding of PA-derived pheromones has provided striking examples of the "costs of sex".

Creatonotos will perhaps be a key object in studying the role of PA-derived pheromones in sexual selection, not just because of the morphogenetic effect of PAs on these species (see above) but particularly because of the peculiar use of coremata: in contrast to most other Lepidoptera, the coremata of *Creatonotos* do not come into play for a short time in close range to the female in the final phase of courtship; instead, the males display the organs independent of the presence of a female (Fig. 6D; see [68, 69, 72]). According to laboratory observations, it seems that the males would attract both sexes to mating sites, but female luring pher-

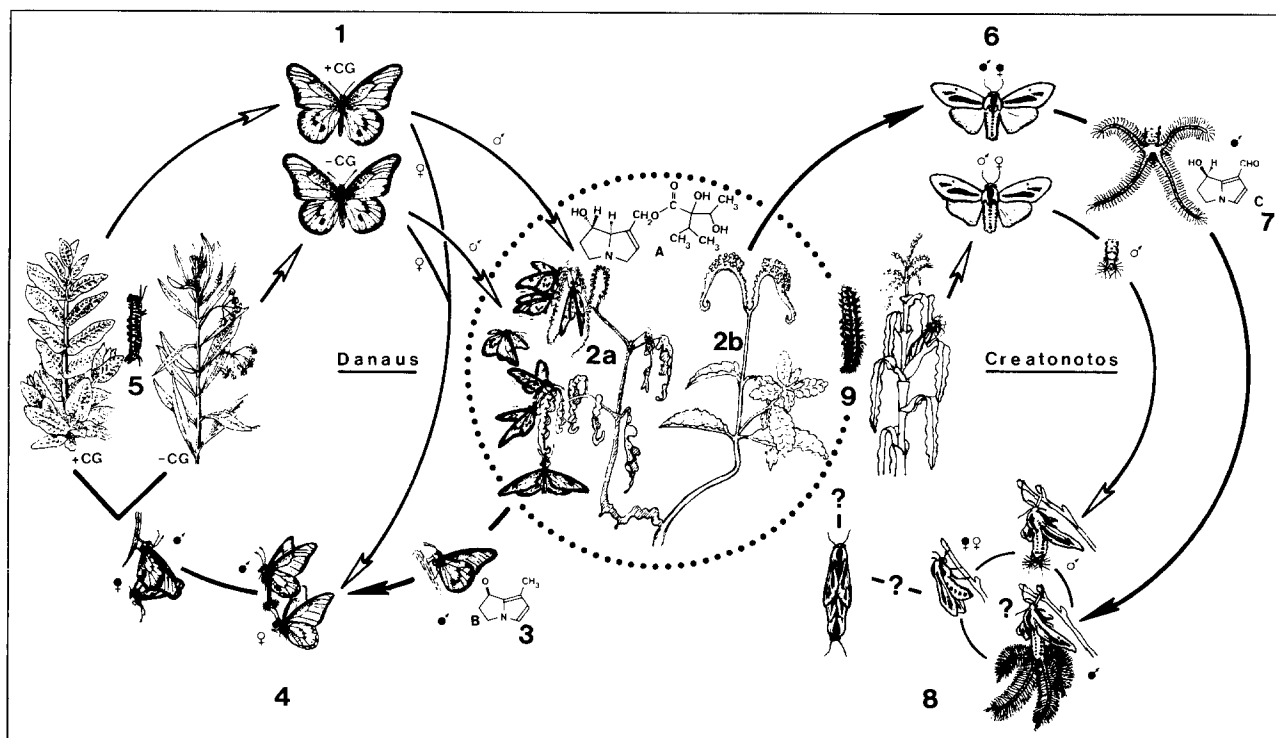


Fig. 7. Simplified schema to illustrate differences and similarities of the role of plants containing pyrrolizidine alkaloids (PAs; center) in the life cycles of *Danaus chrysippus* (Danainae; left) and *Creatonotos gangis* (Arctiidae; right). Thick lines, black arrows and black (filled-in) sex symbols indicate that specimens contain PAs. 1 *Danaus* butterflies either contain cardiac glycosides (CG) (in varying amounts) or lack these substances, depending on the larval food plants (5), asclepiads of various species which can but need not contain cardenolides. The males (rarely females) visit withered PA-containing plants (2a) and take up PAs (e.g., A) which they store. Independent of courtship, the males establish contacts between their abdominal hairpencils and alar glands (3) which are necessary to synthesize the pheromone component danaidone (3, B), required in the final phase of the visually initiated courtship behaviour (4) to be accepted by a female. 6 *Creatonotos* moths contain PAs in varying amounts or lack these substances depending on PAs in larval food (9), various shrubs containing PAs (2b) or not. If PAs (e.g., A) are obtained, they serve as precursors for the pheromone *R*-(-)-hydroxydanaidal (7, C) and also regulate the growth of the coremata (7). Mate-finding and courtship behaviour is not fully understood; however, males display their coremata (8) for a long time, independent of the presence of a female, and females call with luring pheromones. See text and note that the schema excludes certain relevant details, e.g., that there is variation in the amounts of CG, PA, and pheromone in these insects. (See [8, 12, 18, 27, 28, 37, 63] on Danainae, [66, 68–70, 72] on *Creatonotos*.)

omones are also involved [72, 68] (cf. Fig. 7). Field experiments are needed to find whether *Creatonotos* employ a dual mating strategy, like *Estigmene* (where early in the evenings males attract both sexes to leks, later females call males; [73]), or perhaps an even more complex mate-finding (and mate-selection) behaviour. Whatever the case, *Creatonotos* offer a highly promising range of studies in terms of communication mechanisms.

Phyletic Aspects

The pharmacophagous utilization of pyrrolizidine alkaloids by insects calls for ideas on the possible evolution of this peculiar type of insect-plant relationship. Facts such as the wide range of animals

rejecting PAs (which might make these allelochemicals particularly suited as defensive substances for plants and for insects), the apparent link between chemical defence and sexual communication (see above), and the involvement of many PA insects in mimicry all stimulate phyletic considerations; for meaningful speculations, however, we need to know more on the spectrum of insect-PA relationships and understand details of their significance more clearly. (For the controversial discussion on the origin of pharmacophagous PA gathering by Danainae see [74, 19, 52, 27, 28, 75].) In any case, the unrelatedness of the different groups of insects associated with pyrrolizidine alkaloids leaves no doubt that broadly PA utilization and the adaptations involved have polyphyletic origins, with the various consequences that we see having originated from different features.

Vista

We are only beginning to understand the significance of pyrrolizidine alkaloids for insects, and having recognized the basic phenomenology of insect-PA relations, further studies must concentrate on more detailed investigations. Studying the specificity of gathering and utilizing PAs with respect to their molecular structures and carrying out more quantitative analyses considering individual variation appear, among others, most important goals for the future.

The fact that insects can require secondary plant chemicals for their fitness and gather them independently of feeding behaviour shows a peculiar category of insect-plant associations. More important, it demonstrates that the degree of protectedness and the amount of male pheromone is not necessarily fixed for a species but can vary during the life time and depend on the behaviour of the individual – thus mirroring individual traits. In consequence, this phenomenon permits new experimental approaches on some aspects of sociobiology. For instance, the pharmacophagous uptake of pheromone precursors can provide the investigator with specimens of varying but known amounts of pheromones, and thus permit quantitative studies on the role of male pheromones and on female mate-choice, without the need for artificial manipulations such as ectomizing scent and/or receptor organs. Especially the chance to do experiments in the field with *Cretonotos* reared so as to have different and known amounts of pheromone can be expected to make us understand the significance of individually sized and scented coromata. Similarly, the pharmacophagous uptake of defensive chemicals makes quantitative experiments possible on mimicry and learning.

The attraction to PAs of *Zonocerus*, which is a great pest in parts of Africa, might, perhaps, offer a new means of controlling this species. (Following introduction of *Cromolaena*, a PA plant, to prevent soil erosion in Nigeria, the population of *Zonocerus* increased drastically; could PAs have developmental effects on the grasshoppers? (see [40]).

Certainly, pharmacophagy is not restricted to PAs. Collecting of plant fragrances by euglossine bees appears to be pharmacophagy (see [46]), and peculiar feeding habits even of vertebrates perhaps also fall into this category. It is also unlikely that organ-specific and dose-dependent growth regulation by a dietary factor is restricted to *Cretonotos*; even if it was, investigations on the mechanism of organogenesis in this case would be worthwhile and

reveal new insights into nongenetically determined regulation processes during development.

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