Chapter 11
Chemical Ecology of Poisonous Butterflies: Model or Mimic? A Paradox of Sexual Dimorphisms in Müllerian Mimicry

Ritsuo Nishida

Abstract A number of butterfly species are toxic or unpalatable against predators by developing mechanisms either to biosynthesize such noxious elements de novo or to acquire directly from the poisonous host plants for their own defense. Most of these "poisonous butterflies" exhibit aposematically colored wing patterns that are often associated either with Batesian or Müllerian mimicry species to form a "mimicry ring." This review focuses on unpalatable chemical elements potentially operating in three typical mimicry rings: (1) the tiger *Danaus* mimicry ring, (2) the *Idea* mimicry ring, and (3) the red-bodied swallowtail mimicry ring, in association with their mimetic wing patterns. Female-limited polymorphisms are a common feature of the Batesian mimicry but not in Müllerian mimicry, because such diversification is unfavorable for the models. I present here some unique cases of sexual dimorphisms within the putative Müllerian mimicry complexes. A *Danaus chrysippus*-mimicking nymphalid, *Argyreus hyperbius*, is a typical example of the female-limited dimorphic mimics. However, *A. hyperbius* were found to be poisonous with toxic cyanogenic glycosides (linamarin and lotaustralin). Likewise, a pipevine swallowtail, *Atrophaneura alcinous*, which sequesters toxic aristolochic acids, exhibits sexually dimorphic color patterns (male, black; female, smoky brown). A sympatric diurnal zygaenid moth, *Histia flabellicornis*, is mimetic to *A. alcinous* males rather than the females and stores cyanogenic glycosides. The moth is regarded as a Müllerian ally that may have stabilized the wing coloration mutually with those of *A. alcinous* males. On the contrary, a diurnal "swallowtail moth," *Epicopeia hainesii*, mimics the brighter wing color of *A. alcinous* females. Possible adaptation mechanisms on these paradoxical mimicry patterns are discussed.

Keywords Batesian mimicry • Müllerian mimicry • Aposematism • Unpalatability • Sequestration • Defense substance • Pharmacophagy • Sexual dimorphism • Sexual selection • Mimicry ring

R. Nishida (✉)
Discipline of Chemical Ecology, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
e-mail: ritz@kais.kyoto-u.ac.jp

11.1   Introduction

The monarch butterfly, *Danaus plexippus* (Danainae: Nymphalidae), is an exemplar of the model/mimic relationships in butterflies. The larvae feed on milkweed plants (Asclepiadoideae of the family Apocynaceae) and selectively accumulate toxic cardenolides (cardiac glycosides, CGs) from the host plants and pass over to the adults (Reichstein et al. 1968). CGs are powerful heart poison and induce emesis to predatory birds (Brower 1984). If a "hungry blue jay" ingested a monarch butterfly, the sequestered CGs in the butterfly body tissues strongly induced emesis (Brower 1969). After such a noxious experience, the bird would never eat the butterflies with the same wing pattern again. Predatory birds avoid the butterflies primarily because they experience an obnoxious "bitter" taste and/or emesis after ingestion. This results in a visually conditioned aversion toward prey with similar appearance, allowing for the evolution of Batesian mimicry (Brower 1969). Therefore, the monarch is considered to be a typical poisonous model butterfly. The viceroy, *Limenitis archippus* (Nymphalinae, Nymphalidae), was initially thought to be a typical example of Batesian mimicry. However, the viceroy was shown to be fairly unpalatable to some predators (Ritland and Brower 1991; Prudic et al. 2007). Moreover, monarch can sometimes be palatable (or nontoxic) particularly if the larvae grew up on the nontoxic or low-CG milkweed plants (Brower 1969). Thus, the situation between the models and mimics may be interchangeable as in this case, and if both are unpalatable mimicking to each other, it forms an association of the Müllerian mimicry type, rather than Batesian (Rothschild 1979; Huheey 1984).

Unpalatability or distastefulness is strongly linked to the toxicity of the sequestered defense substances, as in human taste (Brower 1984), and thus, the predator effectively avoids toxic prey before ingestion. Visually oriented, avian predators are the most effective selective pressure to contribute to the formation of mimicry butterflies in conjunction with the distastefulness of the model species. The evolution of mimicry is highly dependent on both the visual and chemosensory (gustatory/olfactory) physiology of the predatory animals in addition to the intrinsic toxicity of the model butterfly (Brower 1984; Nishida 2002). However, knowledge on the chemistry of defensive agents stored in each species within the mimicry rings is often scarce (Trigo 2000; Nishida 2002). I highlight chemical backgrounds of acquired defensive elements among some aposematic Asian butterfly species in the following three typical/putative mimicry rings:

(1) The tiger *Danaus* mimicry ring
(2) The *Idea* mimicry ring
(3) The red-bodied swallowtail mimicry ring

The female-limited mimetic dimorphisms are a common feature of the Batesian mimics as in the *Papilio polytes* of the red-bodied swallowtail mimicry ring (Turner 1978). By contrast, Müllerian mimics lack strong sexual dimorphism, presumably due to density-dependent selection, where the divergence of a morph is disadvantageous in the toxic model (Mallet and Joron 1999). However, some exceptional

cases of sexual dimorphisms can be seen within the putative Müllerian mimicry complexes in the above mimicry rings (1) and (3). My particular attention is focused on those sexually dimorphic species where both sexes were confirmed to harbor noxious chemicals – to evaluate their adaptive characteristics within the mimicry rings.

11.1.1 Tiger Danaus Mimicry Ring

Although the monarch butterfly, *Danaus plexippus*, and other milkweed butterflies sequester CGs from the *Asclepias* hosts in the body tissues and become unpalatable to bird predators, some of the butterfly populations often lack CGs as mentioned above. Similar to the American *Danaus* spp., the Old World milkweed butterfly, *Danaus chrysippus* (so-called plain tiger, widely distributed from Africa to tropical Asia) is assumed to be "poisonous" with its conspicuous appearance with a black apex and white subapical spots on the forewing in blight tawny-orange background coloration, likely as a typical model for various mimicry species (Smith 1973) (Figs. 11.1 and 11.2). *D. chrysippus* feeds exclusively on the Asclepiadoideae and presumed to sequester toxic cardenolides from its host milkweed plants during the
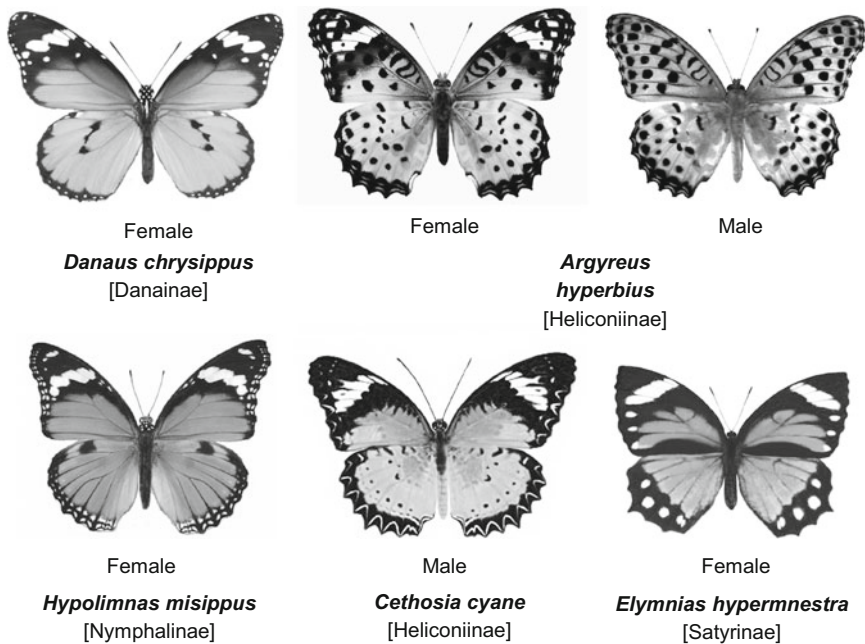

Female
Danaus chrysippus
[Danainae]

Female

Male
Argyreus hyperbius
[Heliconiinae]

Female
Hypolimnas misippus
[Nymphalinae]

Male
Cethosia cyane
[Heliconiinae]

Female
Elymnias hypermnestra
[Satyrinae]

Fig. 11.1 The tiger Danaus mimicry ring. Note that some species in this figure may not occur in the same habitat
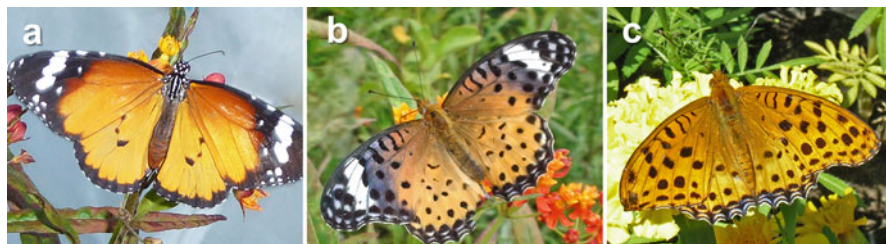
Fig. 11.2 (**a**) *Danaus chrysippus* female, (**b**) *Argyreus hyperbius* female, (**c**) *A. hyperbius* male
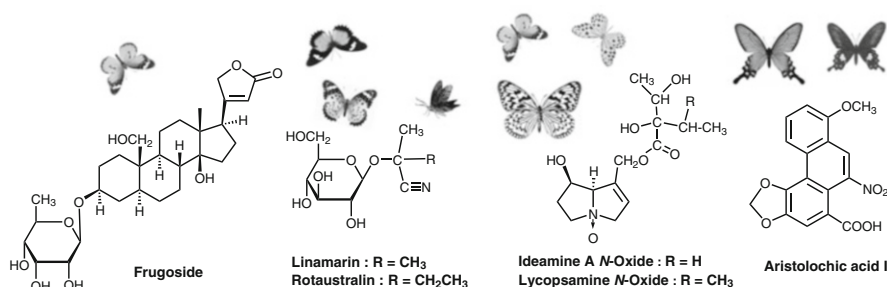

Fig. 11.3 Typical defensive substances in aposematic butterflies

larval stage. However, they are known to be poor and inconsistent sequesterers of CGs (Schneider et al. 1975; Rothschild et al. 1975; Mebs et al. 2005).

In order to confirm the toxicity of a local population of *D. chrysippus* in Okinawa, we examined CG contents in their body tissue. A relatively polar CG, frugoside (Fig. 11.3), was characterized as the major sequestrate in *D. chrysippus* adults raised on *Asclepias curassavica* (Wada et al. unpublished). While calotropin and calactin are CGs known as typical sequestrates in *D. plexippus* raised on *A. curassavica* (A), these less polar CGs were not found as prominent components in *D. chrysippus*, suggesting a selective or differential accumulation of CG species as in the case between *D. plexippus* and *D. gilippus* (Cohen 1985; Mebs et al. 2012). In addition, males of *D. chrysippus* frequently visit plants containing poisonous pyrrolizidine alkaloids (PAs) and pharmacophagously sequester the alkaloids in the body tissues both as a defense substance and pheromone precursors (Edgar et al., 1979; Boppré, 1986) (see also Sect. 11.2). Since *D. chrysippus* males in Okinawa frequently visit PA-containing plants such as *Eupatorium* (Asteraceae) and *Heliotropium* (Boraginaceae), they are assumed to be protected dually both by CGs and PAs. Nevertheless, a more detailed spatiotemporal dimensional survey of sequestered defensive elements is needed further to clarify the "palatability spectrum," because the distribution of *D. chrysippus* is widespread throughout the African-Asian tropical and subtropical regions up to Okinawa in association with their local mimics as discussed below.

There are a variety of butterfly species that closely resemble wing color patterns of *D. chrysippus*, particularly within Nymphalidae to form a "tiger Danaus mimicry ring" in Asian regions (Fig. 11.1):

Danaus genutia (striped tiger) (Danainae: Nymphalidae): This butterfly resembles *D. chrysippus* but with conspicuous black stripes on both fore- and hindwings. These two coinhabiting *Danaus* species, widely distributed throughout India, South Asia, and Japan, are regarded as Müllerian mimics, as both species feed on the milkweed subfamily Asclepiadoideae, although the ability of sequestration of CGs from their host *Cynanchum liukiuense* in Okinawa is unknown. Males of these two species show strong affinity to PA-containing plants and are considered to obtain alkaloids both as defensive measure as well as sex pheromone precursors.

Hypolimnas misippus (danaid eggfly) (Nymphalinae: Nymphalidae): Females are known polymorphic and the plain tiger mimic occurs sympatrically with *D. chrysippus* in Asia (Gordon and Smith, 1998). Since the larvae feed on the "presumably innocuous plant" *Portulaca oleracea* (Portulacaceae), this butterfly is thought to be a palatable mimic.

Elymnias hypermnestra (common palmfly) (Satyrinae: Nymphalidae): Some populations are sexually dimorphic. While the males mimic *Euploea* species, the females mimic *Danaus* spp. (Yata and Morishita 1985). The larvae feed on the palm family Arecaceae (Ackery and Vane-Wright 1984).

Cethosia cyane (leopard lacewing) (Heliconiinae: Nymphalidae): There are several lacewing species mimicking the wing patterns of *D. chrysippus*. Their larvae feed on Passifloraceae, the subfamily of which is known to biosynthesize toxic cyanogenic glycosides (CNs) (Nahrstedt and Davis 1985).

Argyreus hyperbius (Indian fritillary) (Heliconiinae: Nymphalidae): This species is sexually dimorphic and the females have similar patterns to *D. chrysippus* but with additional black spots scattered on the dorsal wings (Fig. 11.2). The female has been considered to be a Batesian mimic (Su et al. 2015). The larvae feed on Violaceae plants similar to many other related species in the tribe Argynnini in Japan. Since the toxicity of this butterfly is unknown, we examined the possible defensive substance in the body as described below.

Among the species listed above, only females are mimetic to *D. chrysippus* in *H. misippus*, *A. hyperbius*, and *E. hypermnestra*, whereas males are considered to be pre-existing morphs at least in the case of the former two, as typical examples of sexual dimorphism. Female-limited wing pattern dimorphism in the butterfly mimicry rings seems restricted to Batesian mimicry, whereas Müllerian mimics lack strong sexual dimorphism (Mallet and Joron 1999). An increase of palatable mimetic pattern relative to a model would weaken the mimetic advantage in Batesian mimicry by negative frequency-dependent selection, whereas an increase of unpalatable mimetic pattern becomes more favorable in Müllerian mimicry by positive density-dependent selection (Turner 1978; Mallet and Joron 1999). If this theory is applicable, both *H. misippus* and *A. hyperbius* would be palatable Batesian mimics of the model *Danaus*. This would also be supported by chemical

constituents in their host plants; both *Portulaca* and *Viola* are presumably "nontoxic" (the former often listed as a local edible wild vegetable). However, the chemical analysis of *A. hyperbius* butterfly bodies revealed the presence of highly toxic cyanogenic glycosides (CNs), linamarin and lotaustralin, both in males and females (raised on wild *Viola yedoensis* as well as cultivated varieties of pansy, *V. tricolor* during the larval stage) in substantial quantities (contents of total CNs: females, 300–400 μg; males 100–250 μg/body) (Nakade et al. unpublished) (Fig. 11.3). Since these CNs were not detected in the host *Viola* plants, *A. hyperbius* seems to biosynthesize these toxic compounds from amino acids, as in *Heliconius* butterflies (Nahrstedt and Davis 1985). It is also known that some of monomorphic *Cethosia* spices store linamarin and lotaustralin (Nahrstedt and Davis 1985).

Here *A. hyperbius* butterfly is determined to be poisonous despite its sexual dimorphism in wing pattern, to form the *Danaus* (male + female) – *Cethosia* (male + female) – *Argyreus* (female) Müllerian mimicry ring. It is noted that a number of *Cethosia* lacewings exhibit a black-spotted wing pattern similar to that of *A. hyperbius* rather than that of *D. chrysippus* which entirely lacks such "leopard"-spotted patterns, suggesting a closer reciprocal interaction between these two taxa within the Heliconiinae in the South Asian regions. We still do not know whether the other sexually dimorphic nymphalids, *H. misippus* and *E. hypermnestra*, store any unpalatable allelochemicals or not.

11.1.2 Idea *Butterfly Mimicry Ring*

A giant butterfly, *Idea leuconoe* (mangrove tree nymph) (Danainae: Nymphalidae), is a primitive danaine species, having a wingspan of 120–140 mm with black markings and veins on the white wing background (Fig. 11.4). Although the butterfly is not aposematic in coloration, compared to other toxic butterflies, it is highly conspicuous in the subtropical forests flying slowly and gracefully under the high predatory avian pressure such as the blue rock thrush, *Monticola solitarius*. The butterfly (originated in Okinawa) accumulates large quantities of PAs (ideamines A, B, and C, lycopsamine, parsonsine) as *N*-oxides from the host *Parsonsia laevigata* (Apocynaceae) during the larval stage (Nishida et al. 1991; Kim et al. 1994) (Fig. 11.3). The total alkaloids often exceed 3 mg/insect, suggesting a high unpalatability to potential predators.

Although males of most danaine butterflies show strong affinity to PAs during adult stage and accumulate PAs by foraging pharmacophagously from various plant sources to obtain defensive agents, as well as pheromone precursors, by visiting PA-containing plants (Ackery and Vane-Wright 1984), *I. leuconoe* and some other related *Idea* species in Southeast Asia likely obtain PAs not pharmacophagously but directly from the apocynad hosts during the larval stage. *I. leuconoe* is highly adapted to PAs not only by sequestering PAs for defense (allomone) but also for specific oviposition cues (kairomone) in females (Honda et al. 1997) and precursors
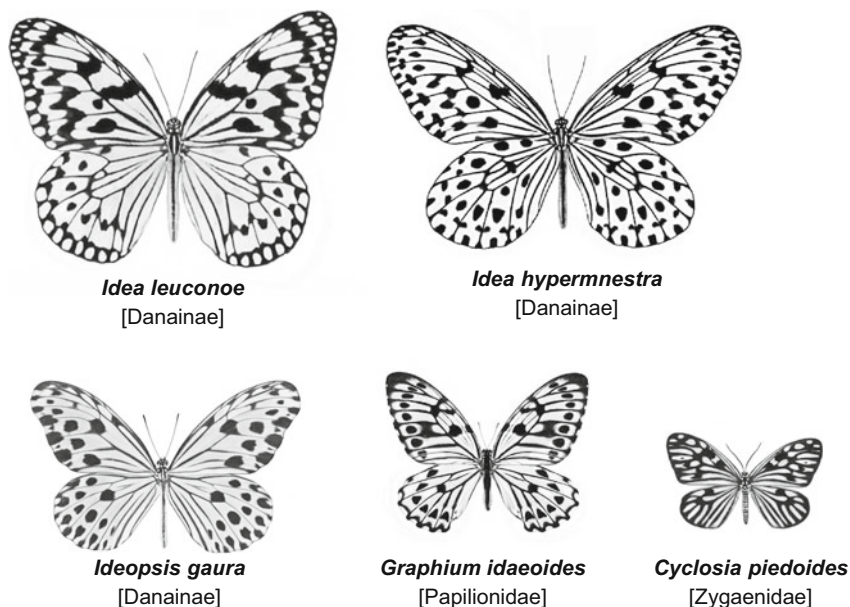
Fig. 11.4 The *Idea* mimicry ring. Note that some species in this figure may not occur in the same habitat

of volatile sex pheromone in males (Nishida et al. 1996). Such a tight linkage with PAs seems to represent an ancestral feature of the Danainae, from which the secondary colonization of the Danainae on PA-free plants (such as Asclepiadaceae) might have taken place with retention of the PA-mediated allomonal and pheromonal systems via pharmacophagous acquisition of the precursors from nonhost PA plants (Edgar 1984; Nishida 2002). *I. hypermnestra* (Fig. 11.4) and *I. lynceus*, both sympatric in Peninsular Malaysia, sequester a series of PA *N*-oxides, tentatively assigned from the hemolymph of the wild adults in the spectrometric analysis (Nishida unpublished). These two species are regarded as the Müllerian mimics in the rainforest habitat.

Beside these two giant *Idea* species, a smaller danaine species, *Ideopsis gaura*, often share the same habitat in Southeast Asia (Fig. 11.4). Its larvae feed on the Apocynaceae, but sequestration of toxic elements from the host is unknown. *I. gaura* males frequently visit PA-containing *Eupatorium* flowers (Asteraceae). A PA *N*-oxide of lycopsamine (or its stereoisomers) was detected by mass spectrometry from the body extracts of *I. gaura* males (captured in Penang, Malaysia) (Nishida unpublished). Thus, these three coinhabiting danaines seem to form a Müllerian mimicry association within the subfamily Danainae.

A Southeast Asian satyrine species, *Elymnias kuenstleri* (Nymphalidae), is also known to mimic *Idea* spp., which is analogous to the relationship between *Elymnias hypermnestra* and *D. chrysippus* in the tiger Danaus mimicry ring (Fig. 11.1). There are several swallowtail butterfly species mimetic to *Idea* spp. or *Ideopsis gaura*, such as *Graphium idaeoides* (Fig. 11.4), *G. delessertii*, and a whitish morph of

Papilio memnon (Papilionidae), which are assumed to be Batesian mimics of *Idea* spp.

The wing pattern of a day-flying *Cyclosia pieriodes* (false *Idea* moth, Zygaenidae) (Fig. 11.4) resembles that of *Idea* spp. or *Ideopsis gaura*. Although the defense substance has not been examined, it is likely that the moth stores some CNs, as many other unpalatable zygaenids are known to biosynthesize/store linamarin and lotaustralin (Holzkamp and Nahrstedt 1994; Nishida 1994). The wingspans between the putative model giant *Idea* spp. (or medium-size *I. gaura*) and minute *C. pieriodes* may be beyond comparison for the insectivorous birds. However, the exquisite wing color pattern may be a more significant factor to develop mimicry beyond their size in the avian vision, probably involving a psychological effect (Wickler 1968; Halpin et al. 2013).

11.1.3   Red-Bodied Swallowtail Mimicry Ring

As quoted by Wallace, the common rose, *Pachliopta aristolochiae*, and many other "red-bodied swallowtails" in the tribes Troidini (Papilionidae) are known as unpalatable models for various Batesian mimicry complexes particularly in the genus *Papilio* (Uésugi 1996) (Fig. 11.5). These troidines feed selectively on the pipevine family, Aristolochiaceae, and sequester toxic aristolochic acids (AAs) (Fig. 11.3) in the body tissues (Euw et al. 1968; Nishida and Fukami 1989; Wu et al. 2000). One of the pipevine swallowtails, *Atrophaneura alcinous*, from mainland Japan exhibits sexual dimorphism to some degree, in that the dorsal wings of males are typically jet black, while the females' are gray or smoky brown with some variations in the degree of darkness; the underside hindwings have long tails and a row of pink or orange spots at the edge in both sexes; the lateral sides of the abdomen are red with a black spot on each segment in both sexes (Fig. 11.5b, e, f).

A day-flying swallowtail moth, *Epicopeia hainesii* (Epicopeiidae) (Fig. 11.5c, d), has a red abdomen and a similar color pattern on the wings with long swallowtail projections as those in *A. alcinous*, although the body size is much smaller than that of *A. alcinous* (Fig. 11.5d–f and 11.6). The brighter gray or smoky brown-color tone and the black veins on the dorsal wings of *E. hainesii* are strikingly similar to those of *A. alcinous* females but not much of males. It suggests a unique case of mimicry in that the moth adopts one of the sexes of the toxic model. Both *A. alcinous* and *E. hainesii* are considered to be more or less sympatric in forests and grasslands in the middle to southern part of Japan. However, *E. hainesii* is distributed further to northern Japan, including Hokkaido, where its putative model *A. alcinous* is absent (Inoue, 1978). In the absence of a model species, there may be no protection from predation (Prudic and Oliver, 2008), unless other defensive measures are present. Since *E. hainesii* is warningly colored with red body even without the presence of the model swallowtail, it strongly implies that this moth is also unpalatable,

Fig. 11.5 The red-bodied butterflies and moths. (**a**) *Pachliopta aristolochiae* female, (**b**) *Atrophaneura alcinous* female, (**c**) *Epicopeia hainesii* female, (**d**) *Histia flabellicornis* female secreting defensive foams containing linamarin (*orange arrow*). (**e**) *A. alcinous* female, (**f**) *A. alcinous* male, (**g**) *E. hainesii* male, (**h**) *H. flabellicornis* male

although their palatability is unknown. The larval host, *Cornus controversa* (Cornaceae), is not known to contain typical noxious elements such as CGs, PAs, and CNs. The moth does not seem to secrete glandular defense substance. Preliminary chemical analysis of the whole-body extracts of *E. hainesii* revealed some unidentified polar compounds on the thin-layer chromatography, which did not match with CNs such as linamarin and lotaustralin (Nishida unpublished). Further studies are needed to substantiate possible unpalatability of *E. hainesii*. There is a
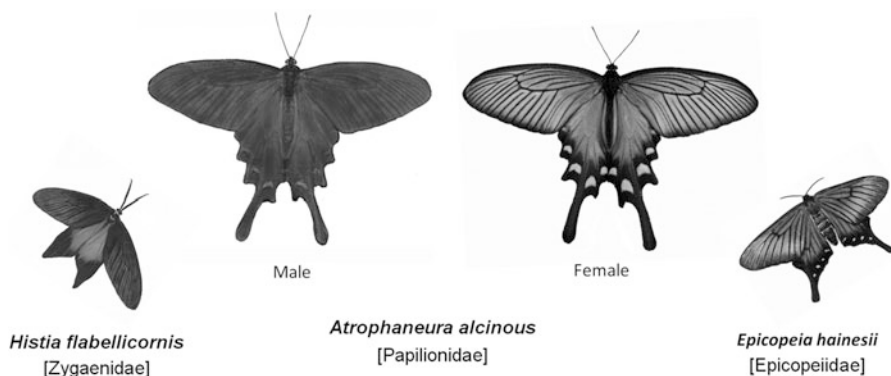
Histia flabellicornis
[Zygaenidae]

Atrophaneura alcinous
[Papilionidae]

Male Female

Epicopeia hainesii
[Epicopeiidae]

Fig. 11.6 The red-bodied swallowtail mimicry ring. Note that some species in this figure may not occur in the same habitat

spectacular array of red-bodied *Atrophaneura/Pachliopta–Epicopeia* mimicry rings distributed in East Asia further to examine in view of Batesian/Müllerian mimicry associations.

Histia flabellicornis moth (Zygaenidae) is a red-bodied diurnal moth resembling *A. alcinous* with black forewing and metallic blue hindwing coloration (larval host: *Bischofia javanica*, Euphorbiaceae) (Fig. 11.5d, h). *H. flabellicornis* is subdivided into many subspecies, and their distribution from Southeast Asia to Taiwan and Okinawa (but not inhabited in Japan's mainland) overlaps that of the red-bodied swallowtails (*Atrophaneura* and *Pachliopta*). The adults secrete toxic linamarin and hydrogen cyanide (HCN) as a foam when disturbed, suggesting the moth to be a Müllerian mimicry ally with *A. alcinous* in Okinawa (Nishida 1994). Although the degree of mimesis is imperfect in size, shape, and coloration, the wing color of *H. flabellicornis* relatively resembles males of *A. alcinous* but less resembles the females. This situation is contrary to the *A. alcinous–E. hainesii* mimicry association pattern, where the mimesis is biased to the females. The metallic blue burnish characteristic to *H. flabellicornis* hindwings may appear less similar to the *A. alcinous* male wing coloration. However, under the strong subtropical sunlight, dorsal wings of *A. alcinous* males (Fig. 11.5f) in Iriomote Island (Okinawa) shine in a metallic blue as in *H. flabellicornis* wings (Fig. 11.5h). *A. alcinous* males may have developed an implementation of structural coloration interacting with *H. flabellicornis* through visual selection by avian predators. Thus, even though the Müllerian association between these two species may be loose, the mimicking pattern may represent a rare case of sexual dimorphism in the toxic model, which might have stabilized the wing morph only in male. As illustrated in Fig. 11.6, the above two cases exemplify a symmetrical relationship of sexual dimorphism in *A. alcinous*, interacting adaptively with either one of the (putative) Müllerian mimic moths (*H. flabellicornis* and *E. hainesii*).

11.2 Discussion

As presented in those three mimicry rings, the chemistry of the unpalatable elements stored in the butterflies is the critical factor to understand the nature of aposematic wing coloration and mimicry – otherwise a conclusion of adaptive coloration must be tentative (Nishida 2002). Besides, "unpalatability" itself is hard to determine (Mallet and Joron 1999) due to the variable ecological and physiological milieus of both the preys and predators. The quality and quantities of defensive substances in presumptive model butterflies often depend on allelochemicals from the host plants, which may result in a variation in palatability, individually or among local communities, which complicates the mimicry association (Nishida 2002). Thus, Batesian and Müllerian mimicries may be interchangeable depending on the "palatability spectrum" of the prey butterflies as well as the "predatory spectrum" of the potential selective pressure (Huheey 1984; Turner 1984). The predatory spectra may involve variations in chemosensory and toxicological sensitivity/susceptibility of the predators. The sensory sensitivity in insectivorous animals may be different greatly among the species, and such variation in response to chemicals or preferences may often be seasonal particularly during highly predacious period and abundance of preys such as breeding seasons (Fink and Brower 1981; Glendinning and Brower 1990). We certainly need to know both prey/predatory spectra in time and space to understand the overall scheme of the existing mimicry rings (Joron and Mallet 1998).

Two instances of sex-limited dimorphisms were presented here in the Müllerian mimicry complexes – the tiger Danaine mimicry ring (*Argyreus hyperbius*) and the red-bodied swallowtail mimicry ring (*Atrophaneura alcinous*). In a toxic model, the diversification of its wing morphology would decrease their fitness due to a "number-dependent selection" as originally stated by Müller (Turner 1978; Mallet and Joron 1999). This widely accepted rule is not supported by the cases noted above. Such paradoxical examples are also known in *D. chrysippus* in Africa (Owen et al. 1994) and *Heliconius numata* in South America (Brown and Benson 1974).

In the *A. alcinous* (male)–*H. flabellicornis* (both sexes) co-mimicking association of the red-bodied mimicry ring, the defensive allelochemicals (AAs and CNs) fulfill the requirement of potential unpalatability that would reciprocally enhance their fitness through Müllerian mimicry. In the case of the *A. alcinous* (female)–*E. hainesii* (both sexes) co-mimicking association, the chemistry of the latter is still unclear, although the moth is suspected to be a Müllerian mimic as mentioned above. The root cause triggering female-limited wing pattern dimorphism in *A. alcinous* may be attributed to an escape from an overload of potential Batesian mimics, similar to the case of polymorphism in *D. chrysippus* in Africa under the high population density of palatable *Hypolimnas* (Smith et al. 1993). Several Rutaceae-feeding black swallowtails such as *Papilio macilentus* and *P. protenor* (females of the former are highly mimetic to *A. alcinous* males) often share the same microhabitat spatiotemporally in the middle part of Japan. Potential higher

predation on females due to behavioral vulnerability may underlie behind this mechanism (Ohsaki 1995 2005). Thus, in addition to the chemistry, further studies are warranted especially on the geographical variations of wing color in both the butterflies and moths but also on the potential interspecific impacts from co-occurring Batesian mimics.

In the *A. hyperbius* (female)–*D. chrysippus* (both sexes) co-mimicking association, both are protected by unpalatable elements (CNs, CGs, and PAs). The black-spotted wing pattern in *A. hyperbius* males resembles that of the co-occurring woodland fritillaries such as *Argynnis paphia* (silver-washed fritillary) and *Argyronome ruslana*. We have recently shown that adults (both sexes) of these fritillary species sequester CNs in their body in substantial quantities (Nakade, Naka et al. unpublished), likely to form a "leopard fritillary mimicry ring," apparently excluding *A. hyperbius* females from the ring. These chemical evidences support that sexual dimorphism is feasible not only in Batesian mimicry but also in Müllerian mimicry, if the other sexual morph is consolidated by a member of other mimetic toxic species, probably to receive better protection than staying in the same color patterns. This would invoke arguments on the sexual selection vs. natural selection. Certainly, additional work is needed to support this hypothesis.

Another type of sexually restrictive feature in the mimicry syndromes can be seen in the specific exocrine organs developed on the wings and/or body, which disseminate odoriferous substances, often accompanied by morphological differences between the sexes. Troidine males such as *A. alcinous* emit characteristic odors from androconial scales on the inner margins of the hindwings (Honda 1980), which represents a structural–chemical dimorphism. Although sex-pheromonal roles of these male-specific scents are mostly unknown, the conspicuous smell of *A. alcinous* males may be attributed to an "odor aposematism," alerting the presence of systemic toxins (AAs) to enhance its visual aposematism (Nishida and Fukami 1989).

In many *Danaus* butterfly species, males pharmacophagously accumulate specific alkaloids from PA-containing plants during adult stage and use these chemicals for their own defense as well as precursors of sex pheromone disseminated from male-specific hairpencil organs (Boppré 1984). In this case, only males become a model with defensive PAs, possibly dually protected also by CGs originating from the larval host plant, representing a sexual "chemical dimorphism" by the sequestered defense substances. In this process, females may also be indirectly protected by visual automimicry, even if they do not have any defensive chemicals (Brower 1969). In the queen butterfly, *Danaus gilippus*, a portion of male's PAs may be transferred to females during copulation as a nuptial gift potentially to protect the female and eventually to eggs and thus to ensure protection of the male's progeny (Eisner and Meinwald 1995).

In contrast, a primitive danaine, *Idea leuconoe*, acquires PAs from the apocynad host during the larval stage (both sexes), and males convert a portion of sequestered PAs to volatile pheromone components to entice females (Nishida et al. 1996). In this hairpencil pheromone system, females assess the quality of protectiveness of a male during the precopulatory behavior (Nishida 2002). PA-derived pheromone

Fig. 11.7 *Idea leuconoe* female in a typical acceptance posture, visually and chemically stimulated by a paper model of male wings with a paper disk treated with the hairpencil extract (male pheromone). The female is arrested, curling her abdomen downward

system thus plays a decisive role in sexual selection of danaine butterflies (Eisner and Meinwald 1987; Nishida 2002). In indoor behavioral tests of *I. leuconoe*, both virgin males and females visually responded to a "fluttering" paper model of *I. leuconoe* (wing image printed on the copy paper) as shown in Fig. 11.7 (Nishida et al. 1996). When the model was scented with crude extracts of hair pencils from mature males (0.1 male equivalent/paper disk), receptive females usually landed on the nearby herbage with abdomen-curling posture (Fig. 11.7). Interestingly, if a fluttering "solid white model + pheromone" was presented instead of the "patterned model + pheromone," many virgin females approached and touched the model, some occasionally exhibiting the typical mate-acceptance posture, whereas none even approached a fluttering "solid black model + pheromone" (Nishida, unpublished). This clearly indicates that the females can select males by both visual and chemical cues and in lesser degrees by vision. If this is the case in nature, non-mimetic males would also be selected by a female by chance, in that the androconial chemical stimulus is sufficient to induce her acceptance, although *Idea* butterflies are basically monomorphic. This suggests an importance of chemical factors together with visual components, which then might facilitate the evolution of a new wing pattern through sexual selection (cf. Turner 1984; Krebs and West 1988).

In addition, males of *Idea* spp. also manipulate the conspicuous white/yellow eversible tufts of hair pencils on the dorsum of the abdomen for defense, by extruding them reflexively to emit a strong odor of phenol and/or *p*-cresol together with other volatiles whenever frightened by predators (Schulz and Nishida 1996). This exemplifies a male-specific aposematic scent operating concurrently with aposematic coloration of defensive organs (Nishida et al. 1996). Interestingly, one of the Idea-mimicking swallowtails, Graphium delessertii, exhibits a conspicuous yellow mark on the inner edge of the hindwings, as though an Idea male in captivity were displaying hair pencils. This could possibly evoke an additional psychological impression upon avian predators. In this case, only males become a template for the mimic.

Batesian or Müllerian mimicry – whichever the case – the evolutionary convergence of morphological characters between the models and mimics seems to have been greatly assisted by chemical elements including wing pigments and subject, concomitantly, to both natural and sexual selections.

References

Ackery PR, Vane-Wright RI (1984) Milkweed butterflies. British Museum (Natural History), London

Boppré M (1984) Chemically mediated interactions between butterflies. In: Vane-Wright RI, Ackery PR (eds) The biology of butterflies. Academic, London, pp 259–275

Boppré M (1986) Insects pharmacophagously utilizing defensive plant chemicals (pyrrolizidine alkaloids). Naturwissenschaften 73:17–26

Brower LP (1969) Ecological chemistry. Sci Am 220:22–29

Brower LP (1984) Chemical defence in butterflies. In: Vane-Wright RI, Ackery PR (eds) The biology of butterflies. Academic, London, pp 109–134

Brown KS, Benson WW (1974) Adaptive polymorphism associated with multiple Müllerian mimicry in *Heliconius numata* (Lepid.: Nymph.) Biotropica 6:205–228

Cohen JA (1985) Differences and similarities in cardenolide contents of queen and monarch butterflies in Florida and their ecological and evolutionary implications. J Chem Ecol 11:85–103

Edgar JA (1984) Parsonsieae: ancestral larval foodplants of the Danainae and Ithomiinae. In: Vane-Wright RI, Ackery PR (eds) The biology of butterflies. Academic, London, pp 91–93

Edgar JA, Boppré M, Schneider D (1979) Pyrrolizidine alkaloid storage in African and Australian danaid butterflies. Experientia 35:1447–1448

Eisner T, Meinwald J (1987) Alkaloid-derived pheromones and sexual selection in Lepidoptera. In: Prestwich GD, Blomquist GJ (eds) Pheromone biochemistry. Academic, Orlando, pp 251–269

Eisner T, Meinwald J (1995) The chemistry of sexual selection. Proc Natl Acad Sci U S A 92:50–55

Euw JV, Reichstein T, Rothschild M (1968) Aristolochic acid-I in the swallowtail butterfly *Pachliopta aristolochiae* (Fabr.) (Papilionidae). Isr J Chem 6:659–607

Fink LS, Brower LP (1981) Birds can overcome the cardenolide defense of monarch butterflies in Mexico. Nature 291:67–70

Glendinning JI, Brower LP (1990) Feeding and breeding responses of five mice species to overwintering aggregations of the monarch butterfly. J Anim Ecol 59:1091–1112

Gordon IJ, Smith DAS (1998) Genetics of the mimetic African butterfly *Hypolimnas misippus*: hindwing polymorphism. Heredity 80:62–69

Halpin C, Skelhorn J, Rowe C (2013) Predators' decisions to eat defended prey depend on the size of undefended prey. Anim Behav 85:1315–1321

Holzkamp G, Nahrstedt A (1994) Biosynthesis of cyanogenic glucosides in the Lepidoptera: Incorporation of [U-^{14}C]-2-methylpropanealdoxime, 2S-[U-^{14}C]-methylbutanealdoxime and D,L-[U-^{14}C]-*N*-hydroxyisoleucine into linamarin and lotaustralin by the larvae of *Zygaena trifolii*. Insect Biochem Molec Biol 24:161–165

Honda K (1980) Odor of a papilionid butterfly: odoriferous substances emitted by *Atrophaneura alcinous alcinous* (Lepidoptera: Papilionidae). J Chem Ecol 6:867–873

Honda K, Hayashi N, Abe F et al (1997) Pyrrolizidine alkaloids mediate host-plant recognition by ovipositing females of an Old World danaid butterfly, *Idea leuconoe*. J Chem Ecol 23:1703–1713

Huheey JE (1984) Warning coloration and mimicry. In: Bell WJ, Cardé RT (eds) Chemical ecology of insects. Chapman and Hall, London, pp 257–297

Inoue H (1978) Genus *Epicopeia* Westwood from Japan, Korea and Taiwan, Lepidoptera: Epicopeiidae. Tyo to Ga (Trans Le Soc Jpn) 29:69–75

Joron M, Mallet J (1998) Diversity in mimicry: paradox or paradigm. Trends Ecol Evol 13:461–466

Kim CS, Nishida R, Fukami H, Abe F, Yamauchi T (1994) 14-Deoxyparsonsianidine *N*-oxide: A pyrrolizidine alkaloid sequestered by the giant danaine butterfly, *Idea leuconoe*. Biosci Biotech Biochem 58:980–981

Krebs RA, West DA (1988) Female mate preference and the evolution of female-limited Batesian mimicry. Evolution 42:1101–1104

Mallet J, Joron M (1999) Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annu Rev Ecol Syst 30:201–233

Mebs D, Reuss E, Schneider M (2005) Studies on the cardenolide sequestration in African milkweed butterflies (Danaidae). Toxicon 45:581–584

Mebs D, Wagner MG, Toennes SW et al (2012) Selective sequestration of cardenolide isomers by two species of *Danaus* butterflies (Lepidoptera: Nymphalidae: Danainae). Chemoecology 22:269–272

Nahrstedt A, Davis RH (1985) Biosynthesis and quantitative relationships of the cyanogenic glucosides, linamarin and lotaustralin, in genera of the Heliconiini (Insecta: Lepidoptera). Comp Biochem Physiol 82B:745–749

Nishida R (1994/1995) Sequestration of plant secondary compounds by butterflies and moths. Chemoecology 5(6):127–138

Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. Annu Rev Entomol 47:57–92

Nishida R, Fukami H (1989) Ecological adaptation of an Aristolochiaceae-feeding swallowtail butterfly, *Atrophaneura alcinous*, to aristolochic acids. J Chem Ecol 15:2549–2563

Nishida R, Kim CS, Fukami H, Irie R (1991) Ideamine *N*-oxides: Pyrrolizidine alkaloids sequestered by a danaine butterfly, *Idea leuconoe*. Agric Biol Chem 55:1787–1797

Nishida R, Schulz S, Kim CS et al (1996) Male sex pheromone of the giant danaine butterfly, *Idea leuconoe*. J Chem Ecol 22:949–972

Ohsaki N (1995) Preferential predation of female butterflies and the evolution of Batesian mimicry. Nature 378:173–175

Ohsaki N (2005) A common mechanism explaining the evolution of female-limited and both-sex Batesian mimicry in butterflies. J Anim Ecol 74:728–734

Owen DF, Smith DAS et al (1994) Polymorphic Müllerian mimicry in a group of African butterflies: a reassessment of the relationship between *Danaus chrysippus, Acraea encedon* and *Acraea encedana* (Lepidoptera: Nymphalidae). J Zool 232:93–108

Prudic KL, Oliver JC (2008) Once a Batesian mimic, not always a Batesian mimic: mimic reverts back to ancestral phenotype when the model is absent. Proc R Soc B 275:1125–1132

Prudic KL, Khera S et al (2007) Isolation, identification, and quantification of potential defensive compounds in the viceroy butterfly and its larval host-plant, Carolina willow. J Chem Ecol 33:1149–1159

Reichstein T, Jv E, Parsons JA, Rothschild M (1968) Heart poison in the monarch butterfly. Science 161:861–866

Ritland DB, Brower LP (1991) The viceroy is not a Batesian mimic. Science 350:497–498

Rothschild M (1979) Mimicry, butterflies and plants. Symb Bot Upsal 22:82–99

Rothschild M, Jv E, Reichstein T et al (1975) Cardenolide storage in *Danaus chrysippus* (L.) with additional notes of *D. plexippus* (L.) Proc Roy Soc London (B) 190:1–31

Schneider D, Boppré M, Schneider H et al (1975) A pheromone precursor and its uptake in male *Danaus* butterflies. J Comp Physiol 97:245–256

Schulz S, Nishida R (1996) The pheromone system of the male danaine butterfly, *Idea leuconoe*. Bioorg Med Chem 4:341–349

Smith DAS (1973) Batesian mimicry between *Danaus chrysippus* and *Hypolimnas misippus* (Lepidoptera) in Tanzania. Nature 242:129–131

Smith DAS, Owen DF, Gordon IJ et al (1993) Polymorphism and evolution in the butterfly *Danaus chrysippus* L. (Lepidoptera: Danainae). Heredity 71:242–251

Su S, Lim M, Kunte L (2015) Prey from the eyes of predators: Color discriminability of aposematic and mimetic butterflies from an avian visual perspective. Evolution 69:2985–2994

Trigo JR (2000) The chemistry of antipredator defense by secondary compounds in neotropical Lepidoptera: facts, perspectives and caveats. J Braz Chem Soc 11:551–561

Turner JRG (1978) Why male butterflies are non-mimetic: natural selection, sexual selection, group selection, modification and sieving. Biol J Linn Soc 10:385–432

Turner JRG (1984) Mimicry: the palatability spectrum and its consequences. In: Vane-Wright RI, Ackery PR (eds) The biology of butterflies. Academic, London, pp 141–161

Uésugi K (1996) The adaptive significance of Batesian mimicry in the swallowtail butterfly, *Papilio polytes* (Insecta, Papilionidae): Associative learning in a predator. Ethology 102:762–775

Wickler W (1968) Mimicry in plants and animals. Wiedenfeld and Nicholson, London

Wu TS, Leu YL, Chan YY (2000) Aristolochic acids as a defensive substance for the aristolochiaceous plant-feeding swallowtail butterfly, *Pachliopta aristolochiae interpositus*. J Chin Chem Soc 47:221–226

Yata O, Morishita K (1985) Butterflies of the South East Asian Islands, vol II. Pieridae and Danaidae. Plapac Co Ltd, Tokyo