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Androconial systems in Danainae (Lepidoptera): functional morphology of *Amauris, Danaus, Tirumala* and *Euploea*

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The morphology of abdominal and alar androconial organs of four species representing four genera of danaine butterflies is described in detail, based mainly on scanning electron microscopy. The findings are discussed with respect to functional significance and phyletic development of the organs.

KEY WORDS:—*Amauris echlea* – *Danaus chrysippus* – *Tirumala petiverana* – *Euploea core amymone* – androconial organs – pheromone glands – sexual communication – scale structure – pheromone-transfer-particles – evolution.

CONTENTS

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INTRODUCTION

Many male Lepidoptera possess specialized epidermal cuticular structures, often connected to glandular cells and considered to be modified non-glandular hairs (scales), which are peculiar to the male sex (Scudder, 1877). Accounts abound on the occurrence and gross morphology of many such *androconial organs* (and in some species their histology), but ethological and chemical investigations have only recently been conducted to elucidate their functional significance. Androconial organs show a wide array of forms, from individual scattered scales, to complex extrusible brush organs on the wings, thorax, legs, or abdomen. Their secretions are usually composed of several components. The suggestion that androconia produce and disseminate pheromones influencing the sexual behaviour of females has been shown to be valid for various species of both moths and butterflies. Male scent organs are usually brought into use at close range to release pheromones capable of persuading a female to accept her prospective mate. However, other or additional functions are likely to be involved, including intra-sexual competition. The subject of pheromone biology in Lepidoptera was reviewed by Boppré (1984a).

The male pheromone system of the Danainae (the Milkweed or Monarch Butterflies) has previously been discussed by Boppré (1977, 1978, 1984a) and reviewed by Ackery & Vane-Wright (1984). The structural and chemical complexity of danaine scent organs may be related to the involvement of most danaine species in large Batesian and Müllerian mimicry rings (see Discussion). This, and other peculiar aspects such as the use of pheromone-transfer-particles, and the biosynthesis of male pheromones dependent on ingestion of noxious secondary plant substances by the adult stage, makes this group of great interest for studying the mechanisms and evolution of sexual communication systems.

The Danainae are currently divided into two tribes, the Danaini and the Euploeini (Fig. 1). The former is almost certainly monophyletic, but the monophyly of the latter remains in doubt (Ackery & Vane-Wright, 1984; Kitching, 1985). All species of Danaini have two types of androconial organs: zones on the posterior part of the hindwings (*alar organs*; Figs 2A–C, 3) microscopically characterized by modified scales and glandular cells, and extrusible brushes (*abdominal organs* or '*hairpencils*') located within the tip of the abdomen which, in their expanded state, form paired, plumose tufts (Fig. 2E). The Euploeini are divisible into two subgroups, the Euploeina and the Itunina. When protruded, the abdominal organs of almost all Euploeina look like bottle-brushes (Fig. 2F). All *Euploea* species have scattered androconia or discrete alar organs on the anterior portion of the hindwings and, additionally, many of the species in this large genus possess forewing alar organs. However, the remaining Euploeina species (belonging to *Idea* and *Protoploea*), and the species of *Anetia* and *Lycorea* which constitute the Itunina, completely lack alar organs. Unlike the 'bottle-brush' abdominal organs characteristic of the Euploeina, the Itunina have plumose hairpencils similar in gross appearance to those of the Danaini.

Hairpencils, which occur in all danaines, are usually composed of several hundreds of hairs. These insert in flexible integumental tubes capable of being protruded (rather like evaginating a finger of a glove) by haemolymph pressure. When everted, the tubes appear through openings between the 8th and 9th abdominal segments; subsequent withdrawal is effected by retractor muscles.

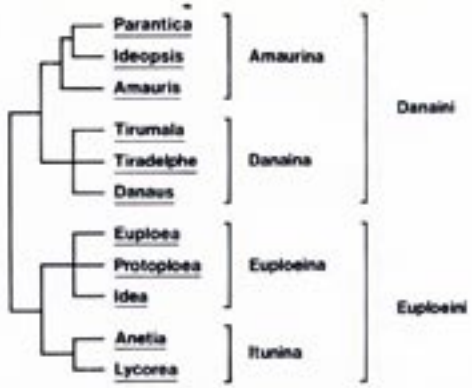


Figure 1. Classification of the eleven recognized genera of the Danainae into two tribes, each with two subtribes, based on the cladistic analysis of Ackery & Vane-Wright (1984).

During courtship flight, males of the Danaini briefly expand their hairpencils close to the female; in *Euploea*, patrolling by males with expanded hairpencils has been observed in addition to hairpencilling during courtship hovering flights (see Discussion). As indicated for *D. gilippus* by Pliske & Eisner (1969), most Danaina disseminate tiny particles (*pheromone-transfer-particles*; Boppré, 1976) during courtship. Stimulation of the females by hairpencil pheromones is essential for successful courtship in various danaines (Pliske & Eisner, 1969; Myers & Brower, 1969; Boppré & Schneider, unpublished). Some components of the species-specific hairpencil bouquet have been identified (summary in Ackery & Vane-Wright, 1984; *cf.* Schulz, 1987, Schulz, Francke & Boppré, 1988). Dihydropyrrolizines, generally used by Danainae as major hairpencil volatiles, are biosynthesized from pyrrolizidine alkaloids which have been actively searched for and ingested by adult males (see Discussion).

Independently of courtship, mechanical contacts are established between the hairpencils and alar organs. In *Amauris* species, only part of each pencil is spread out, fanwise, over the adjacent hindwing patch (Fig. 2G); in *Danaus* and *Tirumala* species the unexpanded, brush-like hairpencils are dipped into the open cavities of the hindwing pockets or pouches (Fig. 2H); in *Euploea* contact behaviour has only once been claimed and has never been verified (see Discussion).

In this paper the integumental structures of the abdominal and alar organs of representative species of three genera of the Danaini and of one species of *Euploea* are described in detail. The species concerned, *Amauris ochlea, Danaus chrysippus, Tirumala petiverana* and *Euploea core amymone,* were chosen for this study because observations and experiments already carried out with these insects permit us to relate a number of structural characteristics to physiological or behavioural functions, and discuss their likely phyletic development. In the future we hope to compare the morphology of all 158 species of Danainae in detail (Boppré, Ackery & Vane-Wright, unpublished).

Although the androconial organs of the species discussed here (and some other Danainae) have been studied previously (Müller, 1877a, b; Illig, 1902; Freiling, 1909; Eltringham, 1913, 1915; Latter & Eltringham, 1935; Brower, Brower &

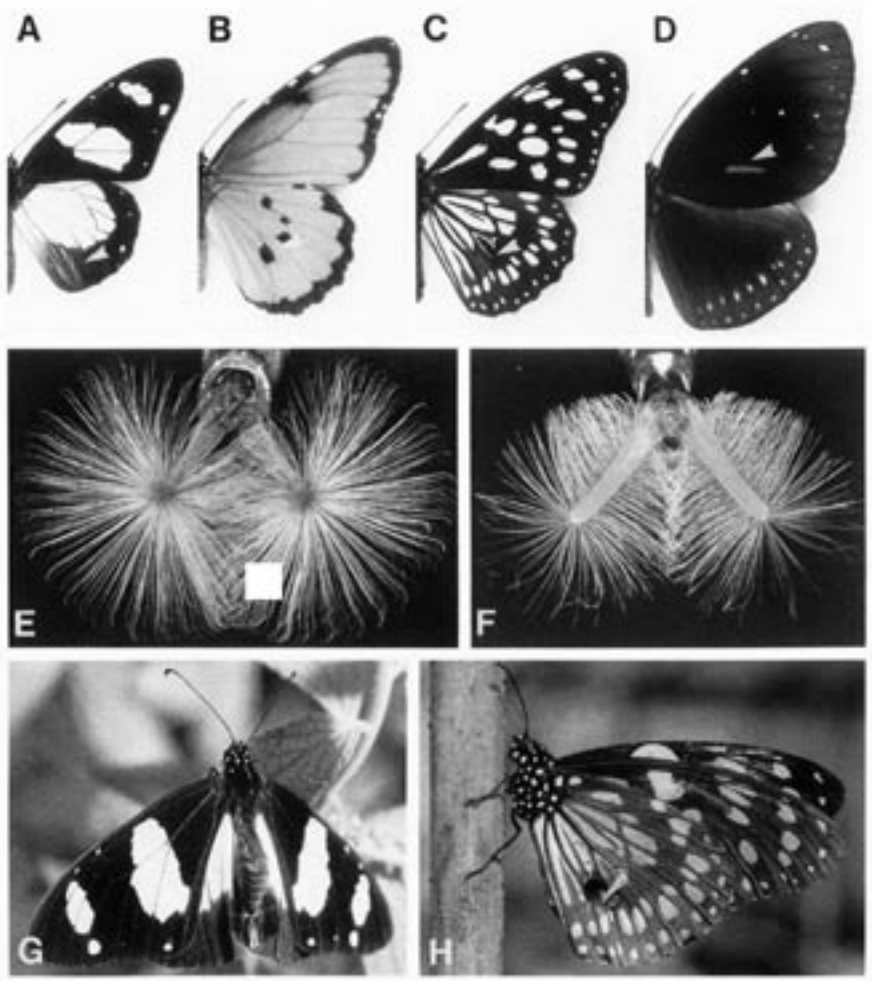


Figure 2. A–D, Set males of A, *Amauris ochlea*, B, *Danaus chrysippus*, C, *Tirumala petiverana* and D, *Euploea core amymone* showing positions of their alar androconial organs (arrows). E–F, Expanded abdominal hairpencils of E, *Tirumala petiverana* and F, *Euploea core amymone*. G–H, Males of G, *Amauris ochlea* and H, *Tirumala petiverana* establishing contact between abdominal hairpencils and alar patches/pouches. In H the pouch has been cut out to demonstrate the insertion of the hairpencils.

Cranston, 1965; Pliske & Salpeter, 1971; Boppré & Fecher, 1977), several structures have been overlooked and others misinterpreted. This has largely been due to insufficient material, the unavailability of modern technical procedures, or the lack of physiological data necessary for functional interpretation of the structures.

MATERIAL AND METHODS

The three species of Danaini were all obtained from Kenya: *Danaus* (*Anosia*) *chrysippus* (L.) subspecies *aegyptius* (Schreber); *Amauris ochlea ochlea* (Boisduval);

and the monotypic *Tirumala petiverana* (Doubleday). Material of *Euploea core* (Cramer) subspecies *amymone* (Godart) was obtained from Hong Kong. The *E. core* assemblage may represent up to five or more biologically distinct allopatric siblings, and separation of the *core* assemblage from *E. algea* (Godart) and other related 'species' remains problematical (see discussions in Ackery & Vane-Wright, 1984).

All four species were cultured at Seewiesen, southern Bavaria. Both field-caught and bred individuals, and androconial organs from dried and freshly killed specimens, were used. Organs of newly emerged males were compared with those of older males which had made contacts between the abdominal and alar organs, in order to assess any resultant morphological changes.

The protrusion and expansion of hairpencils was simulated for macroscopical observation by injecting water into the abdomen of freshly killed males. Photographs of progressive states of hairpencil expansion, closely corresponding to the natural process, were thus obtained.

The structures of abdominal and alar organs were studied principally by scanning electron microscopy (SEM). Dissected organs were glued on aluminium stubs with acetone-celluloid glue or conductive carbon cement (after Gōcke). To see the underside of scales, scales were taken off the wing membrane with sticky tape, which was then glued onto stubs. After desiccation at room temperature, the preparations were kept in a saturated osmium tetroxide atmosphere for 24–48 h and afterwards gold-coated in a Leitz sputtering chamber for 8–10 min, at a discharge current of 1 mA. SEMs (Cambridge Stereoscan IIA, Zeiss Novascan 30) were normally operated at 5 or 20 kV acceleration voltage.

In some cases, organs were washed in alcohol and/or acetone in an attempt to remove apparent secretory material and so reveal the fine structure of the androconia.

Some supplementary data (provided by C.-Chr. Meinecke) were obtained by transmission electron microscopy (TEM): tissues were fixed in cold buffered OsO_4 (Zetterquist), dehydrated in ethanol and embedded in Epon 812. Sections were stained with uranylacetate and lead citrate, or lead citrate only, and studied in a Siemens Elmiscope 1A or 101.

RESULTS

Alar organs

Amauris ochlea (Figs 2A, 3A, 4, 5E–G)

The wing surfaces are largely covered with two kinds of broad, overlapping wing scales, in typical lepidopterous fashion (Fig. 4A). A small area of the hindwing adjacent to vein 1A+2A is specialized to form the *alar patch* (Figs 2A, 3A). In this region (some 9 mm long by 4 mm wide) the wing is thickened, the upper and lower wing lamellae being more widely separated here than elsewhere. The upper surface of the patch is characterized by regular rows of alternately arranged *patch scales* and *cushions*, there being approximately 13 000 of each of these structures (Fig. 4B, C, F). On the underside, the alar organ is only apparent from the thickening of the patch area; the covering scales are unmodified. Unlike the normal wing scales, the smaller patch scales do not

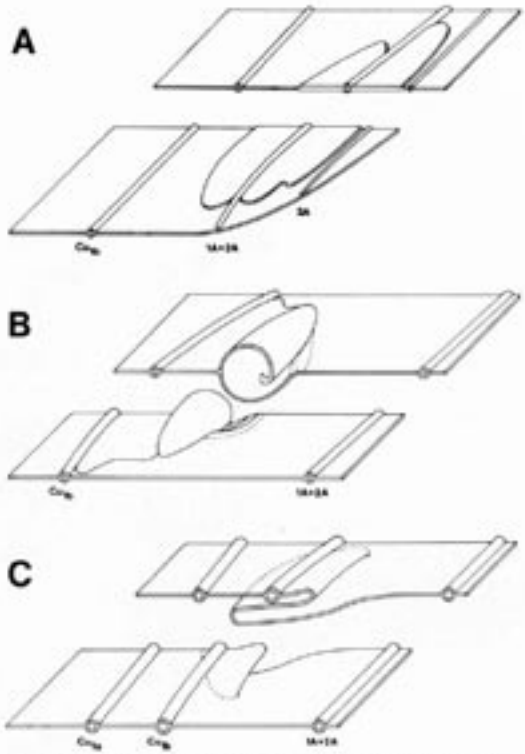


Figure 3. Schematic diagrams of alar organs of male A, *Amauris ochlea*, B, *Danaus chrysippus* and C, *Tirumala petiverana*, showing the differences in gross morphology.

completely cover the wing membrane, which is clearly visible between them (Fig. 4B, F). Patch scales occur at a slightly higher density than ordinary wing scales (400–500/mm^2 *vs.* 250–300/mm^2). Patch scales are club-shaped (Figs 4C, 5E, F), some 65 µm long with the clubbed tip of maximum width of 15–20 µm and up to 9 µm thick, the stems tapering towards the insertion points between adjacent plates (Fig. 4C, F). Patch scales also differ from the normal wing scales in their fine structure. Ordinary wing scales have a closed basal lamella supporting an upper lattice (*cf.* Fig. 5A, B), a structure typical of most Lepidoptera (*cf.* Discussion). In contrast, the patch scales are thickened, generally ovoid in cross-section, with a lattice structure on both upper and under surfaces (Fig. 5E–G). Within the lumen of the scales there is an amorphous substance.

The cushions ("scent cups" of Eltringham, 1915) are rounded, domed structures, with a reticulate surface (Fig. 4G–I); they appear to represent highly modified scale bases. The plates are approximately 20–25 µm in diameter and about 10–15 µm high. Under SEM the surface appears to have small pores, the presence of which has been confirmed by TEM. Each plate is set into a ring-like *cushion socket* (Fig. 4G, I). Histological sections show large secretory cells beneath the plates (Eltringham, 1915; Boppré & Fischer, unpublished).

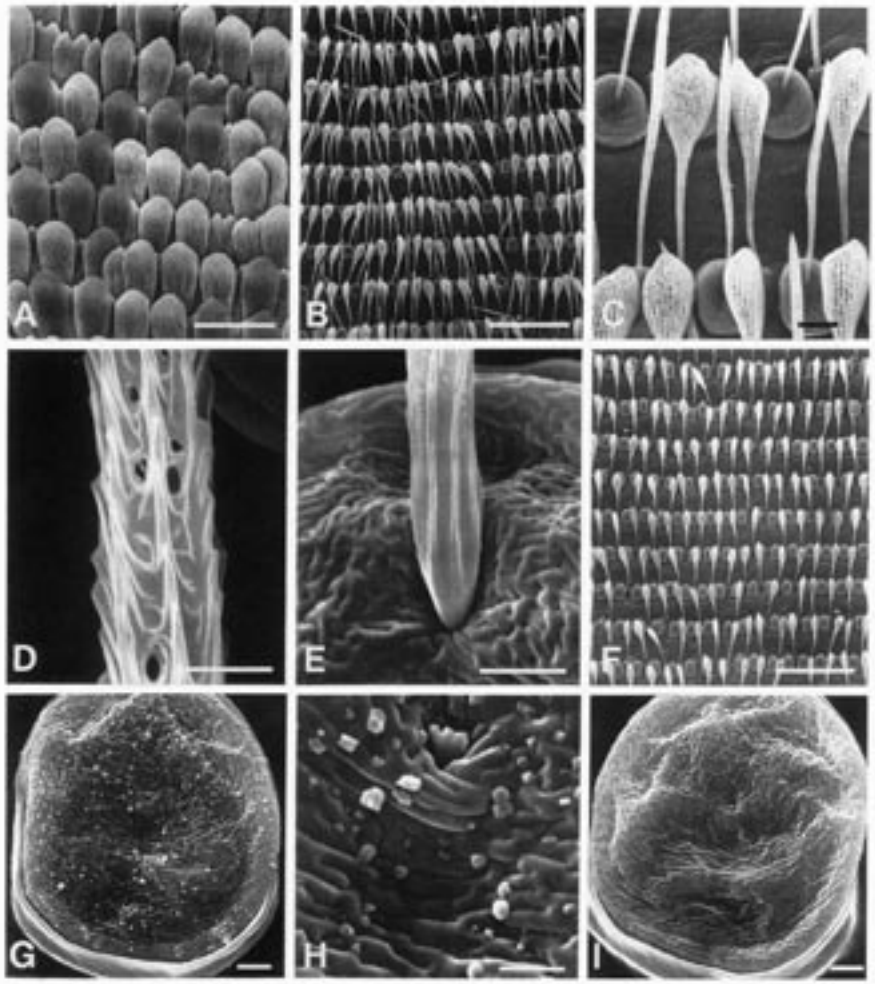


Figure 4. Microscopic characteristics of alar patches of male *Amauris ochlea*. A, Wing scales. B, Part of patch of freshly emerged male. C, Patch scales, cushions, and cushion scales. D, Median part of a cushion scale. E, Connection between cushion scale and cushion. F, part of a patch after black hairs of hairpencils had made contacts (cf. B). G, Cushion with its socket showing crystal-like particles (G, H) which can be washed off (I). Scale bars: A, B, F: 100 µm; C: 10 µm; D, E, G, I: 2 µm; H: 1 µm.

In the centre of each cushion a hair-like *cushion scale* arises. Each is some 75 µm long and 4–5 µm thick, coarsely set with longitudinal ribs and perforated along the outer part of its length by irregularly spaced pores (Fig. 4D). Basally, the cushion scales are constricted (Fig. 4E). Entire cushion scales are only found in freshly emerged males (Fig. 4B), as they are broken off by contact behaviour, after which only the basal stumps of the cushion scales remain (Fig. 4F–I).

SEM examination often reveals crystal-like particles on cushions (Fig. 4G, H)

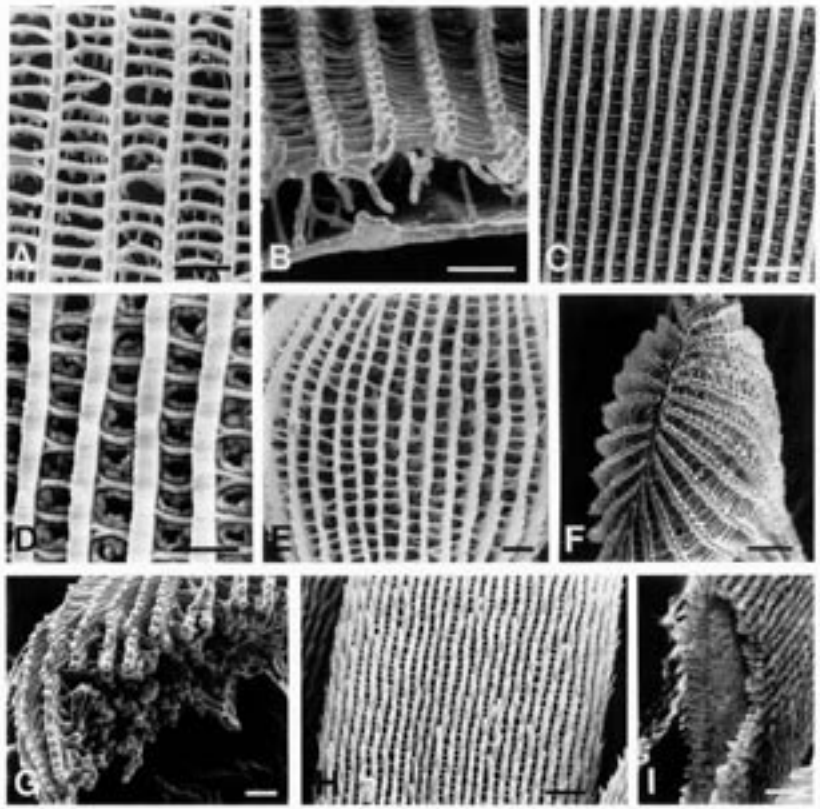


Figure 5. A, B, Wing scales of *Danaus chrysippus* showing typical lepidopterous scale structure. A, Upper side, illustrating lattice. B, Cross-section showing basal lamella, trabeculae, and lattice. C, D, Pocket scales of *D. chrysippus* (note: 'amorphous substance'). E–G, Patch scales of *Amauris ochlea*. H, I, Pouch scales of *Tirumala petiverana*. Scale bars: A–C, E–I: 2 µm; D: 1 µm.

which can be washed off with diethyl ether and other solvents (Fig. 4I); perhaps these particles represent a solidified secretion.

Danaus chrysippus (Figs 2B, 3B, 5A–D, 6)

The alar organs of *D. chrysippus*, termed *alar pockets*, occur posterior but closely adjacent to hindwing vein Cu_{1b}. Each organ consists of a pocket-like fold (partly a duplication) of the upper wing lamella (Figs 2B, 3B). On the underside of the hindwings the pockets form slight bulges patterned with black and white scales. The whole pocket zone is approximately 3–4 mm long and 1 mm wide.

The pockets are formed after eclosion. Before the wings expand, the alar organs are dark, oval patches, grossly similar to the alar patches of *A. ochlea*. During wing expansion, the antero-distal portion of the patch, which is delaminated from the surface, folds up and over the postero-proximal portion, the resultant pocket opening towards the abdomen (Fig. 3B). In the region of the pocket, vein Cu_{1b} is thickened, and the space between the wing membranes is enlarged and filled with haemolymph.

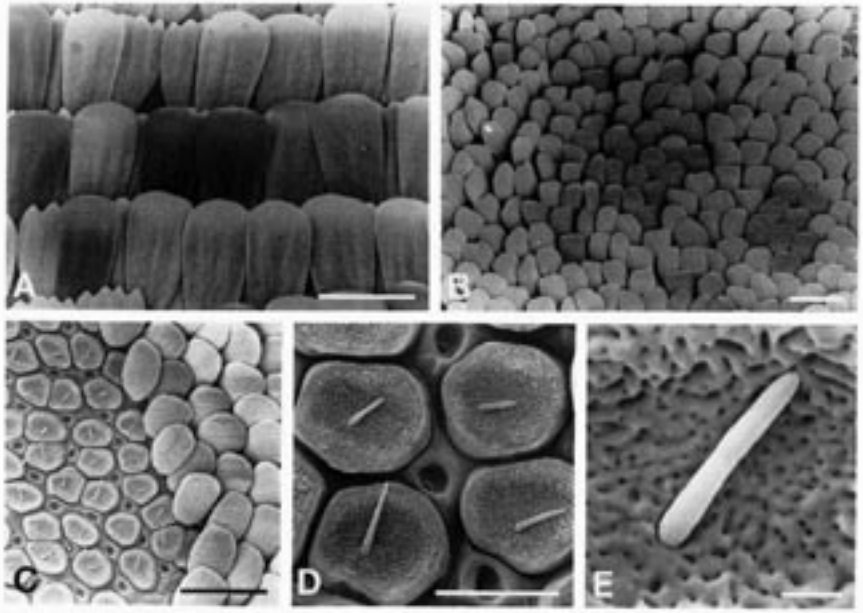


Figure 6. Microscopic characteristics of alar pockets of male *Danaus chrysippus*. A, Wing scales. B, Pocket scales. C, Part of interior of alar pocket partly denuded of pocket scales, showing cushions and cushion scales. D, Cushions showing pores and cushion scales. E, Cushion scale. Scale bars: A–C: 50 µm; D: 20 µm; E: 2 µm.

The wing pockets are lined by uniform, overlapping pocket scales (Fig. 6B). Although similar in shape to ordinary wing scales (Fig. 6A; *cf.* Fig. 5A, B), they are much smaller (approximately 50 µm long, 30 µm wide) and not arranged in regular rows; also, they occur at higher density (1000/mm^2 *vs.* 300/mm^2). In fine structure they resemble the patch scales of *A. ochlea* by lacking a basal lamella and being bilaterally symmetrical, thus having a lattice structure on both surfaces. Similarly to *A. ochlea*, the pocket scales of *D. chrysippus* contain an amorphous substance (Fig. 5C, D).

The pocket scales overlay and completely obscure the 6000 or so cushions occurring within each pocket. The cushions are somewhat irregularly shaped, about 20–25 µm across and 5–8 µm high, and they occupy almost the entire area between the pocket scale sockets (Fig. 6C). In contrast to *A. ochlea*, the plate sockets are less prominent, on the upper surface pores are both numerous and conspicuous (Fig. 6D, E), and the cushion scales are short, being about 7–12 µm long (thus shorter than the width of a plate) and 1 µm thick, and have smooth surface structure (Fig. 6E). The cushion scales of *D. chrysippus* are not broken off during contact behaviour, as the pocket scales form a complete protective covering. Histological investigations have demonstrated the glandular nature of these structures (Boppré & Fischer, unpublished; *cf.* Brower *et al.*, 1965).

Tirumala petiverana (Figs 2C, 3C, 5H, I, 7, 8)

The alar organ of *T. petiverana* occurs in a grossly similar position to that of *D. chrysippus*, lying midway between hindwing veins Cu_{1b} and 1A+2A.

However, instead of being a cupped pocket it forms, projecting below the wing, a deep, clearly visible alar pouch, about 3–4 mm in length (Figs 2C, 3C). On the upper surface, the zone surrounding the opening is about 5 mm in length, 2 mm in width. Close to the pouch, the veins Cu_{1b} and 1A+2A are thickened, as is the haemolymph-filled wing membrane.

The pouch is formed after eclosion. On emergence of the butterfly, the prospective pouch appears as a felted patch (Fig. 7C), covered by a thin transparent membrane. When the wings are being expanded, these patches stretch more than the surrounding wing parts and invaginate to the underside.

The fluffy material which makes the patches at first appear hairy or felted consists of fine threads (Fig. 7B, F) which, in the course of forming the pouches, disintegrate and fill the alar organs as a grey, particulate plug. Removal of this 'dust' reveals that the threads originate from the cushions (Fig. 7E, F) and correspond to the cushion scales of *A. ochlea* and *D. chrysippus*.

The cushions are roughly circular in outline, with lugged sockets and deeply reticulate/pitted upper surfaces (Fig. 7D, E). The cushions are located between pouch scales which have—like patch scales and pocket scales in *A. ochlea* and *D. chrysippus*, respectively—a lattice structure on both sides (Fig. 5H, I).

The undisturbed cushion scales are up to 600 µm long and 3 µm thick, in this species thus being longer than the pouch scales. Their most striking feature is subdivision into a large number of scissile, polyhedral particles, of approximately 0.5–1.0 µm edge-length. Each scale gives rise to large numbers of these

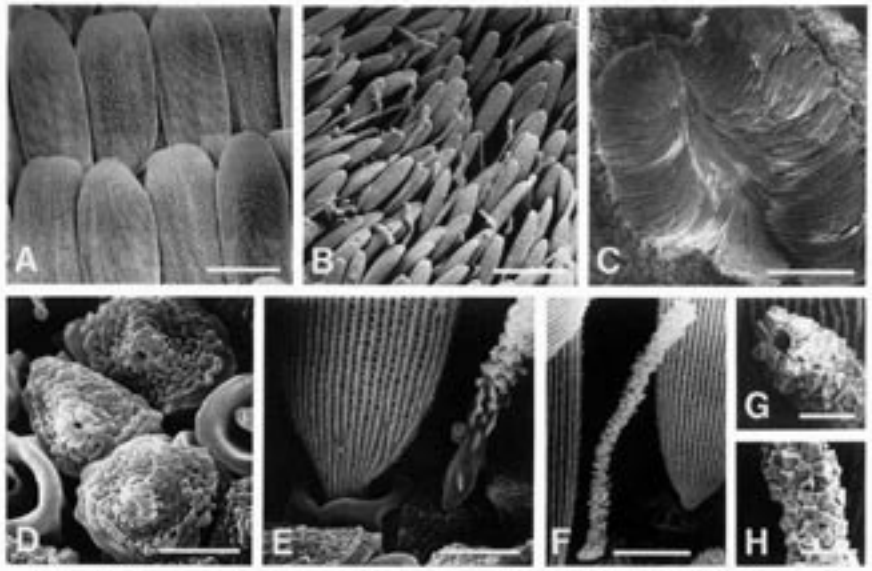


Figure 7. Microscopic characteristics of alar pouches of male *Tirumala petiverana*. A, Wing scales. B, Interior of pouch showing pouch scales and apical parts of cushion scales. C, Alar organ in teneral adult before invagination to form pouch; D, Cushions denuded of scales. E, F, Bases of pouch scales and cushion scales. G, H, Details of particle-generating cushion scales. Scale bars: A, B: 50 µm; C: 500 µm; D, E: 5 µm; F: 10 µm; G, H: 2 µm.

pheromone-transfer-particles (PTPs; Fig. 7F–H), which are taken up by the abdominal hairpencils during contact behaviour (Fig. 2H; *cf.* Fig. 14D–F).

According to a previously unpublished TEM investigation by C.-Chr. Meinecke, the ontogenetic development of PTPs in this species is as follows (see Fig. 8). As in the development of ordinary scales and their sockets, PTP compound threads and their cushions are formed by trichogen and tormogen cells respectively. Development starts about three days after pupation, when the trichogen cells of both threads and other scales form long cytoplasmic processes covered by a thin epicuticle. At this time threads can be distinguished from other scales by their smaller diameter and increased length. More obvious differences occur about two days later, when the epicuticle partly separates from the plasmamembrane. Presumably brought about by continued production of epicuticle at points still adhering to the membrane (Ghiradella, 1974), small bubbles form in an irregular way around each process (Fig. 8A). The bubbles are filled with liquor until the beginning of the seventh day when cytoplasm invades their interior, causing a slight collapse and thus giving them their polyhedral shape (Fig. 8B). During the next day the bubbles are stiffened by addition of cuticle to their walls and by cuticular trabeculae forming interiorly (Fig. 8C). No deposition of cuticle occurs in the lumen of the cytoplasmic processes or on the epicuticle adhering to their membranes. Thus PTPs are linked to each other only by thin epicuticle, which are preformed points of rupture responsible for the friability of PTP compound threads. At the end of the eighth day, large numbers of lysosomes indicate the degeneration of the trichogen cells (Fig. 8D), which are not seen in the adults.

Euploea core amymone (Figs 2D, 9)

In contrast to all Danaini, *Euploea core amymone* has discrete alar organs situated on the forewings in cell Cu_{1b}, approximately on the mid-line and appearing as elongated patches, centrally located, and about 1.5×7 mm in extent (Fig. 2D). In addition, the anterior half of the male hindwings carries modified scales, but no distinct androconial area can be recognized; scales of the hindwings have not yet been studied in detail.

The forewing androconia are composed of two patches of specialized scales which occur diametrically opposite each other on the upper and under wing surfaces. (In contrast, the hindwings only have androconia on the upper surface.) As a result of their respective positions, the hindwing and underpart of the forewing androconial areas are in mechanical contact.

On the upper side of the forewings the androconial scales are widely separated, leaving the wing surface partly uncovered (Fig. 9A, B); on the underside, however, the corresponding area is densely covered (Fig. 9H, I). Ordinary scales appear at a 4× higher density than patch scales on the upperside (Fig. 9D, E), but on the underside the situation is reversed (Fig. 9L, M).

The ordinary wing scales (Fig. 9A, F–H, N, O) are of typical lepidopterous structure although differently shaped on the two wing surfaces. The patch scales on either side of the wings (Fig. 9B, C *vs.* Fig. 9I, K) are not noticeably different in fine-structure but are much more slender and—on the underside—hair-like.

Structures similar to the patch, pocket or pouch cushions of Danaini are lacking in *E. core;* however, the bases of the patch scales on the underside of the

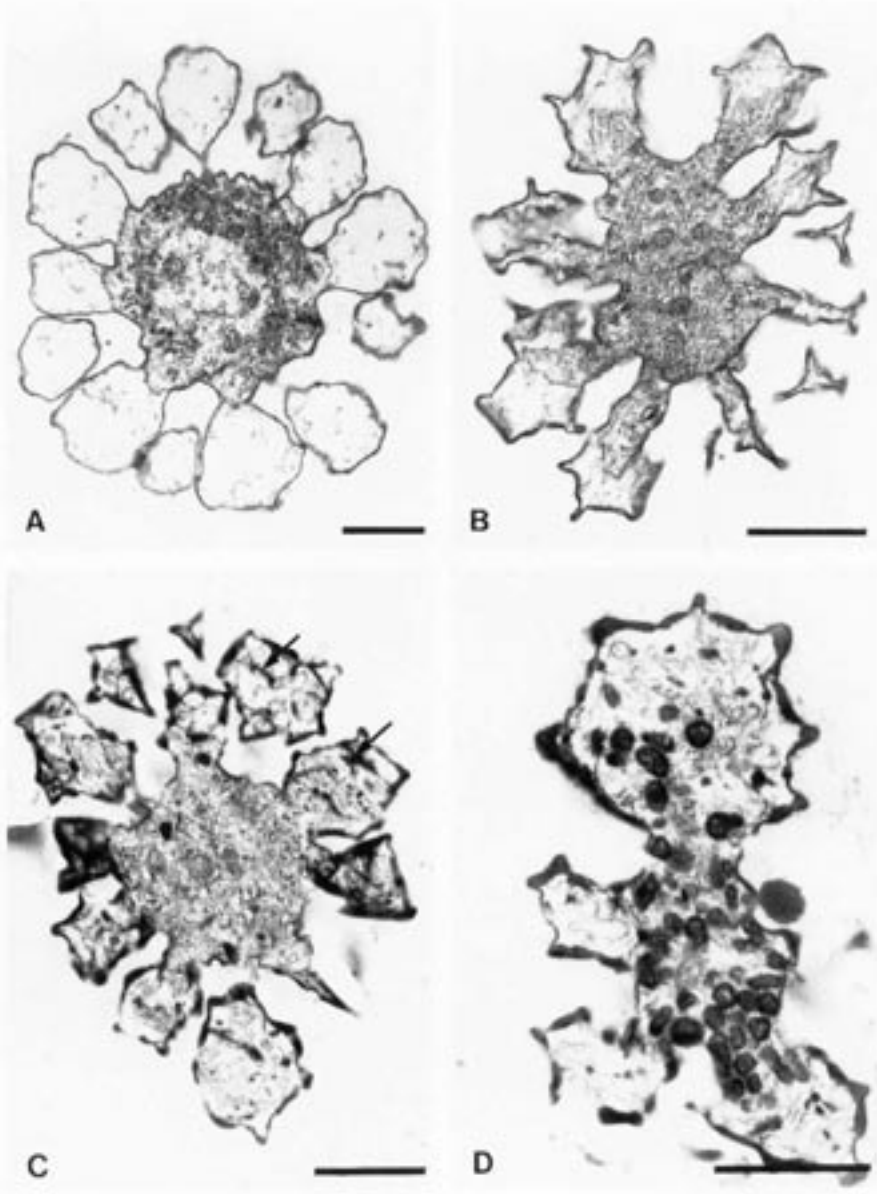


Figure 8. Different stages of the development of particle producing hairs in *Tirumala petiverana*. A, Pupa 5 days old: epicuticular sacs are formed around the cytoplasmic thread. B, Pupa 6 days old: cytoplasm invades epicuticular sacs. C, Pupa 8 days old: epicuticular sacs are stiffened by cuticular trabeculae (arrows). D, Pupa 8 days old: the trichogen cell within the particles begins to degenerate. Scale bars: 1 μm.

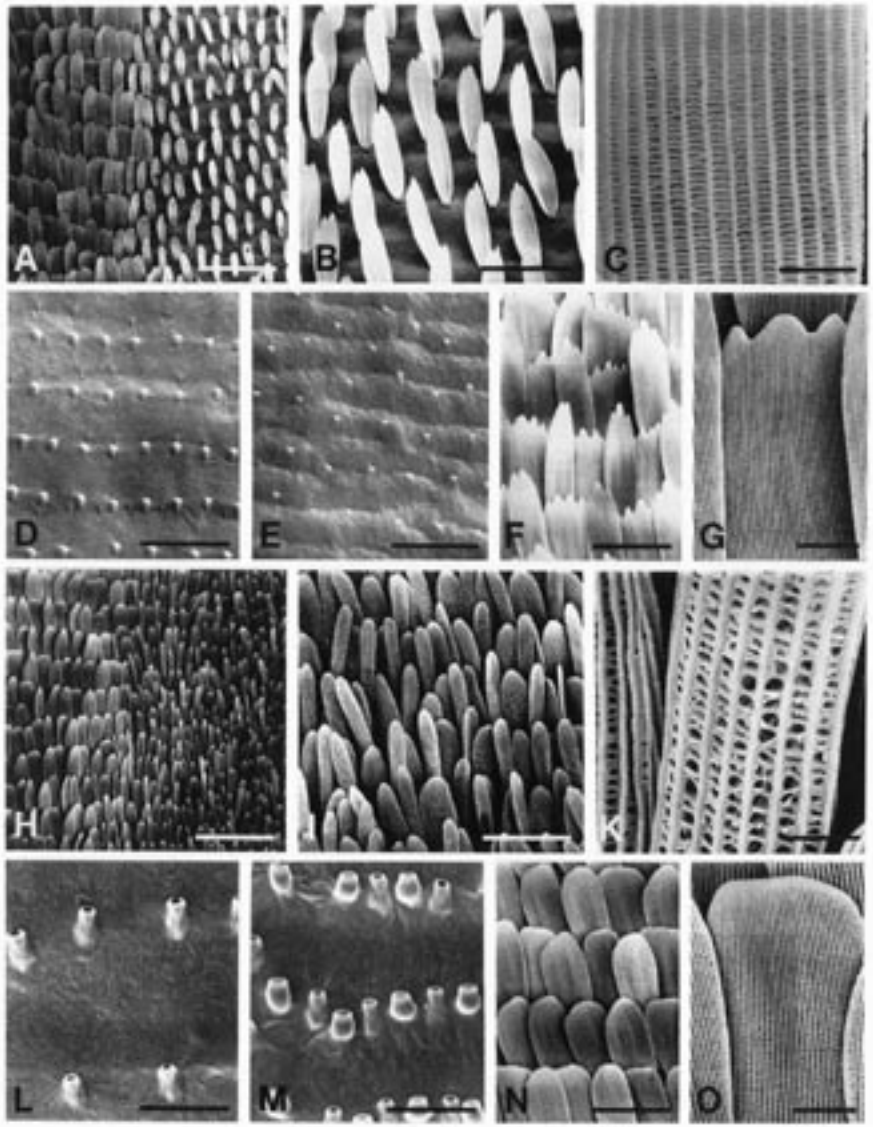


Figure 9. Microscopic characteristics of forewing alar patches of male *Euploea core amymone*. A–G, upperside, ordinary scales (A, left side; F, G) and patch scales (A, right side; B, C), bases of ordinary scales (D) and of patch scales (E). H–O, underside, ordinary scales (H, left side; N, O) and patch scales (H, right side; I, K), bases of ordinary scales (L) and patch scales (M). Scale bars: A, H: 250 µm; B, D, E, F, I, N: 100 µm; C, K: 5 µm; G, O: 20 µm; L, M: 50 µm.

forewing are quite different to bases of wing scales or of patch scales on the upperside (*cf.* Fig. 9L *vs.* 9D, E, M). This indicates that the forewing organs are deployed to the underside and may interact with the hindwing androconia. Glandular cells have been found by light microscopy which are associated with the patch scales on the underside to the forewings. A thorough study, involving TEM and considering both fore- and hindwings is required to fully understand alar androconia in *Euploea*.

Abdominal organs ('hairpencils')

Amauris ochlea (Figs 10–12)

The hairpencils of *A. ochlea*, in their retracted state, lie within a membranous sheath or tube, about 6.5 mm long and 0.6 mm in diameter, situated within segments 5–8 of the abdomen, and opening through intersegmental membrane 8–9 (Figs 10, 11). The basal 0.6 mm of the tube is marked off by a slight, but clearly defined constriction.

Eversion of the hairpencils is effected by increasing haemolymph pressure. Macroscopical examination during progressive protrusion and expansion reveals four different types of hairs—these appear sequentially (Fig. 10). Two bundles first become visible, a white bundle, and an externo-lateral group of *black hairs*. While the *long white hairs* of the white bundle remain closed together like a brush, the black hairs fan out conspicuously (Fig. 10A, B), in nature over the alar patches (Fig. 2G). With a further increase of pressure, the black hairs fold backwards against the sides of the abdomen (Fig. 10C), and the bundles of long white hairs open to form large spheres (Fig. 10E). Now, within each sphere, a cone formed by a third type of hair, the *cone hairs*, becomes visible (Fig. 10F). At still higher pressure the long white hairs fold backwards (Fig. 10H) in a similar fashion to the black hairs, and the cone opens to reveal a fourth hair-type. These *central hairs* form a small sphere (Fig. 10H). During the unfolding of the hairpencils, the outer black and white hairs fan out regularly, but the cone hairs behave as if they were sticky, folding back in groups to form a stellate array (Fig. 10G).

Cross and longitudinal sections of the retracted hairpencils clearly show the distinct arrangement of the hairs (Fig. 11). A fifth hair-type, not distinguishable from the long white hairs macroscopically, is also revealed; it separates the long white hairs and the black hairs. These components are termed *separating hairs*.

All the hairs are inserted within the innermost third of the tube, each type being located in a particular region (Fig. 11A). The central hairs arise basad of the constriction. Arranged concentrically around the central hairs, the cone hairs arise from a narrow band at the constriction zone. Distal to this, the long white hairs, separating hairs and black hairs are successively located (Fig. 11A), the two last named types occurring only at the outer lateral sides of each pencil.

Basally, the thick-walled black hairs (Fig. 12A–D) have a regular, scaly surface sculpture which becomes increasingly differentiated towards the tip, forming rows of sharp, spiny projections; apically, the black hairs can also be seen to have numerous holes. The flattened separating hairs (Fig. 12E–F) exhibit regularly lapped ribs; distally the individual lappets become larger, producing rows of close, rounded projections at the tips. The long white hairs (Fig. 12G–O)

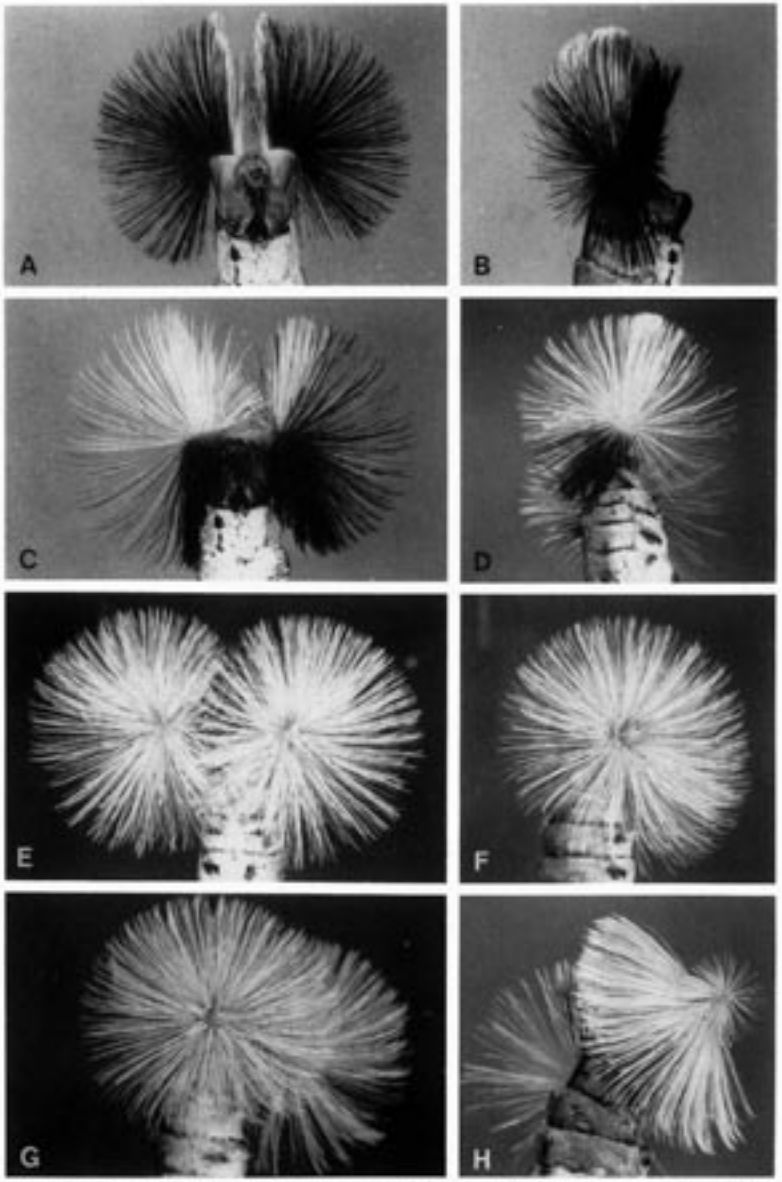


Figure 10. Progressive states of artificially induced hairpencil protrusion and expansion in *Amauris ochlea*. A, C, E, Seen ventrally. B, D, F, G, H, Seen laterally. Magnification: *c.* 3×.

are rather similarly differentiated along their entire length. At the apical end, where many fat, globose nodules occur, the hairs split into longitudinal chains, to produce irregularly shaped pheromone-transfer-particles. The PTPs adhere in masses to the stable basal portions of the mother hairs. The cone hairs

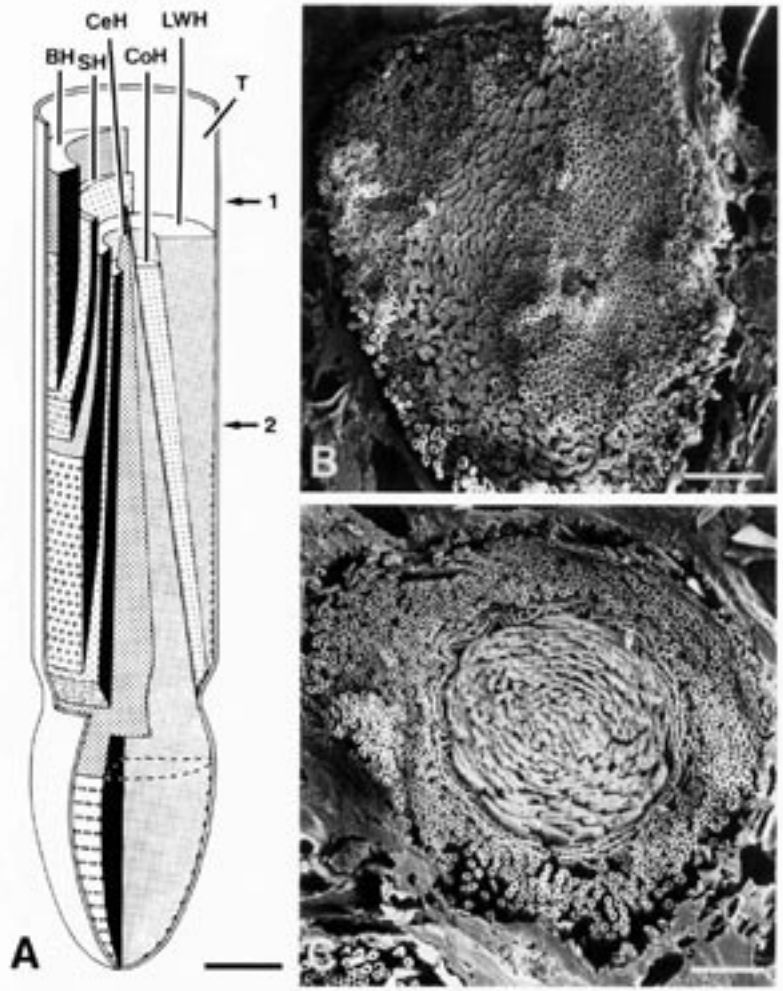


Figure 11. A, Schematic diagram of the proximal third of an abdominal hairpencil of *Amauris ochlea* in its retracted state. The scheme corresponds to a left hairpencil viewed dorsally. The hairpencil is cut open medially (right side in the scheme) and at various longitudinal and cross levels (left side in the scheme) in order to demonstrate the distribution (dots) and the bases (open circles) of the five hair types as well as the asymmetrical arrangement of the black and the separating hairs. B, C, Cross sections through a retracted hairpencil of *A. ochlea* at levels 1 and 2 as indicated in A. Scale bars: A: *c.* 250 μm; B, C: 100 μm. BH: black hair; SH: separating hair; CeH: central hair; LWH: long white hair; CoH: cone hair; T: hairpencil tube.

(Fig. 13P, Q) are flat and smooth, having very wide bases and ending in fine tips. Hairs of this type are filled with densely packed trabeculae. Like the cone hairs, the central hairs (Fig. 12R–U) are also lanceolate; their surfaces are covered with regular scaly projections. In untreated cross-sections these central hairs appear solid. However, after washing cross-sections in diethyl ether, the

hairs appear thin-walled, with spongy trabeculae inside. In osmium tetroxide atmosphere, untreated central hairs become black; this osmiophility (indicating the presence of carbohydrates with double bonds) is also lost by washing. Possibly the soluble material represents solidified secretion.

A summary of the surface and inner structural features and the principal dimensions of the five types are given in Table 1.

Danaus chrysippus (Fig. 13)

The hairpencil tubes of *D. chrysippus* lack a basal constriction, but are located within the abdomen in a similar position to the hairpencils of *A. ochlea*. The organs appear identical to those of its sister-species, *D. gilippus*, which have been described in detail by Pliske & Salpeter (1971).

In the retracted state, each hairpencil appears as a bundle of *particle-budding hairs* surrounded by marginal hairs (Fig. 13A). The 850 particle-budding hairs and 450 *marginal hairs* are both 4.5 mm long and each is about 9 µm or so thick. Both types are thin-walled and have their interiors filled with trabeculae.

The surface structure of particle-budding hairs is distinctly different in the apical and distal parts (Fig. 13D–I). Basally, there are close-set ribs, bearing lappets 4–8 µm long, arrayed in slightly irregular longitudinal rows. The distal tip of each lappet projects outwardly from the hair surface. In the central and apical regions of the hairs are fewer ribs. Apically the ribs are very irregularly aligned, leaving round or oval areas each of which is perforated by several holes about 4 µm in diameter.

SEM micrographs of the central and distal regions reveal many roughly globe-like projections, each attached to the end of a rib lappet. (This matches TEM investigations of *D. gilippus:* Pliske & Salpeter, 1971.) The globes (cuticular outgrowths) eventually break off to generate PTPs. These particles are roughly 2 µm in diameter, and each bears a slight depression which appears to represent the former site of attachment to its mother lappet.

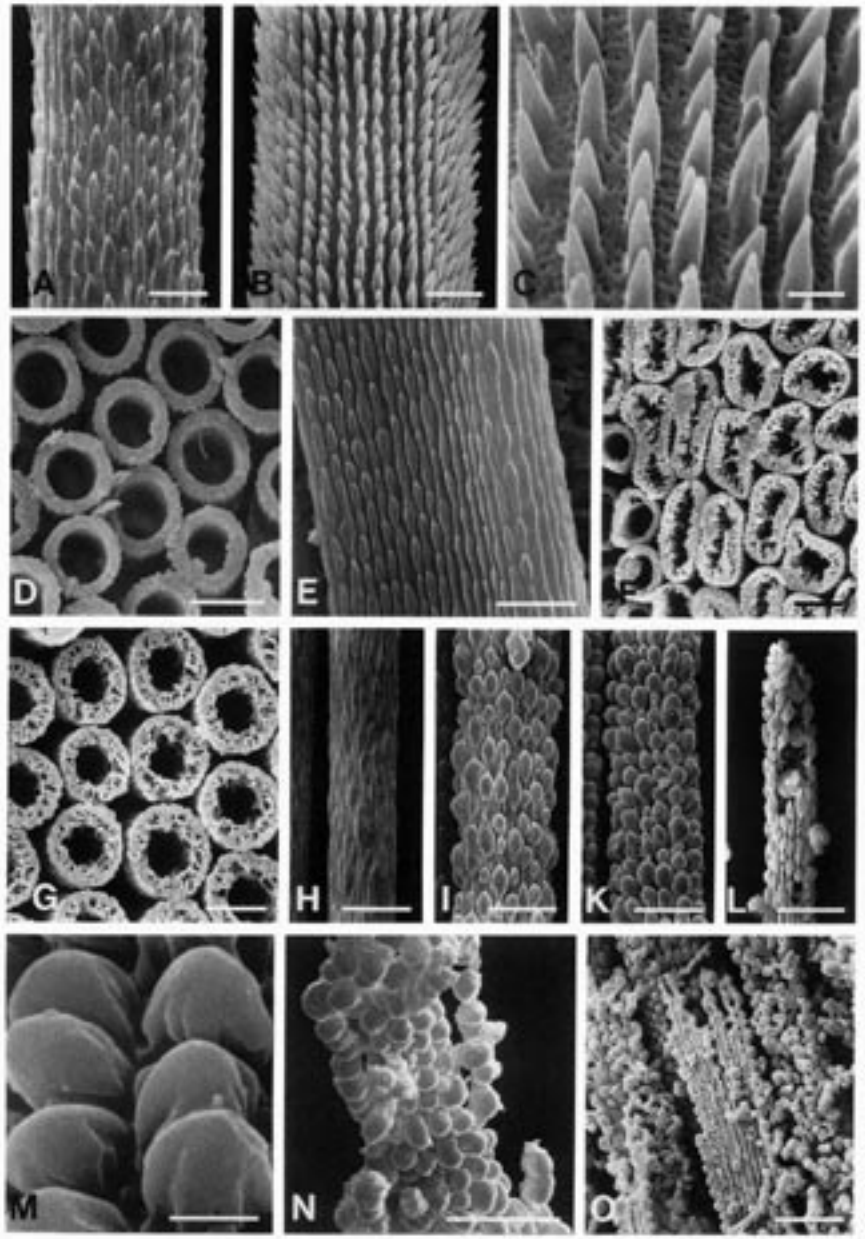
The surface of the marginal hairs also changes from base to tip, but much less strikingly. In general they have relatively smooth longitudinal ribs and highly perforated inter-rib spaces (Fig. 13B, C).

Tirumala petiverana (Figs 2E, H, 14)

In gross morphology, the hairpencils of *T. petiverana* are similar to *D. chrysippus*. However, the hairpencils are relatively simple, having only one hair-type. The 750 hairs (Fig. 14A–C) are 4.5 mm long and 10 µm thick; the surface of each is densely granular while the interior is filled with a mesh-like network of trabeculae. We call these hairs *particle-receiving hairs* because during contact behaviour they become charged with PTPs produced by the plate scales of the alar pouches. The PTPs adhere to the surface of the particle receiving hairs in great numbers (Fig. 14E, F), particularly at the tips, where their presence can even be appreciated by the naked eye: the tip of a protruded but unexpanded hairpencil appears more darkly coloured (Fig. 14D).

Euploea core amymone (Figs 1F, 15)

As already mentioned, the hairpencils of *Euploea* differ macroscopically from those in Danaini by appearing like a bottle-brush; that is the hairs are inserted



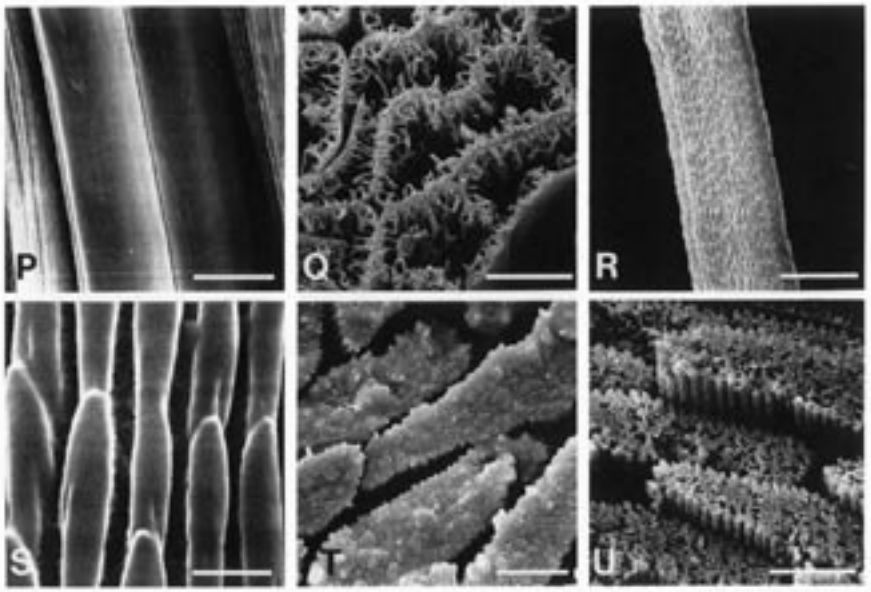


Figure 12. Microscopic characteristics of hairpencil hairs in *Amauris ochlea*. A–D, Black hairs. E, F, Separating hairs. G–O, Long white hairs, basal (H) median (I, K) and apical parts (L–O) showing budding of PTPs (L–O). P–Q, Cone hairs. R–U, Central hairs. Scale bars: A, B, E: 3 μm; C: 1 μm; D, F–L, N, P–R, T, U: 10 μm; M, O: 2 μm; S: 1 μm.

over the entire length of the hairpencil tubes (Fig. 2F). The hairs are uniform in structure (Fig. 15A–C), 4 mm long and 10 μm thick. Inside (Fig. 15D) they are entirely filled with trabeculae. At the base, each hair has longitudinal ribs bearing blunt, thorn-like projections (Fig. 15C). These projections become

TABLE 1. Summary of structural features and principal dimensions of the five hair types making up the abdominal hairpencils of *Amauris ochlea*

	Surface structure	Inner structure	Length (mm)	Thickness (μm)	Number
Black hair	Sharp, raised spines Fig. 12A–C	Round and hollow Fig. 12D	4.5	12	600
Separating hair	Ribs, with slightly raised lappets Fig. 12E	Oval, filled with trabeculae Fig. 12F	4.5	10 × 25	150
Long white hair	Nodulose, generating PTPs Fig. 12H–O	Round, spongy walls Fig. 12G	3.5	15	760
Cone hair	Smooth Fig. 12P	Flat, thin-walled, tapering, fine trabecular network Fig. 12Q	2.1	8 × 60*	80
Central hair	Ribbed with lappets Fig. 13R, S	Flat, tapering, filled with dense trabecular network Fig. 13T,U	2.1	6 × 60*	200

*At base.

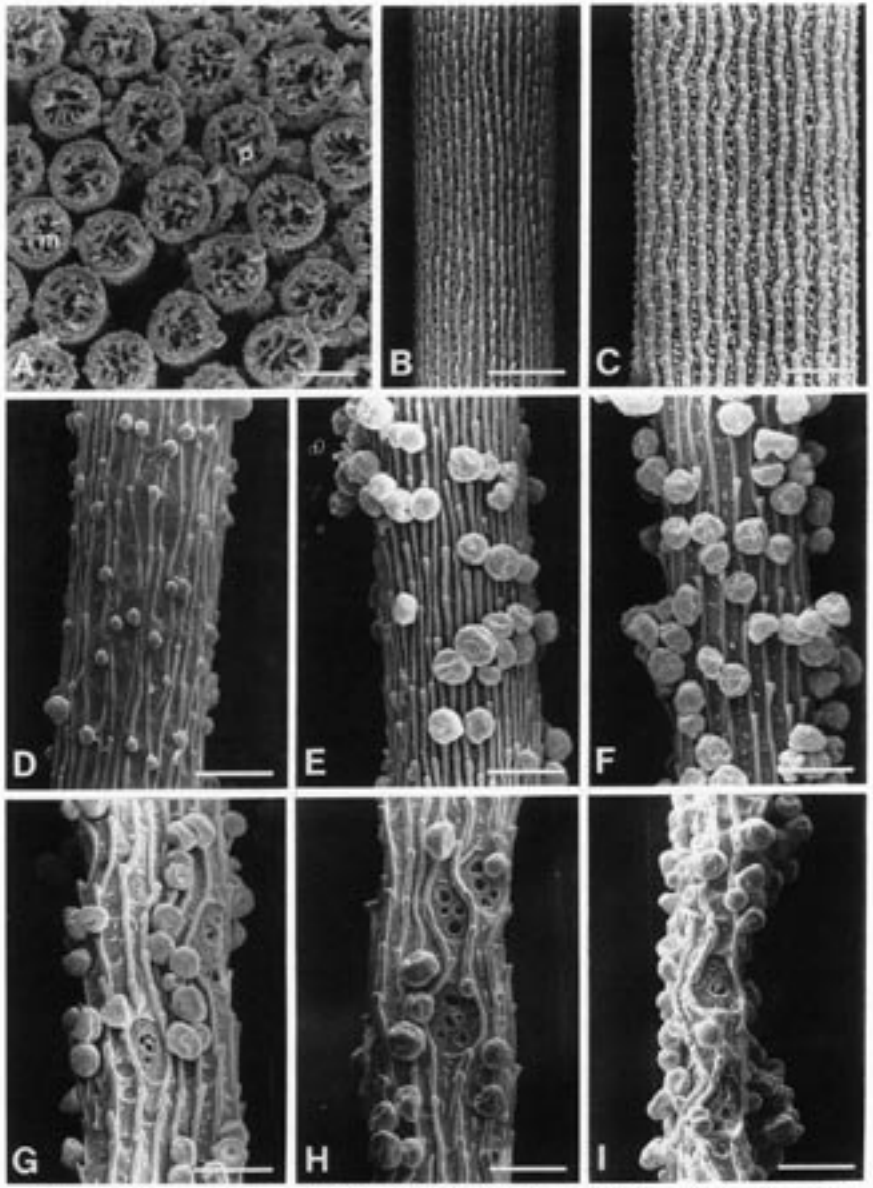


Figure 13. Microscopic characteristics of hairpencil hairs in *Danaus chrysippus*. A, Part of cross section of retracted hairpencil showing particle-budding hairs (p) and marginal hairs (m). B, C, Marginal hairs, basally (B) and apically (C). D–I, Particle-budding hairs at different levels, basally (D) and medially to apically (E–I). Scale bars: A: 10 μm; B–I: 5: μm.

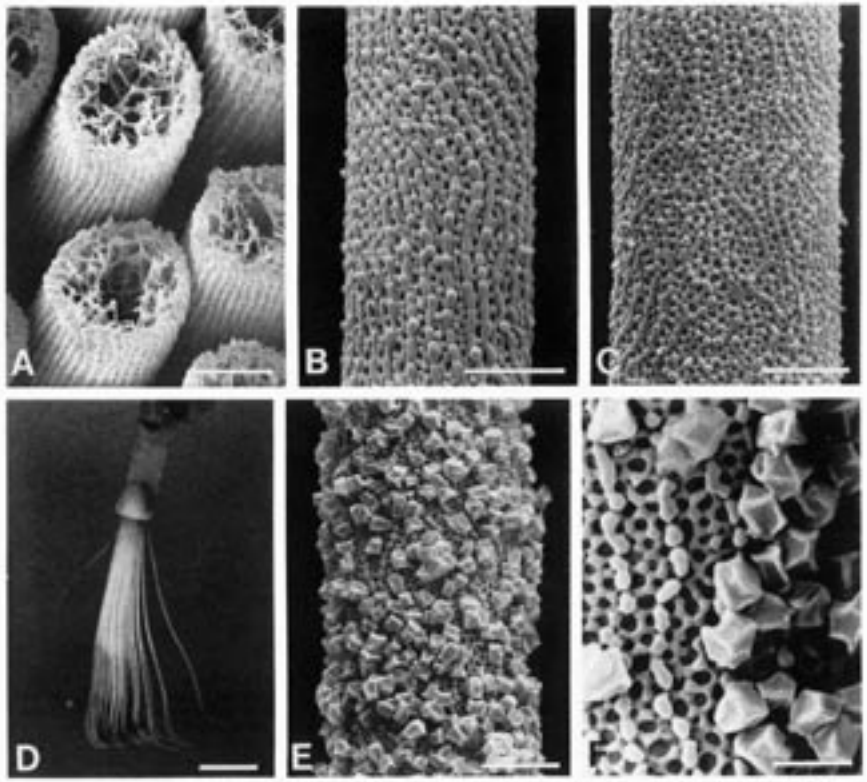


Figure 14. Microscopic characteristics of hairpencil hairs in *Tirumala petiverana*. Hairs of hairpencil of males not yet having established contacts with alar pouches (A–C) and after contact-behaviour (E–F). D, Macrograph of a protruded but not expanded hairpencil showing PTPs at tips of hair. Scale bars: A–C, E: 5 μm; D: 1 mm; F: 2 μm.

increasingly differentiated towards the distal end of the hair, forming ribbed nodules each with one or more raised, outwardly directed spines. Although these projections look as if they might be dehiscent, there are no PTPs in *E. core*, and there is no indication of the occurrence of PTPs in other species of *Euploea*.

DISCUSSION

The androconial organs of danaine butterflies have attracted attention since Müller (1877a) first suggested that hairpencils produced an odour which influences female sexual behaviour. Müller's paper was followed by numerous morphological studies and anecdotal reports on the use of these 'scent organs'. The present experimental phase commenced only recently, with the investigations of Brower *et al.* (1965) into the courtship of *Danaus* (*Anosia*) *gilippus berenice* (Cramer). Here we discuss the functional significance of these complex androconia, their major variations, and possible evolution.

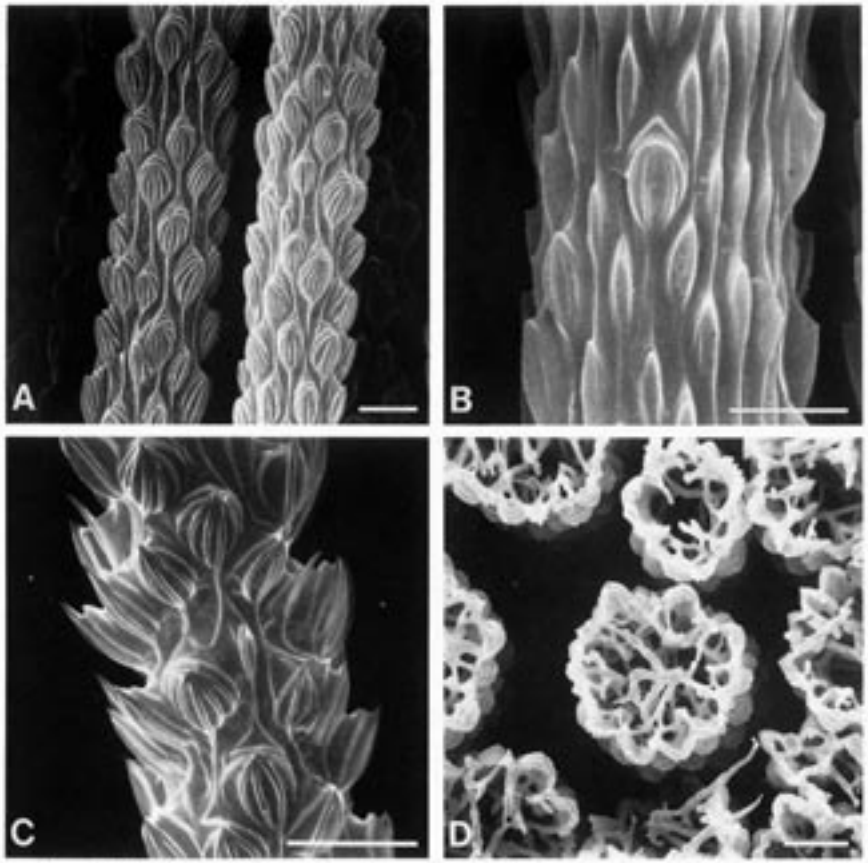


Figure 15. Microscopic characteristics of hairpencil hairs in *Euploea core amymone*. A, Basal, B, median and C, apical part. D, Cross-section. Scale bars: 5 μm.

Major features of danaine pheromone biology

The most characteristic feature of the danaine androconial system is the hydraulically eversible abdominal hairpencil. This organ is known to occur in all 158 currently recognized species of Danainae, with the possible exception of *Tiradelphe schneideri* Ackery & Vane-Wright (still known only from females). Although many groups of moths and some butterflies have coremata opening between abdominal segments 8 and 9, the general form of the danaine hairpencil together with its morphological and functional differentiation, is unique. No member of the co-ordinate sister-groups of the Danainae, that is the Ithomiinae and Tellervinae, has an abdominal hairpencil (Ackery & Vane-Wright, 1984; Ackery, 1987).

In addition to abdominal hairpencils, the majority of Danainae have alar organs, in the form of patches, pockets or pouches. The behaviourally mediated mechanical contacts between them and the hairpencils (at least in Danaini) represent an unusual feature. Within the butterflies, alar and internal abdominal

organs involving similar behaviour are only otherwise thought to occur in the morphine genus *Antirrhea* (Vane-Wright, 1972; DeVries, Kitching & Vane-Wright, 1985). Such pairs of androconial organs, developed on different parts of the body and requiring special behavioural activity to make functionally necessary mechanical contacts, are termed *binate organs*, and appear to be of particular interest. In contrast, pairs of organs which unavoidably make contact by virtue of their respective anatomical positions, we call *dual organs*.

In most Danaini the hairpencils disseminate pheromone-transfer-particles (PTPs). Scent-carrying particles are also found in various other Lepidoptera (Boppré, unpublished), but their occurrence (with few exceptions) throughout the Danaini, and particularly the different modes of particle production in this tribe, appears unique. Thus the male danaine androconial system exhibits several features (e.g. binate abdominal and alar organs, PTPs) which taken separately are not peculiar to this group, but their combination in the milkweed butterflies is outstanding and provides a model system for studies on chemical communication.

These morphological peculiarities, reviewed in detail by Ackery & Vane-Wright (1984) and Boppré (1984a), are reflected both ecologically and physiologically. The hairpencils emit complex species-specific bouquets of volatiles (Schulz, 1987; Schulz *et al.*, 1988; Schulz, Francke & Boppré, unpublished) but, with only few exceptions, dihydropyrrolizines (danaidone, danaidal, hydroxydanaidal) constitute the major pheromone components of all danaines. Dihydropyrrolizines cannot be synthesized by the males *de novo:* the adult males have to sequester pyrrolizidine alkaloids (PAs) which serve as precursors. PAs can be obtained by feeding on the nectar of certain plants, but the Danainae have developed peculiar adaptations to extract PAs from withered or damaged plant parts in a pharmacophagous manner (*cf.* Boppré, 1984b). The utilization by adult males of allelochemics as precursors for biosynthesis of the dihydropyrrolizines essential for courtship success is an ecological feature of the subfamily.

Functional peculiarities of androconial dualism

While the function of the alar organs in danaines is not fully understood, it seems certain that the principal function of the hairpencils is to stimulate the females with pheromones during courtship. As the binate organs are brought into contact independent of courtship in *Amauris* (Lamborn, Longstaff & Poulton, 1911; Lamborn, Dixey & Poulton, 1912; Lamborn & Poulton, 1913, 1918), *Parantica* (Lamborn, 1921), *Danaus* (Brower & Jones, 1965; Seibt, Schneider & Eisner, 1972; Boppré *et al.*, 1978) and in *Tirumala* (Boppré & Fecher, 1977), it is necessary to question both the separate significance of the alar and abdominal organs and the function served or mediated by contact-behaviour. Present knowledge suggests that the two types of organ have quite separate roles, but their functional significance is dependent on the interaction between them. This is not only indicated by the mere occurrence of contact-behaviour itself, but has been demonstrated by both chemical and morphological findings. For example, in *D. chrysippus* physiologically significant amounts of danaidone are only synthesized when hairpencils and pockets have been in contact (Boppré *et al.*, 1978); in *T. petiverana*, contacts are necessary (at least) to transfer PTPs from

the Danaini employ four different mechanisms of particle production, suggests that PTPs are an important feature in pheromone communication. To date, however, the adaptive significance of the use of PTPs remains dubious.

Origin and evolution of danaine androconial systems

The great similarity in gross morphology and fine structure of the abdominal organs found throughout the Danainae, and the alar organs of the Danaini, strongly suggests that the two glandular systems are respectively homologous. If so, both the abdominal and alar systems belong to the groundplan of the Danainae, and the structural differences of the androconial organs observed between species and genera have presumably developed, step-by-step, during the evolutionary radiation of the group. The hairpencils, at least, must be regarded as part of the danaine groundplan, and thus strictly homologous, as they constitute one of the two principal morphological specialisations characterizing the group as a monophyletic unit (the second feature is the ankylose, clubbed foretarsus of the female: Ackery & Vane-Wright, 1984). However, acceptance of this hypothesis of descent with modification poses many interesting problems concerning the origin of the danaine binate system and its subsequent phylogenesis. A thorough discussion would need to take all species into account. Here we present merely a sketch of the possible evolution of the androconia of the four genera treated in the body of this paper. However, although not discussed in detail, we have also taken into account our present knowledge of other danaine genera. We intend to present a more extensive account following further comparative investigations into danaine androconial organs.

Figure 16 reflects the mutual cladogenetic relationships of the four taxa dealt with in this paper, based on the cladistic classification proposed by Ackery & Vane-Wright (1984) for all eleven danaine genera (*cf.* Fig. 1). In order to discuss the anagenetic changes affecting the androconia of the four taxa, we have to consider three hypothetical ancestors within this schema: the ancestor of all Danainae, of all Danaini, and of *Danaus* + *Tirumala*.

The ancestral system

Bottle-brush hairpencils are unique to the Euploeina, occurring in all 75 or so species except a few *Idea* (Kitching, Vane-Wright & Ackery, 1987). The Itunina have the plumose type found throughout the Danaini. As the Itunina are either the sister-group of the Euploeina (Ackery & Vane-Wright, 1984), or represent the sister-group of the Danaini + Euploeina (Kitching, 1985; *cf.* Forbes, 1939), we conclude that the ancestral danaine probably had plumose hairpencils—and we speculate that they were composed of just one hairtype. We assume the latter condition even though a one-hair-type is not universal throughout the Euploeini.

With respect to the alar organs, only *Euploea* species amongst the Euploeini have wing androconia (on both fore and hind wings), whereas all Danaini have hindwing androconia. The Tellervinae and Ithomiinae include a few species with forewing androconia; the Ithomiinae also include a vast majority of taxa with hindwing alar organs. We could thus assume that the original danaine had scattered androconia on both fore- and hindwing upper surfaces. However, no identical androconial systems are found in the Ithomiinae *versus* the Tellervinae

number of important features, indicative not only of probable homology but also of functional similarity. In all cases, they are associated with veins, the wing tissue underlying the androconia being thickened, and they are all composed of cushions bearing cushion scales, together with similarly specialized covering scales.

The cushions of *A. ochlea*, *D. chrysippus* and *T. petiverana* differ in number and packing density but they do not show significant morphological differences. However, the cushion scales differ widely. In *T. petiverana*, in which they generate PTPs, they have an obvious function but the clubbed or minute cushion scales in *A. ochlea* and *D. chrysippus*, respectively, can have no function ascribed to them as yet; they might be functionless rudimentary structures—this is perhaps most likely for *D. chrysippus*, in which they are very small. In *A. ochlea* the cushion scales break off and become lost during contact-behaviour; they were not found on the black hairpencil hairs.

At first sight it might seem paradoxical that the cushions are covered by scales if we were to assume that they are the site of release for a secretion which has to come into contact with the hairpencils. However, the patch, pocket and pouch scales have similar, deeply ribbed upper and under sides and apparently lack the basal lamellae; such scales have not been described previously (Kühn, 1946; Kühn & An, 1946; Downey & Allyn, 1975; Ghiradella & Radigan, 1976). Scales having a similar lattice on both faces and lacking a basal lamella, we term *double-lattice scales*. This atypical structure would seem well-suited for the transfer of a secretion to the hairpencils. Double-lattice scales may thus serve as mechanical protectors for the fragile cushions, yet readily permit the transfer of secretion. These scales might also act as sponge-like reservoirs. Whether or not the presence of the 'amorphous substance' in the lumen (most readily seen in *D. chrysippus*) is indicative of such a reservoir function requires investigation. Double-lattice scales are apparently peculiar to androconial organs in the Danaini.

Although plausible adaptive significance for some of the microscopic features seems obvious, general questions about alar organs remain unresolved. The cushions are covered very incompletely by scales in *A. ochlea*, leaving the glandular area exposed, while in *D. chrysippus* this area is over-layed by scales and additionally protected by a fold. The answer to such puzzling differences might come from more detailed studies on the interaction of hairpencils and alar organs.

Structural adaptations of abdominal organs

Like the alar organs, the abdominal hairpencils in the various species indicate homology and functional similarity. The hairpencils of *Amauris ochlea* seem to represent the highest development of abdominal androconial organs in danaine butterflies. Behavioural studies on males of this species revealed that hairpencils are brought into play in two situations. First, early in the morning, males alight on herbage exposed to direct sunshine, and display their black hairs splayed out on the alar patches (see Boppré, 1977). Secondly, during courtship behaviour, the male hovers above the female and then zooms down, briefly expanding the hairpencils close to the female's head (Boppré, unpublished).

Studies in progress (Boppré *et al.*) reveal that the non-courtship display of hairpencils has an influence on the composition of volatile chemicals in the organ. During courtship the hairpencils emit stimuli necessary for the acceptance

of the male by the female. Petty *et al.* (1977) identified seven volatile substances (probably pheromone components) from *Amauris ochlea* hairpencils which exhibited a topochemical distribution which could be correlated to the morphological differentiation of the organ.

Chemical as well as behavioural information gives circumstantial evidence for the adaptive significance of the different hair types: the lateral position of black hairs and their insertion in the proximal part of the hairpencil tube permits the display of black hairs while the white hairs stay together as closed bundles; separating hairs between black and long white hairs could prevent loss of PTPs from the bundles of white hairs when the black hairs are rubbed on the patches; long white hairs serve a dual function by both producing and disseminating PTPs—which were found on female antennae after courtship by a male (*cf.* Fig. 25.4 in Boppré, 1984a); cone hairs show morphological structures which might prevent uncontrolled evaporation of the chemicals from central hairs; this assumption is supported by the finding that nonanal—a very volatile substance—occurs only on central hairs (Petty *et al.*, 1977). This substance might only be released during the final phase of courtship, after the male has previously stimulated the female with other pheromones.

In contrast with *Amauris*, the hairpencils of *Danaus* and *Tirumala* are more simple, but they are similarly used in establishing contacts with the alar glands as well as stimulating the females with PTPs. Thus, despite a number of detailed differences, they are used in the same context and serve the same function: stimulation of the female with pheromones by means of pheromone-transfer-particles.

In *Euploea*, the bottle-brush hairpencil might be interpreted as an adaptation to enlarge the scent evaporating surface. In this genus the hairpencils are expanded for long periods during patrolling and hovering flights, and PTPs are not employed.

Influences of pheromone-transfer-particles

Both structure and function of the androconial organs are affected by the mode of PTP origin. In the more complex hairpencils of *A. ochlea* and *D. chrysippus*, the separating hairs in the former and the marginal hairs of the latter may prevent loss of PTPs during contact behaviour. In *Tirumala*, PTPs are produced in the alar pouches, which may explain the absence of separating or marginal hairs in the uniform hairpencil of *T. petiverana*. With regard to function, those species that disseminate PTPs expand the hairpencils briefly and only during the final stage of courtship, whereas those without PTPs expand them for long periods during courtship flight. Most Danaini are PTP-disseminators. The Euploeini (and probably *Ideopsis* in the Danaini) lack PTPs. In *Euploea* itself, hairpencils are expanded even when there are no females around (see Boppré, 1984a). Futhermore, when handled, Euploeini expand their bright yellow hairpencils, which might be interpreted as defensive behaviour (*cf.* Ackery & Vane-Wright, 1984: 64). This reaction does not occur in the Danaini except in the genus *Tirumala*, in which PTP loss can be compensated by further contacts with the alar pouches, which function both as production sites and particle reservoirs.

PTPs are found in several groups of the Lepidoptera. This, and the fact that

the Danaini employ four different mechanisms of particle production, suggests that PTPs are an important feature in pheromone communication. To date, however, the adaptive significance of the use of PTPs remains dubious.

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The great similarity in gross morphology and fine structure of the abdominal organs found throughout the Danainae, and the alar organs of the Danaini, strongly suggests that the two glandular systems are respectively homologous. If so, both the abdominal and alar systems belong to the groundplan of the Danainae, and the structural differences of the androconial organs observed between species and genera have presumably developed, step-by-step, during the evolutionary radiation of the group. The hairpencils, at least, must be regarded as part of the danaine groundplan, and thus strictly homologous, as they constitute one of the two principal morphological specialisations characterizing the group as a monophyletic unit (the second feature is the ankylose, clubbed foretarsus of the female: Ackery & Vane-Wright, 1984). However, acceptance of this hypothesis of descent with modification poses many interesting problems concerning the origin of the danaine binate system and its subsequent phylogenesis. A thorough discussion would need to take all species into account. Here we present merely a sketch of the possible evolution of the androconia of the four genera treated in the body of this paper. However, although not discussed in detail, we have also taken into account our present knowledge of other danaine genera. We intend to present a more extensive account following further comparative investigations into danaine androconial organs.

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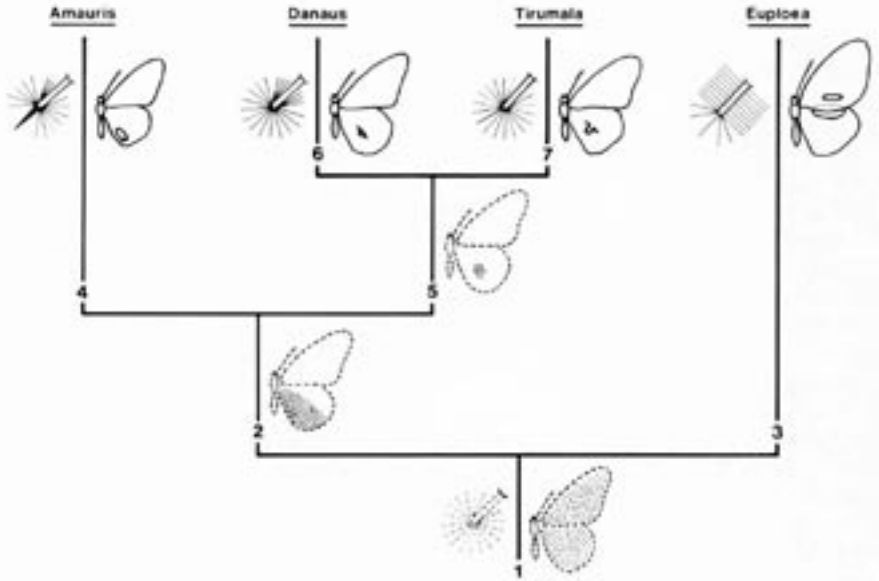

Figure 16. Schema summarizing the postulated cladogenetic relationships and anagenetic developments affecting the androconial systems of the four representative Danainae described in this paper. Dotted sketches represent hypothetical 'groundplan' states. 1: Postulated androconial complement (groundplan) of ancestral danaine: alar organs consisting of scattered scales on upper surfaces of both wings; hairpencils single-haired, plumose. 2: Advances on groundplan in the Danaini: alar organs restricted posterior to cubitus of hindwing, with glandular and double-lattice scales; binate relationship between alar organs and hairpencils; generation of PTPs. 3: Advances on danaine groundplan shown by *Euploea core amymone*: forewing androconia restricted to cell Cu_{1a}, with elaboration to produce dorsal/ventral organ and characteristic dual forewing/hindwing system; hindwing androconia restricted to anterior portion of wing; hairpencils of bottle-brush type. 4: Advances on Danaini groundplan shown by *Amauris ochlea*: hindwing androconia concentrated to discrete patch in cell 2A; hairpencils greatly elaborated to have five types of hairs, including cone, central and PTP-producing types. 5: Advance on Danaini groundplan shown by *Danaus* + *Tirumala*: hindwing androconia concentrated to (evaginated) region of cell Cu_{1a}. 6: Advance on *Danaus* + *Tirumala* condition shown by *Danaus chrysippus*: cubital organ formed as a shallow pocket; hairpencils elaborated to include PTP-producing hairs. 7: Advance on *Danaus* + *Tirumala* condition shown by *Tirumala petiverana*: cubital organ formed as a deep pouch, with cushion scales elaborated to produce PTPs.

versus the Danainae, and so there is little evidence that the various androconia are strictly homologous. As with many secondary features of the Lepidoptera, we are thus drawn into the quicksand of trying to argue and generalize about analogous rather than homologous structures (*cf.* Boppré, 1984a: 260). As a result, although we consider it plausible that the ancestral danaine did have some form of more or less scattered androconia on the upper surfaces of both wings, we do not speculate on their precise structure.

Androconia of the Danaini vs. *Euploeini*

All Danaini are considered to share two basic androconial features not found in the Euploeini: more-or-less concentrated alar organs restricted to the hindwings posterior to the cubitus, and, within these organs, cushions

interspersed with double-lattice scales. Most also exhibit elaborate contact behaviour between these hindwing alar organs and the hairpencils (i.e. they are binate), and most employ PTPs. Ackery & Vane-Wright (1984: 39) argued that the ability to produce PTPs was a specialization fundamental to the Danaini. However, we argue here that there is no compelling reason to consider the various modes of PTPs production throughout the group to be homologous, and it is plausible that the double-lattice alar organ/abdominal hairpencil binate interaction predates the production of PTPs. Perhaps, therefore, the modern Danaini represent not only a clade but also a grade, involving a 3-step evolutionary progression from the groundplan condition: first, the concentration and restriction of androconia to the posterior region of the hindwing; next the development of hairpencil/alar organ contact behaviour for biochemical transfer; and then the evolution of double-lattice scales to facilitate this transfer. At the same time or subsequently PTPs have evolved in a variety of ways.

In contrast to the Danaini, almost all *Euploea* have the hindwing androconia concentrated anterior to the cubital veins. In addition, some species have retained forewing androconia, concentrated in forewing cell Cu_{1b}, with the frequent concomitant development of the 'double-sided' alar organ, having connections between the dorsal and ventral surfaces, and so forming a dual organ with the hindwing androconia (often indicated by a posterior extension to the posterior margin of the forewing). All of these features occur in *E. core amymone*, and systematic evidence favours the idea that such a dual system is part of the *Euploea* groundplan—which therefore also represents a 3-step grade: first, concentration (rather than loss) of androconia to the anterior region of hindwing; followed by or together with concentration of the forewing androconia to cell Cu_{1b}; and then the formation of the dorsal/ventral alar organ of the forewing. (In this fashion we may conceive binate or dual androconial systems as potentially basic to all modern Danainae.) In addition, almost all Euploeina (the subtribe to which *Euploea* belongs) have the bottle-brush type of hairpencil, variously developed. The significance of this basic shift is not clear, but the bottle-brush may be a similarly efficient organ for pheromone dissemination as the plumose type + PTPs.

The androconia of Euploea, Amauris, Danaus *and* Tirumala

Amauris is differentiated beyond the basic condition of the Danaini by the concentration of the hindwing androconia to the area of cell 2A (shown also by *Ideopsis* and *Parantica*, which could therefore be considered an additional synapomorphy for the Amaurina as conceived by Ackery & Vane-Wright, 1984). Further, in *Amauris* there is great elaboration of the hairpencil, with up to five hair types occurring (as in *A. ochlea*), including the development of at least two different types of PTP-producing hairs in different *Amauris* lineages, and the central and cone hairs (in the group to which *A. ochlea* belongs).

Danaus + *Tirumala* differs, with respect to androconia, from the postulated groundplan for the Danaini only by having the hindwing androconia concentrated into cell Cu_{1b}, probably in an evaginated zone.

Danaus differs from the *Danaus* + *Tirumala* condition by the development of the 'pocketed' cubital organ, and the formation of PTPs in the hairpencil, through the multiple transverse fracture of a second type of hairpencil hair.

Tirumala differs from the *Danaus* + *Tirumala* condition by development of the deep 'pouched' cubital organ, and its unique ability to produce PTPs in the alar organs, through elaboration of highly differentiated cushion scales.

With respect to the extraordinary differences between the pheromone systems of *Tirumala* and *Danaus*, a question of outstanding importance is whether the lineage leading directly to *Tirumala* ever had hairpencil PTP-producing hairs or not. If it did, then we have a most extraordinary puzzle—why and how was one 'satisfactory' method of PTP production abandoned and then substituted by another? This problem disappears if we assume that PTP production has evolved separately at least three times within the Danaini (in the Amaurina, *Danaus* and *Tirumala*), but then the need to find a satisfactory explanation for such a set of close parallelisms becomes equally acute. The discovery of the male of *Tiradelphe schneideri* could be of very special importance in this regard since this species most plausibly represents either the sister-group of *Tirumala*, or of *Danaus* + *Tirumala*.

Ecological context of danaine pheromone biology

We have discussed the androconial system of Daninae only in the context of inter-sexual selection, although intra-sexual functions of male scents are being increasingly considered (*cf.* Boppré, 1984a). To date there is **no indication that danaine androconial secretions play a role in male-male interactions**, although one might imagine that the common occurrence of dihydropyrrolizines in all Danainae indicates some intra-sexual function(s) for these compounds, particularly since their synthesis requires so many adaptations. However, the use of androconial organs in intra-sexual communication amongst the Danaini is unlikely because, as already discussed, the pheromone-transfer-particles appear to dictate the use of hairpencils only in close-range, inter-sexual situations. In contrast, it would be worth investigating possible roles for pheromones in male-male interactions of *Euploea;* patrolling with expanded scent organs might serve to attract females but could also, for example, repel other males. Thus, intra-sexual (in addition to inter-sexual) functions of male pheromones remain a challenging field for further studies.

As outlined above and discussed in more detail by Boppré (1984a), allelochemics affect the courtship success of male danaine butterflies directly and significantly. In addition, the pyrrolizidine alkaloids are stored and afford chemical protection against predators. The speculation by Conner and Eisner (in Eisner, 1980; Conner *et al.*, 1981) that PA-derived pheromones may allow females to assess the alkaloid content of males and therefore their degree of protection, and Brown's (1984) finding that PAs can be transferred to the female during copulation via the spermatophore, shed some light on the so far puzzling phenomenon of a correlation between chemical communication and chemical defence (see also Boppré, 1986). However, the precise role of dihydro-pyrrolizines, used by so many species, produced in such high quantities and requiring so much 'costly' effort, is still not fully understood.

This survey taken together with behavioural and chemical data (*cf.* Ackery & Vane-Wright, 1984; Boppré, 1984a; Schulz *et al.*, unpublished), strongly suggests that the male pheromone system in Danainae was elaborated under high selection pressure. As previously hypothesized (Boppré 1978, 1984a; *cf.* Brower, 1963, 1970), the male androconial systems might have evolved as a consequence of the unpalatability of the Danainae and the appearance of Batesian mimics.

Chemical communication would have become necessary due to selective pressures by Batesian mimics upon a visual communication system in proto-danaines. The pheromone biology was apparently differentiated and elaborated morphologically as well as chemically during the development of advantageous Müllerian mimicry (*cf.* Boppré, 1978).

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