Visual and chemical signalling in butterflies: functional and phylogenetic perspectives

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[Plates 1 and 2]

SUMMARY

The two major signal functions for adult butterflies are protection against predators and intraspecific communication. Protective use of colour patterns (e.g. aposematism, crypsis) is mainly directed against visually hunting vertebrate predators. Mate location in butterflies is effected through behavioural adaptations of both sexes, and long-range visual searching by males. Recognition and selection of mates at close-range is largely controlled by species-specific male pheromone components, although other signals (e.g. colour, movement) play the initial role. Increased elaboration of pheromones for specific signalling has been hypothesized where the design of visual signals is constrained by defensive functions involving pattern convergence (co-mimicry). Male pheromones are also important for trait signalling, often being decisive in female mate choice.

The general context of visual and olfactory signals in butterfly communication is discussed using specific examples, including *Amauris*. Their low divergence in colour patterns contrasts with great differentiation in the chemistry of their scent-organ volatiles. Models for the evolution of chemical signalling systems in these mimetic butterflies are compared and assessed.

A more complete understanding of the evolution and function of butterfly signalling systems will require renewed effort in a number of areas. These include integrated studies on the use and significance of colours and scents, the measurement of colour, chemical analyses of pheromone bouquets, genetics, and receptor physiology. All of this work will need to be put in a better comparative framework through cladistic analysis of a wider variety of biologically contrasted butterfly groups, including previously studied cases lacking a rigorous phylogenetic perspective.

1. INTRODUCTION

Adult signalling in true butterflies (Papilionoidea), a group of about 14 500 species of day-flying or crepus-cular ditrysian Lepidoptera (Scoble 1992), involves all common signal modalities used by animals. However, only visual (including the ultraviolet range) and olfactory signals have been investigated to a signifi-cant extent (for reviews, see Silberglied (1984), and Boppré (1984)). Even so, our understanding of but-terfly signalling by vision and scent remains fragmen-tary.

Signalling by adult butterflies serves two major functions: protection and intraspecific communica-tion. Signal systems designed to give protection against predators include both high signal-to-noise ratio stratagems (e.g. warning coloration, false warn-ing coloration, deflection marks) and low signal-to-noise ratio stratagems (crypsis, disruptive patterns). Requirements for intraspecific communication include not only aggregation behaviour (both protective and sexual), mate location, pre-mating recognition (or isolation), mate acceptance, and mate refusal, but also intrasexual competition. In general, visual and chemi-cal signals form integrated systems. Understanding the physiological, ecological and historical significance of these factors provides an outstanding challenge for comparative biology.

2. MATE LOCATION AND MATE RECOGNITION VERSUS PROTECTIVE SIGNALLING

In butterflies mate location is largely mediated at long range by visual cues (Magnus 1958; Silberglied 1984; Rutowski 1991; Scoble 1992), involving the response of males to a variety of moving or settled objects. Because of their apparently weak specificity, visual cues must be placed in a broader context, whereby sexually active individuals adopt typical searching behaviours, such as patrolling, perching, stream-following or hill-topping by males (Scott 1974), or flying in appropriate areas by females, thereby bring-ing conspecifics into proximity (cf. Rutowski 1991). Behavioural displays (e.g. fluttering, hovering) must also be considered as visual signals or releasers. Long-

range protective signalling in butterflies (and many day-flying moths), primarily used to deter vertebrates (Rothschild 1985), is largely visual too. This potential conflict over the use of visual signals has apparently resulted, at least in some cases, in sex-limitation of colour patterns employed for different functions (Vane-Wright 1975; Turner 1978).

Mate location in butterflies differs starkly from the system seen in moths. For the moths, which mostly fly at night, visual cues are generally ineffective, except for optomotor responses. Typical for moths is the release of female sex-attractant pheromones to assemble males from long distance. Our present understanding of phylogenetic relationships within the Lepidopters (see review by Scoble (1992)) favours the view that the ancestral mate-location strategy of the true butter-flies did not rely on pheromone assembly, but was almost certainly visual, as in most butterfly species today. Whether or not this has come about by historical accident or by some yet unperceived need or limitation affecting the Papilionoidea, this singular fact has had a number of consequences for butterfly biology (Silberglied 1984), such as the frequent occur-rence of female-limited pattern polymorphism (Vane-Wright 1981), or the elaboration of male pheromone systems (see below).

As a result of selection for Müllerian or Batesian mimicry, many butterflies share the same conspicuous patterns and, to a greater or lesser degree, the same general behaviour and even microhabitat (e.g. Papa-georgis 1975). As this selection for pattern conformity affects the long-range visual system used for specific communication, many warningly coloured butterflies appear to have a problem of distinguishing them-selves, one from another, by sight alone. The extent to which males waste time chasing inappropriate objects is unknown, but heterospecific pursuits are often observed. Brower (1963; cf. Boppré 1978, 1984) suggested that species belonging to mimicry complexes should be characterized by elaboration of chemical means for specific communication, and noted that the males of many aposematic butterflies have complex exocrine glandular systems (androconial organs). As pointed out by Boppré (1978, 1984), closely related species in Müllerian mimicry rings, because of their common behavioural history, possibly face even greater problems than unrelated Müllerian mimics or those involvesd in Batesian mimicry; this would apply, *a fortiori*, to groups of closely related co-mimics such as members of a single genus, which will all tend to have very similar courtship behaviour.

Because male pheromones usually only come into play in a late phase of courtship, elaboration of male scent signals is not an alternative to mate location by visual means but an addition. Furthermore, it is now accepted that male scents can serve functions other than species recognition (see below). Thus the general elaboration of male pheromonal systems seen in Müllerian co-mimics recurs in cryptic or otherwise non-mimetic species of butterflies, as well as in other ditrysian Lepidoptera (Boppré 1984; Birch *et al.* 1990; Phclan 1992), and appears to represent a response to problems other than visual mimicry alone (Vane-

Wright 1972). Nevertheless, where the design of visual signals is constrained by protective functions involving convergence or non-divergence of colour patterns (e.g. mimicry or crypsis), increased dependence on male pheromones for species recognition seems a reasonable expectation largely borne out by circumstantial evi-dence. But is there any evidence that colour patterns, by themselves, can play a role in specific communica-tion and speciation among butterflies, where mimicry may or may not be a constraint?

3. SPECIES RECOGNITION

Many non-mimetic butterflies have bright patterns affecting one or both sexes. Anecdotal evidence sug-gests that butterflies can respond to differences in colour of other butterflies. Regrettably, the precise properties and functions of visual signals have rarely been investigated.

Magnus (e.g. 1958) carried out a now classical series of behavioural experiments on the response of male *Argynnis paphia* (Nymphalidae: silver-washed fritillary; plate 1, figures 9–11) to a variety of visual stimuli, clearly demonstrating, despite varying sensiti-vity to different colours, that a wide range of colours and patterns can elicit the first phase of courtship. Visual long-range orientation of the male butterflies only serves to bring the potential mates together. At close range, female pheromones come into play and are necessary to initiate male courtship behaviour (probably a rare exception in butterflies). Receptive *Argynnis* females release a pheromone (cf. Magnus 1958; Treusch 1967), perception of which makes males no longer responsive to colour (M. Boppré, unpublished observations). This temporal sequence involves not only different signal modalities but also a complete switch between them. Furthermore, although receptive females react to approaching males by directing their abdominal glands towards them (Treusch 1967), apparently in a visually oriented response, this behaviour might be strongly influenced by perception of male forewing pheromones.

The late Bob Silberglied and his collaborators examined the role of colour and pheromone differ-ences in the semi-species *C. eurytheme* and *C. philodice* (Pieridae: Coliadinae: clouded yellow, or alfalfa but-terflies; plate 1, figures 22–27). As a result of suitable legume hostplants being spread by agriculture, these two formerly allopatric species now occur together throughout much of the U.S.A. (Silberglied & Taylor 1973). They readily produce F_1 hybrids when at high population density, but nonetheless remain distinct.

Males use only visual cues to locate females (hidden females are not detected, even at close range), and can be attracted to paper dummies, especially those contrived to be similar to the yellowish-green of the female underside. However, otherwise attractive dum-mies additionally made uv-reflectant are repellent to males, rather than attractive. The dorsal wing surfaces of male *eurytheme* are brilliantly uv-reflectant, in contrast to male *philodice* and the females of both species, which do not reflect uv (plate 1). As a result, wing display by *eurytheme* males inhibits approach by
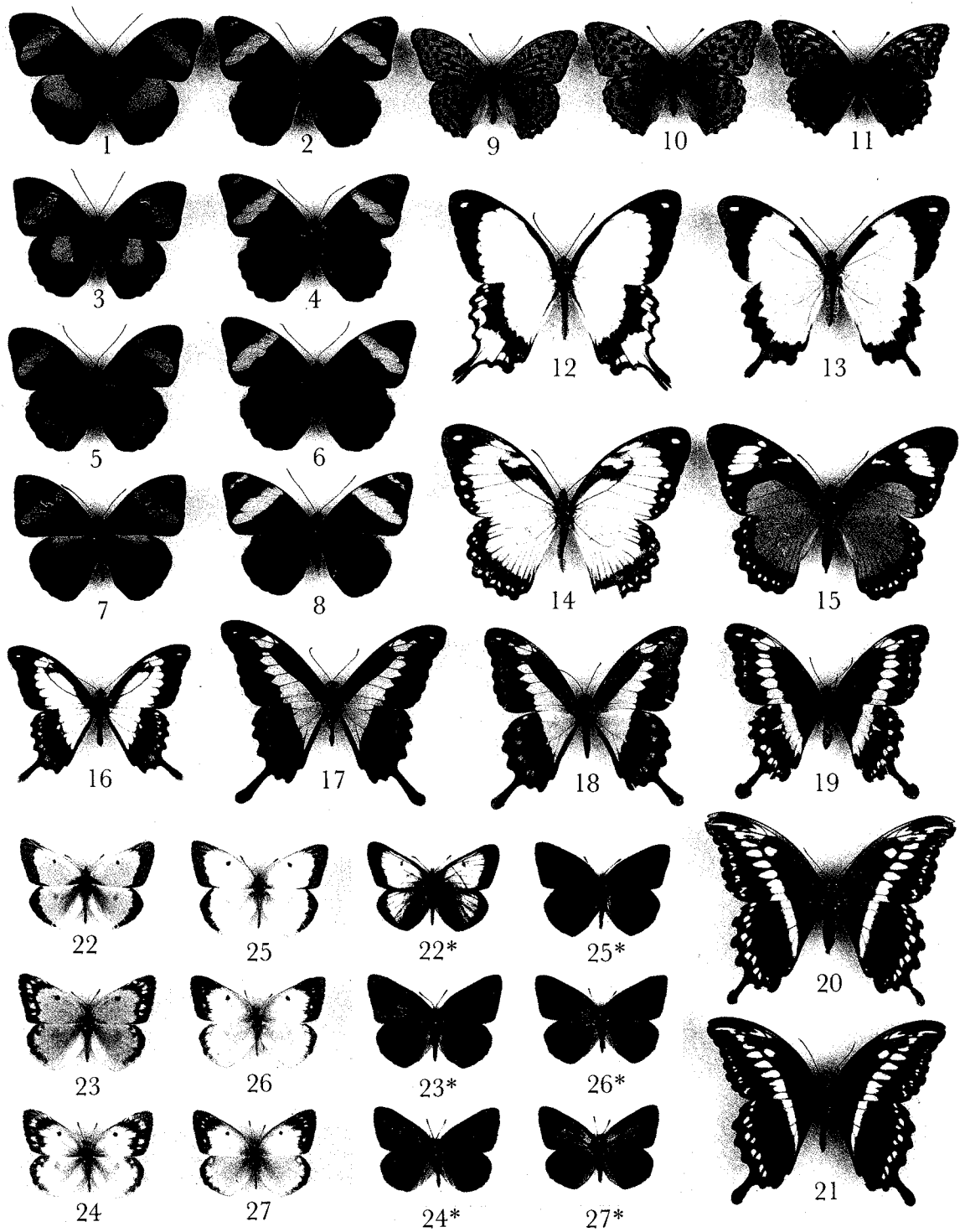
Plate 1. Figures 1–8: *Nessaea* species, (1) *N. obrina* male, (2) *obrina* female, (3) *N. batesii* male, (4) *batesii* female, (5) *N. hewitsoni* male, (6) *hewitsoni* female, (7) *N. aglaura* male, (8) *aglaura* female. Figures 9–11: *Argynnis paphia*, (9) *paphia* male, (10) *paphia* male-like female, (11) *paphia* 'valesina' female. Figures 12–21: *Papilio* species, (12) *P. dardanus* male, (13) *dardanus* male-like female from Comoro Islands, (14) *dardanus* tail-less male-like female from Pemba, (15) *dardanus* 'Danaus chrysippus-like' female, (16) natural male hybrid of *P. dardanus* × *P. phorcas*, (17) *P. phorcas* male, (18) *phorcas* male-like female, (19) *phorcas* 'constantinus-like' female, (20) *P. constantinus* female, (21) *constantinus* male. Figures 22–27: *Colias* species in visible light, (22) *C. eurytheme* male, (23) *eurytheme* male-like female, (24) *eurytheme* 'alba' female, (25) *C. philodice* male, (26) *philodice* male-like female, (27) *philodice* 'alba' female. Figures 22*–27*: ultraviolet reflectance images of the corresponding *Colias* specimens (22–27). 0.44 × natural size.
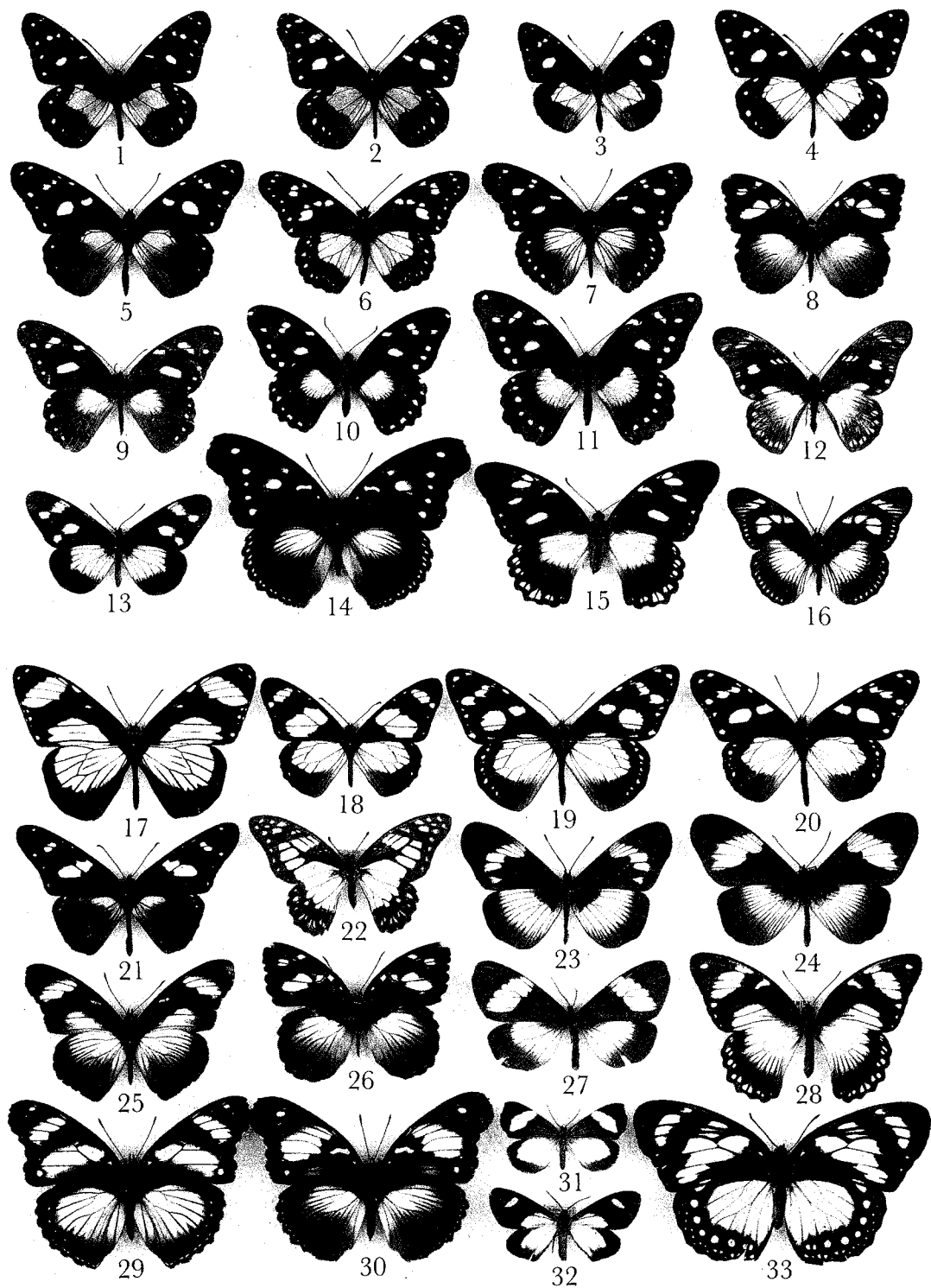
Plate 2. Figures 1–16: members of the putative *Amauris echeria* mimicry complex from eastern Africa, (1) *Amauris echeria*, (2) *A. ellioti*, (3) *A. albimaculata*, (4) *A. crawshayi*, (5) *A. inferna*, (6) *Pseudacraea deludens* [Nymphalidae], (7) *Hypolimnas anthedon* f. 'mima' [Nymphalidae], (8) *Aterica galene* f. 'dimorpha' [Nymphalidae], (9) *Papilio zoroastres* [Papilionidae], (10) *P. echerioides*, (11) *P. jacksoni*, (12) *Graphium almansor* [Papilionidae], (13) *Acraea johnstoni* [Nymphalidae], (14) *Hypolimnas dinarcha*, (15) *Papilio dardanus* f. 'cenea', (16), *Pseudacraea lucretia*. Figures 17–33: members of the putative *Amauris niavius* mimicry complex from eastern Africa, (17) *Amauris damocles*, (18) *A. ochlea*, (19) *A. tartarea*, (20) *A. dannfelti*, (21) *A. hecate*, (22) *Graphium philonoe*, (23) *Acraea aganice*, (24) *A. macarista*, (25) *Hypolimnas anthedon* f. 'anthedon', (26) *Aterica galene* f. 'albimacula', (27) *Nyctemera acraeina* [Arctiidae], (28) *Papilio dardanus* f. 'hippocoonides', (29) *Hypolimnas deceptor*, (30) *H. bartelotti*, (31) *Nyctemera leuconoe*, (32) *Pitthea famula* [Geometridae], (33) *Euxanthe wakefieldi* [Nymphalidae]. 0.44 × natural size.

other males of both species (Silberglied & Taylor 1978).

Judged by their behavioural responses, females are indifferent to the visible colour distinction between males (*eurytheme* are normally orange, *philodice* yellow). For *eurytheme* females, however, UV reflection is an integral part of the normal mate-recognition signal system, whereas *philodice* females seem unaffected by male colour altogether. Acceptance of *philodice* males by their females depends on the presence of a pheromone bouquet including three *n*-hexylesters (Silberglied & Taylor 1978; Grula & Taylor 1979). Male pheromones also form part of the signal system for *eurytheme*, acceptance by females depending on a male bouquet including variable quantities of two or perhaps three behaviourally relevant volatile hydrocarbons (Sappington & Taylor 1990).

4. COLOURS AND SCENTS IN SPECIATION

In this section, three examples of extremely closely related butterfly species (belonging to *Colias, Nessaea, Papilio*) are discussed, each involving a pair of species that co-exist in a broad area of sympatry, but also occur separately, outside the area of overlap.

In *Colias* we have seen that male *eurytheme* and *philodice* use their different UV reflectance and pheromone components for intra- and even interspecific signalling. Speciation, however, is a process involving a sequence of transformations made through time. A comparison of two species in isolation, even if they are sister-taxa, can never be sufficient to resolve many of the key issues involved because the direction of the transformational sequence cannot be inferred. As no phylogenetic scheme is currently available for *Colias*, we are unable to place the evolution of their particular signal differences (UV reflectance, pheromones, behaviour) in a rigorous comparative context. For example, we would wish to know if the differences observed in the two *Colias* evolved in sympatry by reinforcement, or in allopatry. In the latter, did they evolve intrinsically, without influence by other coliadinine butterflies, or do they represent changes brought about by direct selection during previous speciation events? Unfortunately, where we do have some understanding of phylogenetic relationships, as in the next two cases, we have little or no experimental knowledge of the relevant communication mechanisms.

Nessaea is a colourful (but not mimetic) neotropical nymphalid genus, currently including four species (Jenkins 1989; plate 1, figures 1–8). The blue-banded females are almost identical in appearance, but the males differ in the presence and extent of orange and blue bands on the hindwing upperside (Vane-Wright 1979*a*). In particular, the partly sympatric sister-species pair *N. obrina* (= *ancaea*) and *N. hewitsoni* differ sharply: *obrina* has a bright orange hindwing discal band, while *hewitsoni* has a bright blue submarginal band instead.

By analogy with *Colias*, we might expect the unique bright blue pigmentary coloration of all *Nessaea* butterflies to be a strong visual attractant for males.

For female *obrina*, male orange might be a specific releaser for courtship to proceed, whereas for female *hewitsoni* the presence of orange might be expected to evoke mate refusal. A putative *obrina* × *hewitsoni* male hybrid, with both orange and blue hindwing markings, was recorded by Vane-Wright (1979*a*). On the present hypothesis, such an orange-marked hybrid would be unacceptable to *hewitsoni* females (although it might be acceptable, by the same token, to *obrina*). In the lower Amazon, where *hewitsoni* does not occur, *obrina* is sympatric with *N. batesii*, a very similar orange-marked species considered by Vane-Wright (1979*a*) to be the sister of (*hewitsoni* + *obrina*). Although *Nessaea*, like *Colias*, lacks complex androconial organs, we anticipate that male pheromones will prove to play a key role in the specific communication of these butterflies also.

Three widely distributed African swallowtails, *Papilio dardanus, P. phorcas* and *P. constantinus* (plate 1, figures 12–21), comprise a monophyletic group, with *dardanus* and *phorcas* as sister-species (Vane-Wright & Smith 1991). The males of these two polytypic species differ starkly in appearance throughout their ranges, male *dardanus* being largely creamish yellow with a restricted black pattern, while *phorcas* males are black with a wide apple-green discal band crossing both wings.

This system is of exceptional interest, particularly the evolution of polymorphic Batesian mimicry and its genetic control, *Papilio dardanus* presenting an outstanding example of this phenomenon (Sheppard 1975; Clarke *et al.* 1985). Here we simply wish to concentrate on the observation that, in a speciation event leading to a sex-limited mimetic species (*dardanus*) and a non-mimetic species (*phorcas*), extraordinary divergence in male coloration appears to have occurred from the putative ancestral condition (similar to the yellow, narrow-banded *constantinus*). In the laboratory, hybrids between all three species can be obtained (Clarke *et al.* 1991), but in nature such hybrids appear to be very rare, and have only actually been found for *dardanus* × *phorcas* (Vane-Wright & Smith 1992).

In a peripheral isolate of *Papilio dardanus* from Pemba island (where neither *phorcas* nor *constantinus* occur), Cook *et al.* (1993) have demonstrated that male *dardanus* differentiate between female morphs (cf. Magnus 1958, on *Argynnis*). Males are highly attentive to the common black and white '*hippocoonides*' mimetic form, but tend to be repelled by yellow, male-like '*trimeni*' females. In this population the distinctive male-like '*trimeni*' coloration appears to be useful, however, as a mate-refusal signal, reducing harassment of impregnated females by actively courting males. Unfortunately, no investigations have yet been undertaken into communication amongst the three species where they are all sympatric (in various parts of mainland east Africa).

Although disparate at first sight, there are some striking similarities between the *Colias, Nessaea* and *Papilio* examples. Semi- or sister-species show marked autapomorphic colour differences particularly affecting one member of each pair (UV absorption in *C.*

philodice, suppression of orange in *N. hewitsoni*, production of unique green coloration in *P. phorcas*). Although all three species pairs lack complex androconia, the significance of pheromones has been demonstrated for *Colias*, and they seem likely to be important for *Nessaea*; in the *Papilio* example, it is probably significant that the sister taxon *constantinus* has obvious androconia, whereas *phorcas* and *dardanus* do not. Finally, we note that male hybrids of *dardanus × phorcas*, although somewhat intermediate in appearance, fail to produce the bright apple green colour unique to *phorcas*. If the green is a specific releaser for *phorcas* females, then isolation of *phorcas* from *dardanus* may be assured, but the reverse may not (cf. discussion of the supposed *Nessaea* hybrid, above). Interestingly, there is a suggestion that, where the two *Papilio* sister-species overlap, some introgression may occur (Clarke *et al.* 1991; Vane-Wright & Smith 1992), but the 'cohesion' (Templeton 1989) of both entities as separate species seems beyond doubt.

These examples do not appear to be atypical. Unfortunately, few rigorous cladistic analyses as yet exist for the many butterfly genera which include sympatric species showing marked differences in male coloration.

5. MALE PHEROMONES IN SPECIATION OF MÜLLERIAN CO-MIMICS

From what we have seen so far, it would appear that specific communication in butterflies normally involves an integrated sequence of signals operating in at least two modes: usually sight and scent, in addition to gross behaviour patterns. In many cases tactile, contact-chemoreception or audio signals can also be involved at some stage.

Due to the species-specificity of female sex-attraction, male pheromones in moths need not exhibit species-specificity. If, as already suggested, pattern convergence for mimicry or crypsis constrains the use of colour, butterflies may of necessity place greater or even total reliance on male olfactory signals for specific communication. The aposematic Afrotropical milkweed butterfly genus *Amauris* provides a potentially outstanding example of the elaboration of pheromonal communication amongst a closely related group of Müllerian co-mimics.

Amauris butterflies are primary models for several classical mimicry complexes. Two principal pattern types occur within the 12 *Amauris* found on the African mainland. One is black with large white discal areas on both wings (the *niavius* pattern; plate 2, figures 17–33), the other is black with a buff or yellow hindwing discal area and small white or yellow forewing spots (the *echeria* pattern; plate 2, figures 1–16). In Kenya, a dozen or so species of Lepidoptera of different families (including day-active moths) share the *naivius* pattern, while a similar number look like *echeria*. Two of the best known polymorphic mimics, *Papilio dardanus* and *Hypolimnas anthedon* (= *dubius*), have morphs corresponding to both of these principal *Amauris* patterns.

Low divergence in *Amauris* colour patterns contrasts

with marked differentiation in androconial morphology (Ackery & Vane-Wright 1984; Boppré & Vane-Wright 1989), and particularly with the wide array of volatiles associated with the abdominal hairpencils (Schulz *et al.* 1988, 1993). In the six Kenyan *Amauris* analysed, a total of 138 volatiles have been separated (and mostly identified) from extracts of male hairpencils. The complex bouquets exhibit specificity, not only at species level but also at higher taxonomic level, permitting replication (with one exception) of the previously accepted phylogenetic relationships of a total of seven *Amauris* species by cladistic analysis of the pheromone gland chemicals alone (Vane-Wright *et al.* 1992). In combination with a morphological data set, an improved standard tree was obtained. Based on this analysis, a number of alternative models for the evolution of specific male scent organ volatiles in these butterflies can be considered.

Perhaps the simplest and most unambiguous chemical signalling system would be the production, by each species, of one or more unique signal substances not found in any other members of the group. Such an extreme pattern of specificity would have the result that cladistic analysis of the chemical data would fail to recover any hierarchical structure. Hierarchical structure would result if, at each speciation event, new chemicals were added to one daughter lineage without concomitant loss of older pheromone components. At this extreme, only certain terminal species in each lineage would be found to have unique compounds. Other than in these species, recognition would be based on unique bouquets, the more recent species having more and more complex bouquets or aromas. An alternative extreme would be successive simplification of an originally complex signal, such that only the oldest or stem species in any clade possessed unique signal substances, more recent species having simpler and simpler bouquets.

In reality, all three of these processes may have been involved in the evolution of the complex *Amauris* scent bouquets (Schulz *et al.* 1993). All species examined produce unique compounds, varying from three unique substances each in *A. tartarea*, *A. albimaculata* and *A. ochlea*, to as many as 19 in *A. niavius* and even 21 in *A. echeria*. We as yet lack data for the other five African mainland species, and we have no evidence that any of these substances is behaviourally relevant. None the less, the possibility clearly exists that each species could have its own unique chemical 'badge' or 'logo'. Equally, because evidence of phylogenetic relationships seems so well preserved in the 'pheromone record', we also have to consider the additive or subtractive models. Given that there is no clearly increasing or decreasing trend in the total number of compounds (ranging from 32 to 59: Schulz *et al.* 1993) with hierarchical level of the species represented (line figure 1*h*), we conclude that additions may often be conserved across successive speciation events and that, equally, a roughly corresponding number of losses also occur.

We can model this balanced additive and subtractive scheme with a simple type of matrix (line figure 1*a*). Each speciation event adds a new signal substance

(a)

zero	1000000
one	1100000
two	1110000
three	0111000
four	0011100
five	0001110
six	0000111
seven	0000011

(b)

(c)

outgroup	10000000
tartarea	11000001
niavius	11100001
echeria	01110000
hecate	00111000
albimaculata	00001100
damocles	00000110
ochlea	00000110

(d)

(e)

zero	1000000
one	1100000
two	0110000
three	0011000
four	0001100
five	0000110
six	0000011
seven	0000001

(f)

(g)

zero	10000
one	11000
two	01000
three	01100
four	00100
five	00110
six	00010
seven	00011

(h)

tartarea 32 (3)
niavius 48 (19)
echeria 52 (21)
hecate 43 (18)
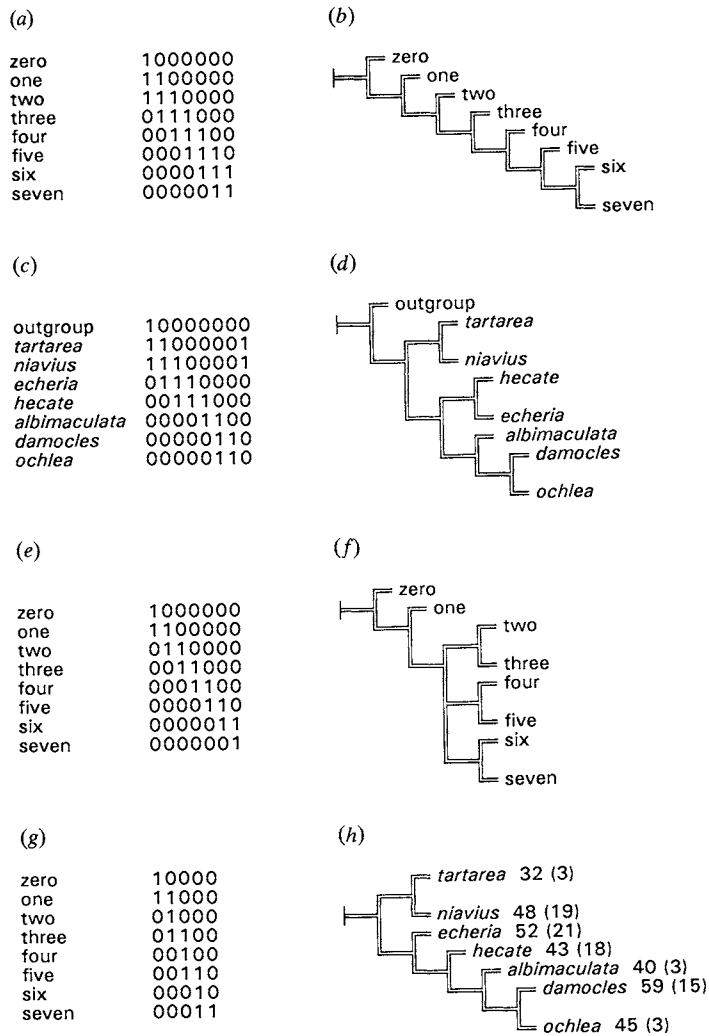albimaculata 40 (3)
damocles 59 (15)
ochlea 45 (3)

Figure 1. (a) Matrix for three-step turnover model of pheromone bouquet accumulation and decay in successive speciation events, from species 'zero' to 'one' through 'seven'; (b) maximum parsimony cladogram derived from the seven characters and eight species in (a), with 'zero' specified as the outgroup; (c) model for seven *Amauris* species based on (a), modified and labelled (including one extra character), to produce cladogram (d); (d) topology for *Amauris* modelled by matrix (c), corresponding to the result from analysis of real pheromone bouquet data (Vane-Wright *et al.* 1992; figure 5); (e) matrix for two-step variant of (a); (f) maximum parsimony cladogram derived from (e); (g) matrix for minimum-step variant of (e), which generates a maximum parsimony cladogram corresponding to (b), not (f); (h) standard cladogram for *Amauris* species in (d) annotated to show total number of pheromone bouquet volatiles, with number of unique substances in parentheses, for each species (data from Schulz *et al.* (1993)). In addition, see text.

(notated as '1' in each character column) to the terminal lineage. In turn, each substance is subsequently lost after the third speciation event. If the first species (with only a single signal substance) is specified as the functional outgroup, then the most parsimonious cladogram for such a matrix successfully specifies the phylogenetic sequence implicit in the model (line figure 1b). Simple manipulation of this matrix (line figure 1c), labelled here to represent the seven African *Amauris*, generates the topology (line figure 1d) actually found by cladistic analysis of their scent-organ volatiles (Vane-Wright *et al.* 1992).

Note in this model how *A. hecate* is removed from its presumed correct association with the *A. ochlea* group by increased rate of signal loss at two subsequent speciation events. If we make a more extreme model based on such a two-step process (line figure 1e), then we are unable to recover the chained topology; depending on the number of terminals in the analysis, we either get a nearly symmetrical cladogram (line figure 1f) or a totally unresolved 'bush'. However, if we refine this model to permit only minimum step changes (a single addition or loss at each step, not both at once: Vane-Wright 1979b), cladistic analysis of this type of matrix (line figure 1g) again gives the fully chained topology (line figure 1b) as the most parsimonious interpretation.

Exploration of such models may help us to consider how changes in patterns of gain and loss affect our ability to reconstruct phylogenies from scent data alone. More significantly, these models may also help us assess or predict patterns of inheritance, and consider the physiological problems faced by organisms as they respond to new signals, or switch off their

responses to the old. With respect to *Amauris*, until physiological and ecological assays are devised to test the biological significance of the various findings, we cannot say much more about our results at present. They do, however, set some limits to the 'signalling environment' (Otte 1989) in which we presume the evolution of communication within whole communities of these chemically protected butterflies has occurred.

6. MATE RECOGNITION VERSUS MATE SELECTION SIGNALS

Species recognition is desirable for both sexes, to avoid grossly maladaptive heterospecific matings. However, signals used in sexual communication of butterflies have to fulfil a variety of additional requirements, partly different for the sexes because of their basically different interests. For selection of mate quality, females are expected to recognize not only (inheritable) traits but also the social status of males. Intrasexual communication of males (e.g. territorial or agonistic interactions) can also be important in limiting the male choice available to females. Unfortunately, such eco-ethological aspects have received only minor attention in research on lepidopteran pheromones, even in the well-investigated lures of female moths (cf. Boppré 1991).

Quality and quantity of chemicals appear to ensure that pheromone bouquets can provide relatively accurate and reliable transmission of information, able to change during the adult life of a butterfly. Chemicals may convey information not only on species and sex, but also on age and mating status. Visual signals appear less differentiated, fixed through adult life, unless fading or darkening of pigments occurs. However, different behavioural displays have the potential to alter or control the information encoded in a visual sign. Even though individual signal components of such a system may be cross-reactive (non-specific), in combination the integrated system will usually carry an unambiguous message. Considering the evolution of multi-functional signals then poses basic problems of determining primary and secondary functions, and identifying the selective pressures involved. For example, in the seemingly clear case of *Colias* (above) male scents mediate species recognition but the pheromones involved might originally have been trait signals. Similar considerations might apply to visual signals in two species of *Pieris*: Wiernasz (1989) found evidence for female choice affecting forewing colour pattern elements in male *P. occidentalis*, but went on to suggest that the same pattern components may be part of the mate recognition system whereby *occidentalis* females avoid mating with the closely related *P. protodice*.

(a) Mimicry, species recognition and individuality: milkweed butterflies as a paradigm

The milkweed butterflies (Danainae; cf. Ackery & Vane-Wright (1984) for a general account) exemplify many aspects of butterfly signalling by the sheer

multiplicity of factors involved in their visual versus chemical communication, including a direct linkage between chemical protection and chemical communication on the one hand, and mimicry (Batesian and Müllerian) on the other. The complexity of sexual communication in danaines (see Boppré (1984, 1986, 1990) for details and references; cf. Eisner & Meinwald (1987) and Schneider (1987)) is not typical for butterflies, but many of the elements can be recognized in other butterflies, and in various moths, and this facilitates an evolutionary approach to understanding signals and their functions.

With respect to danaine pheromone biology, in addition to species-specific male secretions (as discussed in detail for *Amauris*, above), dihydropyrrolizines occur as common hairpencil components. Their biosynthesis is dependent on pyrrolizidine alkaloids (PAs) which the adult males have to gather actively, usually from decaying parts of certain plants and independent of nutrient uptake. Pharmacophagy with respect to PAs results in the occurrence of dihydropyrrolizines in varying amounts (up to 500 μg per male). In addition to serving as precursors for the biosynthesis of pheromone components, PAs are stored and act as the principal known defence substances of the butterflies (although supplemented by cardenolides from larval hostplants in species of *Danaus*), thus being responsible for the occurrence of mimicry. During mating, males transfer to the females large amounts of previously accumulated PAs in their spermatophores. It is very plausible to assume that dihydropyrrolizines serve as signals to tell the female how much nuptual gift (in the form of defensive chemicals) she can expect to receive from a prospective mate.

Thus, Danainae give some insight into the costs that signals may entail, and at the same time they provide examples of sequential synergistic visual and chemical signalling, as well as of dual roles for chemical signals: species and trait recognition. The dependence on plants for positive chemicals and pheromone precursors implies temporal sexual dimorphism (females are auto-mimics until they have mated) and great variation as well as dynamic intra-individual differences in defensive potency among males and mated females. In parallel, there is individuality in male courtship signals.

It is tempting to speculate that the sequence of evolution of intraspecific signalling in danaines began with the utilization of PAs from plant sources, leading to the use of dihydropyrrolizines for trait signalling. Subsequent evolution of mimicry (implying constancy in visual signals) has increased selection for additional pheromone components to mediate species-specificity.

In many danaine genera, males do not release their pheromones just into the air but disseminate pheromone-containing cuticular particles onto a female's antennae. The extended hovering flights, characteristic of the courtship behaviour of most danaines and shown prior to hairpencil expansion, might relate to employing such 'pheromone transfer particles' which are available in limited quantity only. The considerable investment involved in gathering PAs in quantity also adds to this energetically expensive behaviour,

perhaps resulting in male coyness with respect to expenditure of valuable resources and increased relevance of visual cues.

There are several groups of moths which also utilise PAs as male pheromone precursors or for defence. However, the polyphyletic 'PA-insects' differ greatly; e.g. several taxa employ dihydropyrrolizine pheromones but exhibit different mating strategies. Thus, the very same compounds serve as signals with apparently quite different messages, and comparative behavioural and sensory physiological studies in an ecological context should permit insight into the design and evolution of both signals and receivers.

7. SIGNAL FUNCTION AND EVOLUTION

Among the examples given above, females of *Argynnis paphia*, *Colias eurytheme*, *C. philodice*, *Papilio dardanus* and *P. phorcas* are all polymorphic, whereas the males of these species are monomorphic in every case. The male colour patterns of the *Colias*, *Nessaea* and *Papilio* species are monomorphic and distinctive throughout their vast ranges, whereas the female patterns are not. This reflects a broad generalization about the Papilionoidea: males show greater inter- but less intraspecific variation than females, suggestive that male visual signalling is strongly constrained by a need for specific recognition (e.g. male–male interactions? cf. Silberglied 1984), whereas female colour patterns are not. Paradoxically, behavioural evidence suggests that, while males may be sensitive to differences in colour signals, in most cases females are insensitive even to gross differences in male colour (uv reflection of male *C. eurytheme* notwithstanding; Silberglied 1984). This in turn relates to an unsolved mystery of butterfly evolution (Vane-Wright 1984): how is the stabilizing process that apparently affects male colour pattern released during speciation, so that shifts to new stable patterns in descendent lineages can occur? Dramatic, punctuational changes can happen to male butterfly colour patterns during speciation, but how these changes take place is simply not understood.

In an attempt to clarify some of these questions, Vane-Wright (1979*b*) introduced the idea of pathway analysis, whereby alternative models for the evolution of colour patterns could be considered, either through the accumulation or loss of separate morphs, or by selection of modifiers affecting particular morphs (disruptive and directional selection; cf. discussion by Nijhout (1991) on gradual versus discontinuous pattern change). Where the selective advantages of different female colour patterns can be linked to protective signalling (mimicry, crypsis) or recognition functions (e.g. mate attraction or avoidance; Vane-Wright 1984; Cook *et al.* 1993), this pathway-modelling approach has proved useful (e.g. Vane-Wright & Smith (1991) on *Papilio dardanus*). With respect to the evolution of male patterns, however, the mystery remains unsolved.

If we turn to the evolution of olfactory signals, where we have an adequate phylogenetic framework and as we have already seen, we may be able to

consider the evolution and decay of male bouquets in an analogous way to pathway models for visual signals. The discovery of many qualitative (discontinuous) differences between *Amauris* species offers hope that we will be able to reconstruct, through comparison between populations, subspecies and species, an intelligible pattern of trans-demic to trans-specific male pheromone change. With respect to the question of evolution of recognition signals by selection, or as a by-product of incidental processes, species such as *Amauris nossima* in Madagascar, which appears to have evolved in complete geographic isolation, are likely to be of particular interest (Vane-Wright *et al.* 1992). Studies on genetics, as exemplified by the work of Grula & Taylor (1979) using *Colias* hybrids, and Sappington & Taylor (1990) on qualitative variation in *Colias* pheromones, suggest further possibilities for investigating the transformational evolution of male pheromone bouquets.

8. CONCLUSIONS AND PROSPECTS FOR FUTURE WORK ON THE EVOLUTION AND DESIGN OF BUTTERFLY SIGNALLING SYSTEMS

We have presented observations and hypotheses regarding what we believe are principal issues for understanding the evolution and design of butterfly signalling systems. Given the limitations of present knowledge about key functional processes and phylogenetic patterns, an attempt to draw general conclusions must be premature. However, we offer the following generalizations and ideas, based on evidence presented here and on other knowledge, in the hope of focusing debate:

1. Mate location is normally effected by male visual search.
2. Conflict can occur in the visual mode between protective and specific functions, and this is probably a factor in the elaboration of male pheromone systems, notably in mimetic or cryptic species.
3. Although male visual signals are under strong stabilizing selection within species, sexual selection by females does not provide a convincing explanation for this (cf. Silberglied 1984; Vane-Wright 1984).
4. Female colour patterns are often variable or even polymorphic, despite the existence of male preferences.
5. Female pheromones, if used at all, function at close-range only.
6. While intraspecific signalling in males involves both species and trait recognition, visual signals seem unlikely candidates for trait signalling, whereas pheromone bouquets may serve both functions.
7. During phylogenesis, signal function of a pheromone component may transform from trait to specific recognition, and so provide a mechanism for a steady-state evolutionary model, in which pheromone components are both supplemented and lost through successive speciation events.
8. In the case of the Danainae, where visual signalling

is constrained by Müllerian mimicry, complex male pheromone systems probably convey information on quality of nuptial gift (status) and even genetic quality (signal complexity), as well as species recognition.

Four major desiderata we believe would carry the subject most forward are:

1. Revitalisation of work on the function of colour and pattern, particularly in the tradition cut short by the tragic death of Bob Silberglied. This would involve an integrated approach to colour (including uv) and scent in relation to species-recognition and mate selection (cf. Silberglied 1984). Renewed effort is also needed to assess patterns for environmental contrast or matching (Papageorgis 1975; Endler 1984) with respect to protective signal functions, and measuring the physical properties of butterfly colours to assess potential signal content (cf. Huxley 1975; Endler 1990).

2. Greater effort on butterfly pheromone chemistry, to include new emphasis on population and individual variation in pheromone components (cf. Sappington & Taylor 1990).

3. In addition to more work on the mate selection in individual species (e.g. Smith 1984; Wiernasz 1989), we need a systematic general approach mediated through hybridization of populations, subspecies and closely related species differentiated with respect to physiologically relevant signals. Studies on the inheritance of functional wing pattern elements (e.g. Sheppard *et al.* 1985; Brakefield & van Noordwijk 1985; Nijhout *et al.* 1990) and pheromone components (e.g. Grula & Taylor 1979) are likely to be far more revealing if linked to phylogenetic reconstructions (Nijhout 1991). Groups for study should be selected so as to reflect a variety of different visual signalling needs or 'environments' (e.g. mimicry versus crypsis versus neither, crepuscular versus fully diurnal, forest versus steppe, etc.).

4. Ignorance on the physiology of signal receivers must also be overcome. It would be useful, for example, to renew the type of work on electrophysiology of butterfly colour perception pioneered by Swihart (e.g. 1967), and further eco-physiological studies on chemoreceptors are also required. This research would be comparative in approach, to obtain ideas on the evolution of receptors which could then be linked with ideas about the evolution of signals.

Finally, we would like to make a general plea for further research on the biology and systematics of butterflies in an evolutionary framework. In tropical regions several hundred butterfly species can coexist within a single hectare. This diversity, coupled with the wealth of biological and evolutionary problems entailed, makes the study of butterfly signalling systems a particularly exciting and worthwhile challenge.

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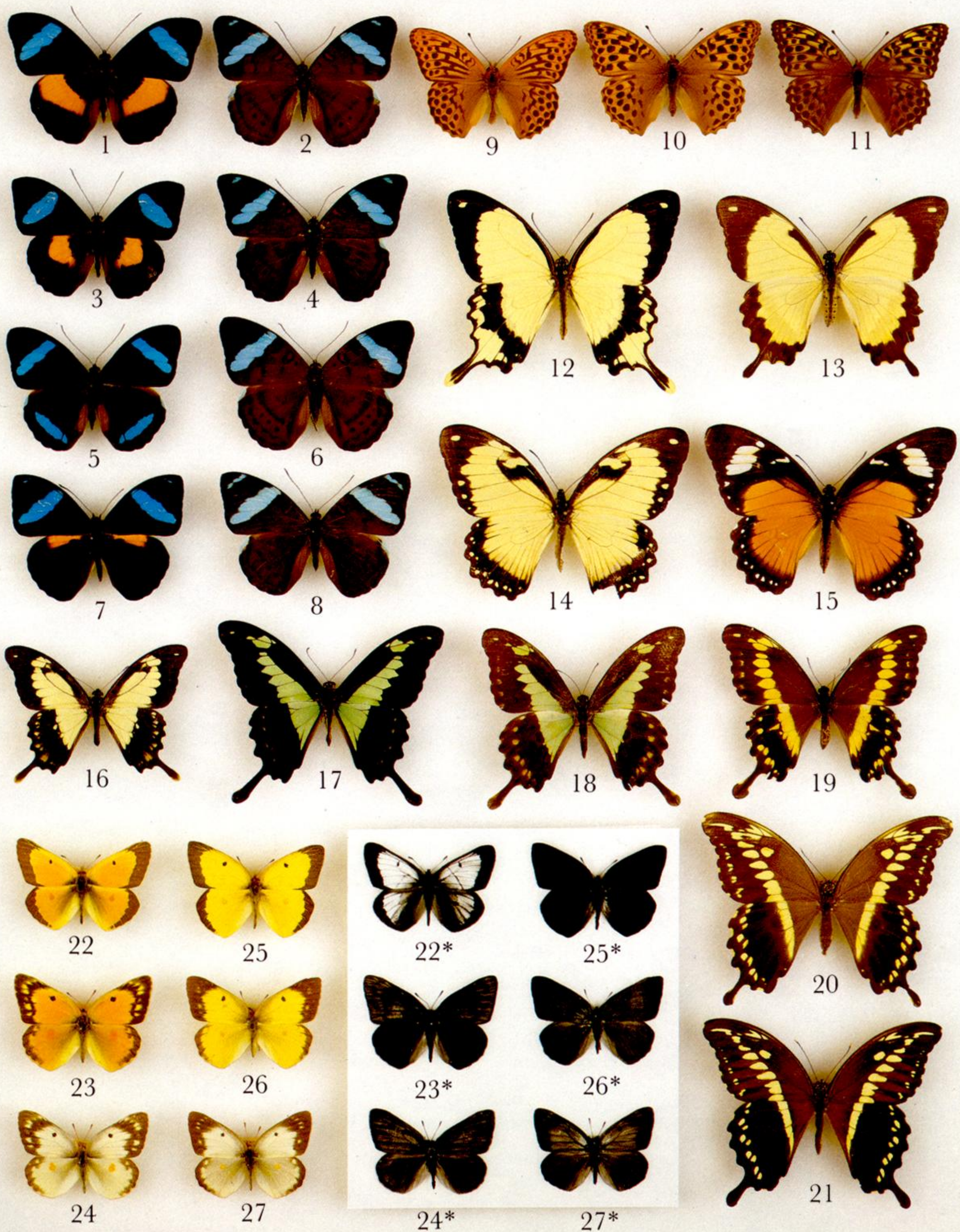Plates 1 and 2 were printed by George Over Limited, London and Rugby

Plate 1. Figures 1–8: *Nessaea* species, (1) *N. obrina* male, (2) *obrina* female, (3) *N. batesii* male, (4) *batesii* female, (5) *N. hewitsoni* male, (6) *hewitsoni* female, (7) *N. aglaura* male, (8) *aglaura* female. Figures 9–11: *Argynnis paphia*, (9) *paphia* male, (10) *paphia* male-like female, (11) *paphia* 'valesina' female. Figures 12–21: *Papilio* species, (12) *P. dardanus* male, (13) *dardanus* male-like female from Comoro Islands, (14) *dardanus* tail-less male-like female from Pemba, (15) *dardanus* 'Danaus chrysippus-like' female, (16) natural male hybrid of *P. dardanus* × *P. phorcas*, (17) *P. phorcas* male, (18) *phorcas* male-like female, (19) *phorcas* 'constantinus-like' female, (20) *P. constantinus* female, (21) *constantinus* male. Figures 22–27: *Colias* species in visible light, (22) *C. eurytheme* male, (23) *eurytheme* male-like female, (24) *eurytheme* 'alba' female, (25) *C. philodice* male, (26) *philodice* male-like female, (27) *philodice* 'alba' female. Figures 22*–27*: ultraviolet reflectance images of the corresponding *Colias* specimens (22–27). 0.44 × natural size.
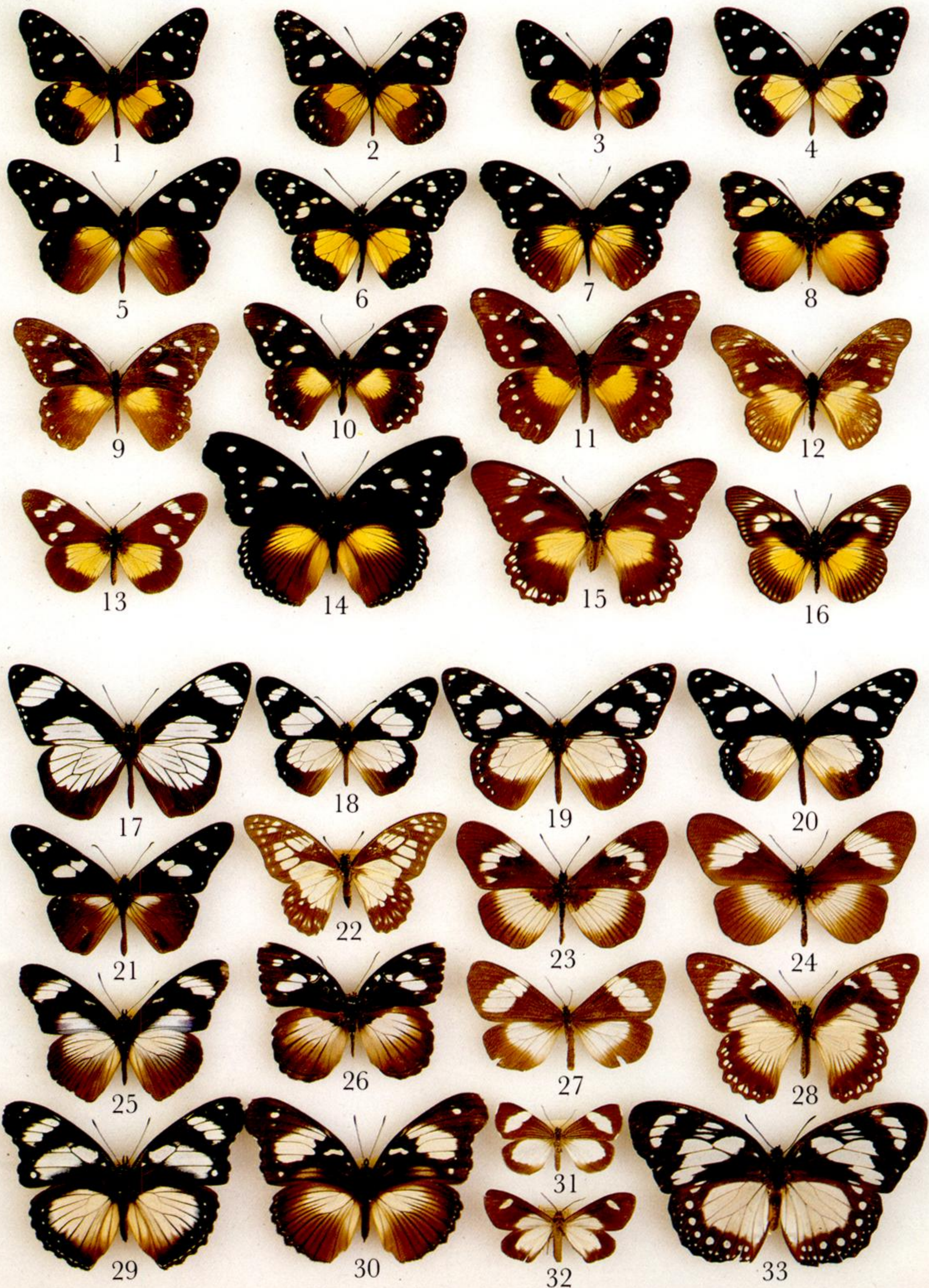
Plate 2. Figures 1–16: members of the putative *Amauris echeria* mimicry complex from eastern Africa, (1) *Amauris echeria*, (2) *A. ellioti*, (3) *A. albimaculata*, (4) *A. crawshayi*, (5) *A. inferna*, (6) *Pseudacraea deludens* [Nymphalidae], (7) *Hypolimnas anthedon* f. 'mima' [Nymphalidae], (8) *Aterica galene* f. 'dimorpha' [Nymphalidae], (9) *Papilio zoroastres* [Papilionidae], (10) *P. echerioides*, (11) *P. jacksoni*, (12) *Graphium almansor* [Papilionidae], (13) *Acraea johnstoni* [Nymphalidae], (14) *Hypolimnas dinarcha*, (15) *Papilio dardanus* f. 'cenea', (16), *Pseudacraea lucretia*. Figures 17–33: members of the putative *Amauris niavius* mimicry complex from eastern Africa, (17) *Amauris damocles*, (18) *A. ochlea*, (19) *A. tartarea*, (20) *A. dannfelti*, (21) *A. hecate*, (22) *Graphium philonoe*, (23) *Acraea aganice*, (24) *A. macarista*, (25) *Hypolimnas anthedon* f. 'anthedon', (26) *Aterica galene* f. 'albimacula', (27) *Nyctemera acraeina* [Arctiidae], (28) *Papilio dardanus* f. 'hippocoonides', (29) *Hypolimnas deceptor*, (30) *H. bartelotti*, (31) *Nyctemera leuconoe*, (32) *Pitthea famula* [Geometridae], (33) *Euxanthe wakefieldi* [Nymphalidae]. 0.44 × natural size.