Chemistry at the Solanaceae/Ithomiinae Interface

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CHEMISTRY AT THE SOLANACEAE/ITHOMIINAE INTERFACE[1,2]

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ABSTRACT

The secondary chemical constituents of 42 species in sixteen genera of Solanaceae, of five species in three genera of Apocynaceae-Parsonsieae, and of 142 species in 45 genera of Ithomiinae (Lepidoptera: Nymphalidae) whose larvae feed on these plants, have been analyzed and compared in a standardized manner. Large orb spiders (*Nephila clavipes*), which cut field-caught adult Ithomiinae out of their webs, were used to assay fractions for defensive substances eventually present in or derived from larval food plants; the fractions were applied externally to the palatable nymphaline butterfly *Biblis hyperia*. All extracts and fractions from Solanaceous plants were inactive, not defending *Biblis* against predation by *Nephila*. The principal defensive compounds of adult Ithomiinae (also found in eggs and occasionally in Apocynaceae used by some Ithomiinae larvae) are dehydropyrrolizidine alkaloid (PA) monoesters and their N-oxides, stored in up to 20% of dry weight. These compounds are obtained from a variety of natural sources by the adults after emergence from the pupa (freshly hatched individuals of both sexes in 26 genera were readily eaten by *Nephila*), especially from decomposing Boraginaceae-Heliotropoideae and flowers of Compositae-Eupatorieae, for which they serve as pollinators. *Trichogonia*, *Adenostemma*, and many *Eupatorium* flowers were confirmed as rich sources of the alkaloids, showing characteristics indicating long periods of coevolution in obligatory mutualism with the ithomiine pollinators. The highly diversified and variable Solanaceae toxins seem not to be stored and used for defense by the Ithomiinae, but may be important in mediating larval feeding preferences and oviposition in the butterflies, which show appreciable chemical specificity in host plant preference. Patterns of ithomiine larval use of the Solanaceae at the generic level, however, do not indicate parallel phylogenetic diversification of the two groups. An alternative hypothesis points to sequential adaptation by radiating lines of Ithomiinae to different classes of chemical toxins in the already diversified Solanaceae. By obtaining their PAs, necessary for their defense and reproduction (pheromone synthesis), from a source stabilized by mutualism, the Ithomiinae avoid dependence on the Solanaceae chemicals, constantly destabilized by divergent selection in the antagonistic system. Quantitative determination of PA concentrations in parts of different Ithomiinae and Eupatorieae permits the drawing of a diagram for the flow of these substances in natural ecosystems.

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[1] Presented in part at the Second International Symposium on the Biology and Systematics of the Solanaceae (Missouri Botanical Garden, August 1983), at the Second Gordon Research Conference on Chemical Aspects of Plant-Herbivore Interactions (Ventura, California, January 1983), at the Jardim Botânico do Rio de Janeiro (April 1983), at the 35th and 36th Annual Reunions of the Sociedade Brasileira para o Progresso da Ciência (Belém, Pará, July 1983 and São Paulo, July 1984), and at the first meeting of the International Society of Chemical Ecology (Austin, Texas, June 1984). For a preliminary communication, see Brown (1984); detailed analysis of the insect side of the interface is presented in Brown (1985).

[2] I am especially grateful to Prof. João Vasconcellos-Neto for providing the fundamental biological background for the analysis of the interface and the testing of chemical fractions with *Nephila clavipes*. An earlier version of this paper was read and criticized by T. M. Lewinsohn, W. W. Benson, L. E. Gilbert, M. Rothschild, and J. A. Edgar. R. F. D'Almeida, O. H. H. Mielke, and Gerardo Lamas M. stimulated my interest in the Ithomiinae, and special attention to the interface with Solanaceae was infused by W. W. Benson, L. E. Gilbert, and B. A. Drummond III. W. G. D'Arcy, M. Nee, M. Whalen, D. Costich, L. A. F. de Carvalho, and especially S. Knapp were most helpful with systematic aspects of the Solanaceae, and H. F. Leitão Fo with the Compositae. Silvana Aparecida Henriques assisted greatly in extraction and fractionation (Table 3), and José Roberto Trigo and Renata S. C. Dias helped in field study of the Ithomiinae/Solanaceae interaction. T. Eisner provided information and reprints about the *Utetheisa*/PA/*Nephila* interaction first studied by him and his students in Florida. R. Forster and D. A. Monteiro facilitated access to field sites in the Fazenda Santa Elisa, Instituto Agronômico de Campinas. A. and C. Kascheres provided important services in NMR and mass spectra at the Instituto de Química, UNICAMP, and I. Valio and L. Sodek permitted extensive use of the Micronal colorimeter in the Departamento de Fisiologia Vegetal, UNICAMP. Chemistry of the Solanaceae was lucidly presented to me by W. Evans, K. Schreiber, D. Lavie, and J. G. Roddick. I especially thank L. P. Brower, J. A. Edgar, and M. Rothschild for help in understanding chemical ecology, pyrrolizidine alkaloids, and bioassay techniques. FAPESP contributed to the original installation of the Laboratório de Ecologia Química (project 74/0544) and Rhodia S. A./FUNCAMP to its reinstallation. The CNPq provided a research fellowship.

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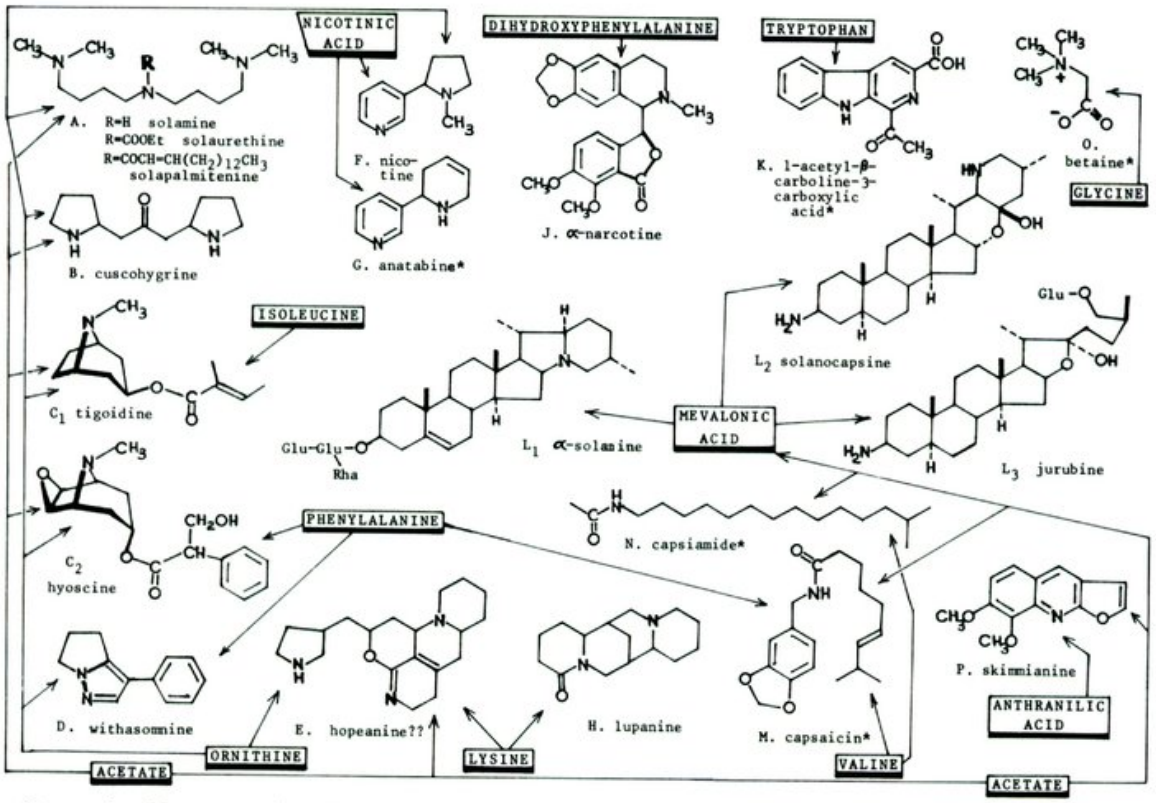


FIGURE 1. Representative alkaloids known from the Solanaceae, with probable biosynthetic pathways; see Table 2 for occurrence in genera. Asterisked alkaloid types are not known yet in leaves of natural Ithomiinae host plants.

Plants in the cosmopolitan family Solanaceae are widely known for their toxic qualities derived from an exceptionally diverse suite of alkaloids (Fig. 1) and steroidal bitter principles, terpenes, saponins, and phenolic glycosides (Fig. 2; Evans, 1979; Schreiber, 1979; Harborne & Swain, 1979; Kirson & Glotter, 1982). Such a rich larder of potential poisons could be expected to repel, deter, or intoxicate almost all herbivores while causing a few to become specialists; the specialists might be predicted to sequester the Solanaceae poisons and use them in their own defense against predators. In fact, the herbivorous insects found on Solanaceae leaves in the Neotropics are often restricted to the family and some of them are brightly colored as if to suggest unpalatability (Table 1). These form ideal systems for the investigation of biochemical ecology and coevolution (Brown, 1980).

One of the most important groups of Solanaceae herbivores in the Neotropics is butterfly larvae of the nymphalid subfamily Ithomiinae (Drummond & Brown, 1987). The brightly colored adults are regarded as prime distasteful movers in regional insect mimicry complexes (Brown, 1979, 1987; Brown & Benson, 1974). They even pull in other less abundant and perhaps less protected, but typical aposematic butterflies such as Heliconiini (Brown, 1972a; Brown & Benson, 1974), Danainae-Itunini (Brown, 1987), Acraeinae (Brown & Benson, 1974), and Troidini (which are usually central models of their own mimicry rings), as well as myriad Batesian mimics (Satyrinae, Nymphalinae, Charaxinae, Riodininae, Pierinae, Dismorphiinae, Hesperiidae, Castniidae, Geometridae, Dioptidae, Arctiinae and Pericopinae—the last two possibly distasteful Müllerian mimics—and members of other insect orders such as Odonata and Homoptera). Adult Ithomiinae were involved in the original proposals of Batesian and Müllerian mimicry and have been shown to be rejected by a variety of vertebrate predators (Bates, 1862; Belt, 1889; Brower & Brower, 1964; Haber, 1978; Coimbra-Filho, 1981). One known exception (Brown & Vasconcellos-Neto, 1976) involves complex learning behavior by a single tanager population (*Pipraeidea melanonota*), which squeezes out fatty abdominal contents of individuals in winter reproductive diapause dur-

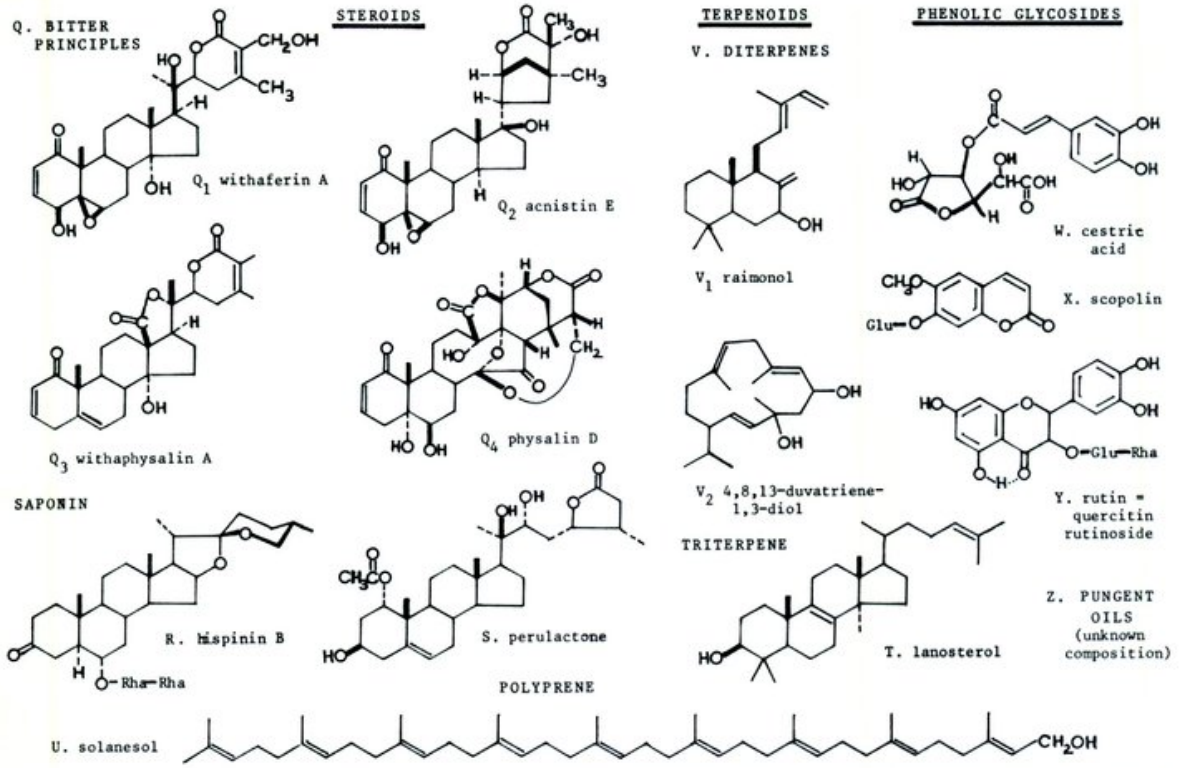


FIGURE 2. Examples of non-nitrogenous secondary compounds found in Solanaceae used as larval hosts by Ithomiinae. Carotenoids and other pigments are not included; for flavonoids, see Harborne & Swain (1979). See Table 2 for distributions.

ing the cold of early morning, treating them as it does fruits with a bitter rind. Similar learned behavior has been observed in jays (Corvidae) eating ithomiines in Costa Rica (R. Hagen, pers. comm.), as well as in orioles and grosbeaks attacking wintering monarchs (*Danaus plexippus*) in central Mexico (Calvert et al., 1979; Fink & Brower, 1981). Like most aposematic insects, Ithomiinae have tough and resilient bodies; surviving individuals squeezed by the tanager were often captured in flight one or more days after attack. They often remain on the forest floor for weeks after death, avoided by predatory and scavenging ants. Even the giant tropical orb spider *Nephila clavipes*, which often clutters the airspace of ithomiine colonies with broad sticky webs and takes most aposematic butterflies with typical rapaciousness (Vasconcellos-Neto & Lewinsohn, 1982, 1984), cuts out all Ithomiinae from its web rapidly (10 sec.–2 min.) after contact with any part of the body or wings. Other spiders, especially flower-frequenting Thomisidae (crab spiders), may take Ithomiinae regularly, however (Drummond, 1976; pers. obs.).

It has been suggested frequently that the protection of adult Ithomiinae against predators is due to alkaloids or other toxic chemicals sequestered and stored by the larvae from Solanaceae and passed on to the adult (Brower & Brower, 1964, p. 154; Young, 1972, p. 291; Drummond, 1976, p. 268, 1981, p. 63; Brown, 1980). A good precedent for this suggestion exists in the storage of cardiac glycosides (Fig. 3A) by larvae of Danainae—sister-group to the Ithomiinae (Ackery & Vane-Wright, 1984)—which are transmitted to adults and help protect them against avian and other predators (Brower et al., 1967; Brower, 1969; Brower & Glazier, 1975). Nevertheless, no evidence has been obtained yet for the presence of any Solanaceae secondary chemical sequestered naturally into the tissues of any herbivore. Indeed, all results reported by Rothschild (1973) showed metabolism and excretion of Solanaceae alkaloids by specialist herbivores; a later experiment showed retention of tropanes in *Manduca sexta* (tobacco hornworm) (Rothschild et al., 1979), but this herbivore does not normally encounter these insecticidal compounds in nature. Since Solanaceae alkaloids and steroids are relatively stable compounds, abundantly available to Ithomiinae through the larval food plant and toxic enough to be eminently suit-

TABLE 1. Important herbivores specialized to Solanaceae (other than *Nicotiana*) in Brazil. (Source: personal observations and A. G. d'Araújo e Silva et al., Quarto Catalog dos Insetos que Vivem nas Plantas do Brasil (1968).)

Insect Order	Family and Subfamily	Genera	Occurrence on Solanaceae	Degree of Specialization (Sp. Level)	Damage to Plants	Aposematic?
ORTHOPTERA	Acrididae: Romaleinae	*Chromacris* and others	Frequent	Low to moderate	Heavy	Many are
THYSANOPTERA	Various families		Infrequent	Low to moderate	Moderate	Rarely
HEMIPTERA	Miridae		Frequent	High	Moderate	Some are
	Coreidae	*Phthia, Hypselonotus*	Frequent	High	Moderate	Most are
	Lygaeidae + Pyrrhocoridae		Infrequent	Moderate to high	Light	Yes
	Pentatomidae[a]	*Runibia*	Infrequent	High	Light	Yes
	Tingidae	Many genera	Infrequent	High	Light	Some are
HOMOPTERA	Cicadellidae	Many genera	Infrequent	Moderate	Moderate	Many are
	Membracidae	Several genera	Infrequent	Moderate	Light	Some are
	Aleyrodidae		Moderate	Low	Moderate	Not
	Aphididae	Several genera	Frequent	Moderate	Moderate	Rarely
	Coccoidea: various families		Moderate	Low	Moderate	Not
LEPIDOPTERA	Hepialidae	*Phassus*	Rare	Moderate	Heavy (borer)	Unlikely
	Geometridae	*Hammaptera*	Rare	High	Light	Probably
	Arctiidae (including Ctenuchinae)	Various genera	Infrequent	Moderate	Moderate	Yes
	Noctuidae	*Gonodonta*	Rare	High	Moderate	Yes
	Pyralidae: Chrysauginae	Several genera	Rare	High	Light	Some are
	Sphingidae	*Manduca, Hyles*	Infrequent	High	Heavy	Adults + Larvae
	Nymphalidae: Ithomiinae	Over 30 genera	Frequent	High	Heavy	Adults yes
COLEOPTERA	Chrysomelidae: eight subfamilies	About 20 genera	Frequent	Usually high	Heavy	Most are
	Coccinellidae	*Epilachna*	Infrequent	Moderate	Moderate	Yes
	Curculionidae: Brachyderinae, Cleoninae	6 genera	Infrequent	Moderate	Light	Rarely
	Scarabeidae	Many genera	Rare, flowers	Low	Moderate	Rarely
	Meloidae	*Epicauta, Lytta*	Frequent	Moderate	Heavy	Some are
DIPTERA	Agromyzidae and others	Several genera	Rare	Moderate	Light	Not

[a] Many Pentatomid predators are often found patrolling Solanaceae; they do not attack the plants, but the herbivores.

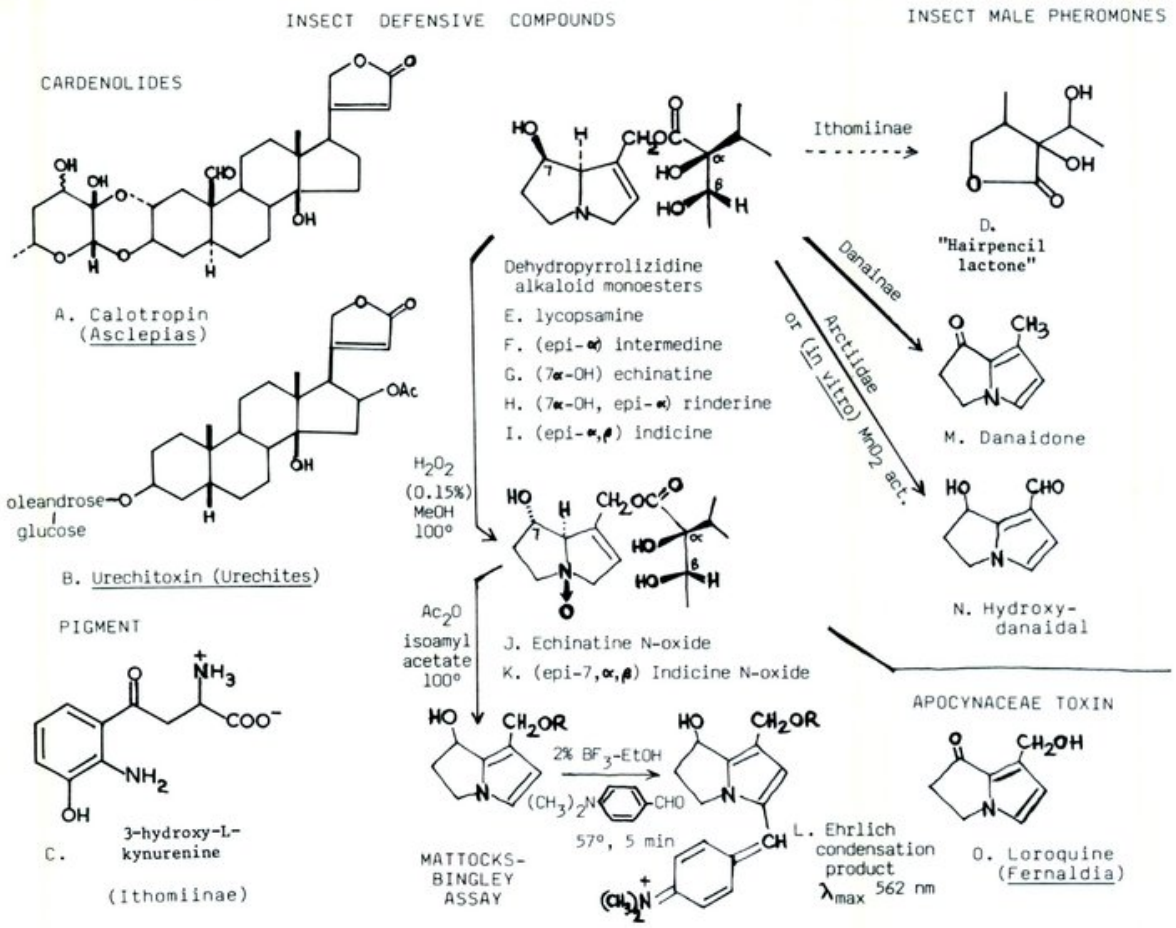


FIGURE 3. Danaine and ithomiine pheromones and defensive compounds, including cardiac glycosides (A, B), a pigment (C), male sex pheromones (D, M, N), dehydropyrrolizidine alkaloids (E–I) and their N-oxides (J, K), the reaction sequence and final product (L) of the Mattocks-Bingley assay, and Apocynaceae toxins (B, E, O). Compounds M, N, O are dihydropyrrolizines with carbonyl conjugation. See text for explanations.

able for protection against predators, their absence in Solanaceae-feeding insects was quite a disappointment (Rochschild, 1973). The only known "secondary chemicals" isolated from the Ithomiinae before this work were the amino-acid yellow pigment 3-hydroxy-*L*-kynurenine (Fig. 3C; Brown, 1967; Brown & Domingues, 1970) and a pheromonal lactone related to some esterifying acids of pyrrolizidine alkaloids (Fig. 3D), as well as the presumed precursors of the latter (Fig. 3E, F) (Edgar et al., 1976).

Especially interesting to find in adult Ithomiinae were these latter compounds (dehydropyrrolizidine alkaloids, or PAs, Fig. 3E–K), since they have been shown to be very important in both defense and reproduction of the butterflies in the sister group Danainae (Edgar & Culvenor, 1974; Schneider et al., 1975; Schneider, 1977; Rothschild & Marsh, 1978; Boppré, 1978; Edgar et al., 1979; Edgar, 1982). Unlike the rather sporadically stored cardenolides (Rothschild et al., 1975; Dixon et al., 1978; Malcolm & Rothschild, 1983; Fig. 3A, B), PAs are universally present in field-captured adult Danainae, which actively seek them out and sequester them (Boppré, 1984) from flower nectar, plant exudates, and even other insects (Bernays et al., 1977). Some danaines (*Euploea*) also obtain PAs from apocynaceous larval food plants (Parsonsieae: *Parsonsia*; Edgar, 1982). Larvae of a primitive ithomiine isolated in the New Guinea region, *Tellervo zoilus*, feed on these same plants; adults contain PAs presumably derived from *Parsonsia* (Edgar, 1982), as do adults of a primitive Central American ithomiine (*Tithorea tarricina*) whose larvae feed on *Prestonia* (Apocynaceae: Parsonsieae) containing the same PA found in the butterflies (Fig. 3E; Edgar, 1982; Edgar & Harvey, in prep.).

The purpose of this paper is to examine in detail, by chemical analysis and field bioassay,

three basic questions related to the specific interaction between Neotropical Ithomiinae and their Solanaceae larval host plants. These questions derive from conventional aspects of insect-plant coevolutionary theory, especially as applied (with much success) to aposematic insects on poisonous plants (Ehrlich & Raven, 1965; Benson et al., 1976; Brown, 1980):

(1) To the extent that host plant specificity may exist in the Ithomiinae/Solanaceae relationship at generic and specific levels, how does it relate to chemical mediation of cues for oviposition and larval feeding?

(2) How might such chemical specificity reflect mutual interaction of these two groups over long evolutionary time, leading to diversification in both groups as a function of reciprocal selective pressures—that is, what is the evidence for coherent patterns of biochemical or "parallel diversification" coevolution?

(3) To what extent do the Ithomiinae use the poisonous secondary chemicals of their Solanaceae larval hosts for their own defense against predators in any stage of their life cycle?

A preliminary phylogenetic (Brown, in prep.) and chemical (Brown, 1984, 1985) survey of the Solanaceae/Ithomiinae interface, accompanied by an efficient spider bioassay for predator-defense compounds, has indicated surprisingly that, while the first question seems to merit an affirmative response, the other two questions must be answered by "little or no evidence in favor." The Ithomiinae apparently colonized the Solanaceae well after the generic diversification of this plant family in the New World, tolerating and using the diverse secondary chemicals (Figs. 1, 2) for oviposition cues to regulate specificity, but not for defense. Efficient chemical protection of adult Ithomiinae is generally unrelated to toxins encountered by larvae but not stored; instead, the adults seek out PAs in flower nectar and decomposing leaves, using them not only in pheromone synthesis (Fig. 3D) but also in defense, exactly as in the Danainae. The results of this survey are reported here, along with details of chemistry, bioassay, host plant specificity, and alternative ecological and physiological factors in the relationship.

MATERIALS AND METHODS

STUDY SITES

Field observations of interactions between Solanaceae and their herbivores were undertaken principally in two very different artificial tropical forest systems in the Fazenda Santa Elisa, Instituto Agronômico de Campinas, São Paulo (600 m elevation): Monjolinho (a forest garden of native plants) and Amarais (an old eucalyptus forest with natural under- and middlestory) (see Brown et al., 1981, for photos, maps, and description). Supplementary observations were made in Sumaré, SP (similar to Amarais, 550 m); Martinho Prado, SP (floodable riverine forest, 400 m); Serra do Japi, Jundiaí, SP (native seasonal and montane forest on rocky soils, 850–1,150 m); Serra Negra, SP (humid montane forest, 1,000 m); Bertioga, São Vicente and Mongagua, coastal São Paulo (perhumid tropical forest, near sea level); Poços de Caldas, Minas Gerais (montane forest, 1,200 m); coastal Rio de Janeiro and Espírito Santo (disturbed tropical rain forest, sea level to 800 m); Goiânia, Goiás (riverine thicket, 800 m); various parts of Rondônia, SW Brazil (seasonal forest on rich soils, 100–650 m); various parts of NE and S Pará, Brazil (tropical forest, sea level to 800 m); the region north of Manaus, Amazonas, Brazil (disturbed tropical rain forest on poor soils, 50 m); and in northern Venezuela (deciduous and cloud forest, sea level to 1,400 m). Some limited data have also been obtained in Mexico, Panama, Colombia, Ecuador, and Peru.

JUVENILE BIOLOGY

Ecological observations in the field were conducted with the aid of binoculars and suitable recipients for living adults and larvae (for details see Brown & Benson, 1974, and Brown, 1972b). Identification is secure for adult ithomiines, at least 90% certain for juveniles, and secure for Solanaceae host plants at the genus or section level but still uncertain in a few cases at the species level. Early stages of ithomiines were brought into the laboratory when necessary and reared to adults in glass or plastic dishes on separated leaves of natural or experimental host plants, kept subhumid. At least one fertile egg could usually be expressed from any field-captured reproductive female ithomiine by careful pressure on the abdomen with thumb and forefinger, working backwards slowly from the fourth segment. Such eggs were kept with expected or potential food plants until hatching (3–9 days). A large number of potential food plants could be found in Monjolinho or other areas in Campinas; many others were cultivated as available in my garden.

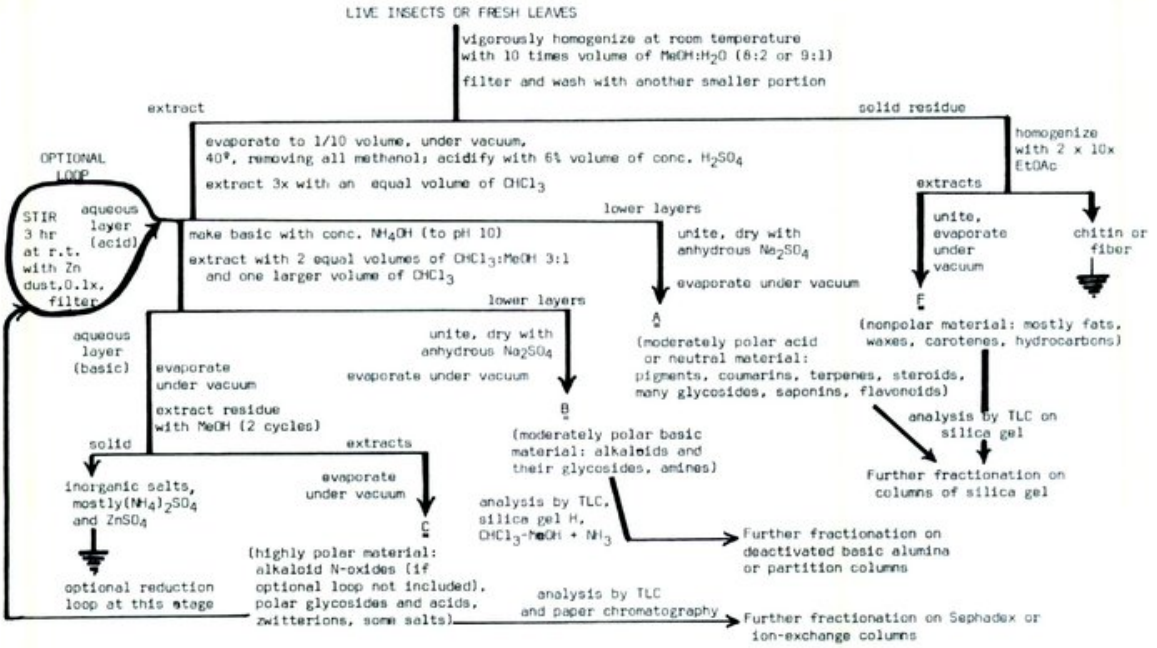


FIGURE 4. Mild and rapid standardized fractionation scheme for fresh leaves, flowers or insects, adapted to alkaloid-containing materials, giving fractions for chemical and ecological analysis and assay.

CHEMICAL FRACTIONATION

A standard extraction and fractionation was developed for all plant or animal material (Fig. 4). Leaves or flowers of plants observed to be used by Ithomiinae larvae or adults were separated from petioles or peduncles and maintained fresh until extraction; juvenile and adult Ithomiinae were kept alive in dishes or envelopes until the moment of extraction. Thus, all extractions were performed on fresh and recently functioning living tissues. The method was adapted to rapidly remove all organic substances of moderate to high polarity and at the same time deactivate enzymes in the living material; stable nonpolar compounds were removed afterwards. The initial use of heat, aeration, acids, bases, or acetone (which can condense with primary amines) was avoided. Exhaustive extraction was sacrificed in favor of rapidity; the more labile polar compounds (especially glycosides) were immediately and efficiently dissolved by vigorous maceration in the first extracting solvent added to fresh material. The leaves, flowers, or insects were divided if necessary into pieces of no more than 2 cm maximum linear dimension by scissors, already under or falling directly into methanol-water 8 : 2 (usual for leaves to extract less chlorophyll) or 9 : 1 (for adult insects), using about ten times the volume in milliliters as the fresh weight of material to be extracted. The extraction was immediately completed with scissors (if less than 40 ml) or with a kitchen homogenizer (Braun "Mini-Pimer" with a two-blade metal propellor) introduced into the suspension from above. Within a minute, leaves were reduced to tiny fragments and insects to pieces of chitin, partly coated with a fatty layer. The extract was homogenized for 1–3 minutes and filtered under suction; the insoluble material was washed and stirred with a further amount of aqueous methanol, up to half the original volume. Nonpolar compounds (fats, waxes, carotenes, some terpenes, and free flavonoids), insoluble in aqueous methanol, were immediately extracted from the residue with two portions (each 5× original fresh weight) of ethyl acetate, giving a "fat fraction" (F) and leaving only polymeric organic fiber or plaque (cellulose, lignin, chitin).

The filtered and united aqueous-methanolic extracts were evaporated under vacuum at less than 40°C until all the methanol was removed. The resulting aqueous suspension (at times after filtration, giving a chlorophyll or nonpolar fraction F′) was acidified with conc. sulfuric acid (roughly 6% of the total volume, making it about 2 N = 1 M in acid) and extracted three times with an equal volume of chloroform. If occasional emulsions encountered at this or later stages (especially in some plant extracts) did not separate after 6 hours, they were filtered or broken

with MeOH. The organic layers were united, dried with anhydrous granular sodium sulfate, filtered, and evaporated under vacuum to dryness to give a relatively more polar but nonalkaloidal fraction (A), often including some chlorophyll from leaves (usually little soluble in aqueous methanol) plus terpenoids and steroids (including most cardiac glycosides, withanolides, and saponins), flavonoids, coumarins, and organic acids (Fig. 2). Half or all of the aqueous phase was then made strongly basic (pH above 10) with conc. ammonium hydroxide and extracted with two equal volumes of chloroform-methanol 3 : 1 and one to three volumes (1.5×) of chloroform; this gave effective partition between water-methanol 8 : 1 and chloroform-methanol 6 : 1, obviating salting out of polar organic constituents from the upper layer. The organic layers were combined, dried, and evaporated as above to give a moderately polar alkaloid fraction (B) containing glycoalkaloids, tropanes, pyrrolizidines, and simpler bases (Figs. 1, 3). The remaining aqueous phase retained alkaloid N-oxides, very polar glycosides, more polar acids, and salts and sugars; it was directly evaporated and extracted with methanol (two cycles with filtration) to give a polar extract (C) and leave insoluble organic salts, mostly ammonium sulfate. If the alkaloids corresponding to the N-oxides were desired, the other half of the acidic solution (after $CHCl_3$ extraction, 2 N in H_2SO_4) or fraction C after dissolution in 2 N H_2SO_4 was reduced by stirring for 2 hours at room temperature with an excess of zinc dust (usually in grams equal to one tenth of solution volume), filtered (paper cone, gravity funnel), alcalinized with an excess of conc. NH_4OH (until all zinc salts dissolved as the zinc-ammonia complex), and extracted as above with $CHCl_3$-MeOH (2×) and $CHCl_3$. If this gave a much greater weight of alkaloid than in unreduced extracts, significant amounts of N-oxide were indicated. The complete extraction and fractionation scheme is illustrated in Figure 4.

Crude or purified fractions were analyzed by NMR spectra in $CDCl_3$ + 1% TMS, on the Varian T-60 of the Instituto de Química, UNICAMP, or eventually by IR spectra (in $CHCl_3$ or KBr) on a Perkin-Elmer Infracord. Mass spectra were performed by Mrs. Concetta Kascheres on the Varian MAT 311A instrument of the Instituto de Química, at 70 eV, probe temperature 60–105°C. Chromatographic analysis on TLC plates (coated microscope slides) used silica gel H (no binder) and varying amounts of chloroform-methanol or benzene-ethyl acetate, plus 1% NH_4OH for alkaloidal fractions; other systems used ethanol, acetone, and amines. Separations on adsorption columns (silica gel or alumina activity III were usual) followed results of the TLC analysis and used careful gradient elution. Alkaloids were purified by partition chromatography with the system ethanol-chloroform-water (usually 1 : 9 : 1), stationary (upper) phase 0.75× (V/W) on Celite 545 and including phenol red as indicator (Brown & Kupchan, 1962) or chloroform and buffer solutions.

DETERMINATION OF PAS

Dehydropyrrolizidine alkaloids (PAs) were quantitatively assayed in fresh plant or insect material (they are destroyed after death or drying), cut up under absolute methanol (at least 10×), and, after at least one day, colorimetric determination on aliquots (usually 1/20, preferably containing no more than 100 μg PA; 40 μg gave initial absorbance off scale (> 2.0) and the Beer's law curve became unreliable above 150 μg) with a modification of the Mattocks (1967, 1968)-Bingley (1968) method. The assay is very sensitive as modified (< 0.1 μg is easily seen), very accurate for N-oxides (± 1%), reasonably accurate for total PA (± 10%), and extremely specific and applicable on total plant or insect extracts with minimal interference by other components; the final product of Ehrlich condensation shows λ_{max} 561.5 nm (in isoamyl acetate-ethanol-acetone about 1 : 1 : 2 with 2% BF_3) with $\epsilon \sim 57{,}000$ (Fig. 3L). For total PA determination, the aliquot is treated with 0.5 ml of freshly prepared 0.15% methanolic H_2O_2 (from 200 : 1 dilution of 30% aqueous H_2O_2 stabilized with 5 mg/ml of $Na_4P_2O_7$) and heated in a boiling water bath for 30 minutes, followed by drying with hot air for 5 minutes. This procedure destroys about 40 ± 5% of the total alkaloid N-oxide formed, but milder methods of oxidation give incomplete reaction or difficulties in the following steps. The resulting completely dry N-oxide (or an aliquot of the original extract air-dried at 80°C) is taken up in 1.0 ml isoamyl acetate, treated with 0.125 ml acetic anhydride, and heated 2 minutes in the boiling water bath. After being cooled to room temperature, the solution is treated with 1.0 ml of a solution of absolute ethanol : 20% BF_3-methanol : *p*-dimethylaminobenzaldehyde (50 : 5 : 1) and heated at 56–59°C for five minutes. Absorbance is read against a blank which passed through all the reactions, at 561.5 nm, after di-

lution to 3.8 ml with anhydrous acetone; further dilution with acetone gives a linear decrease in absorbance. At room temperature absorbance reaches a maximum several hours after the last reaction, about 5% above the reading immediately after it, and then diminishes; it may be maintained for many days at 4°C. Each microgram of PA in the original aliquot contributes to the absorbance 0.05 (N-oxide) or 0.03 (total base) (standard curves with echinatine and lycopsamine samples, Fig. 3G or E, provided by J. A. Edgar). Determinations were routinely done in racks of 40 13 × 100 mm glass test tubes, always doing total base and N-oxide determinations in the same batch to equalize the initial aliquot sampling and give an internal control on abnormal results. Separate pipets and bulbs should be used for each reagent in the series; water must be rigorously eliminated from the test tubes before the Polonovski reaction (Ac_2O-isoamyl acetate).

PAs with a carbonyl group conjugated with the unsaturated ring (7-ketones, or 9-aldehydes or carboxylates, such as Fig. 3M–O) or with no double bond in the ring did not give any blue color with this method. Dihydropyrrolizines (with the nucleus of Fig. 3M, N) without carbonyl conjugation could be directly determined on aliquots to an accuracy of about ± 10% using the final reaction in the series. They were usually destroyed in the first reaction step (oxidation) if present in the extract.

NEPHILA BIOASSAY

Fractions were bioassayed for predator-defense compounds, following observations of Vasconcellos-Neto and Lewinsohn (1982, 1984), with natural populations of the giant tropical orb spider *Nephila clavipes* (L.). This predator, common from December through July in Campinas (the same period as that of maximum ithomiine density in the same places) and present in small numbers year round, cuts out of its web, alive and unharmed, any field-captured ithomiine that is introduced naturally or experimentally, after contact with any part of body or wings. If the butterfly struggles excessively (ithomiines generally remain quiet, awaiting liberation), it may be attacked or wrapped in silk, but the spider does not suck it (Vasconcellos-Neto & Lewinsohn, 1982, 1984). *Nephila* spiders are among the most abundant, effective, and aggressive potential predators of flying insects in Campinas and throughout the more seasonal Neotropics, and can be safely regarded as very important in the evolution of predator defense mechanisms in the Ithomiinae. Vertebrate predators have not been used yet in the bioassay, but they were shown to reject an ithomiine by Brower & Brower (1964), to reject almost all ithomiines by Haber (1978), to reject two ithomiines by Coimbra-Filho (1981), and to accept only fatty abdominal contents of ithomiines in Brazil by Brown & Vasconcellos-Neto (1976).

Living individuals of the palatable nymphaline butterfly *Biblis hyperia* (Cr.), readily attacked and eaten by the spider, were painted with aqueous solutions, suspensions, or emulsions of fractions or compounds (corresponding to 0.5–1.5 butterfly, 2 g fresh weight of leaves, or 0.2 g fresh weight of flowers), covering the entire body, legs, antennae, and wing bases to at least half the radius, and let dry. Still alive, they were thrown into part of the web of an adult, non-satiated, and healthy spider (as judged by web structure), from a distance of 0.5–1.5 m on the side opposite the spider, between 1200 hours and 1500 hours on a warm sunny day. The spider normally advanced immediately to attack the butterfly (delays of up to 5 minutes can occur if the spider is "dozing" or distracted). If the *Biblis* was punctured, sucked, and then cut out or wrapped in silk by the spider, or (to still its struggles) was bitten, wrapped in silk, cut out, and taken to another part of the web and sucked, the test was regarded as negative. All such tests were repeated at least twice with different spiders and different individuals of *Biblis*. A positive test consisted of the spider's drawing back from the animal, at times inspecting various parts with its sensory palps but not biting or sucking, and eventually cutting out the living butterfly by breaking all necessary strands (usually cutting them with the third pair of legs), manipulating the entire insect and letting it drop unharmed. This test, usually repeated at least three times with at least two *Biblis*, was regarded as strong evidence for effective predator-deterrent compounds in the extract.

Biblis is common year round in most parts of the Neotropics, not especially fast- or high-flying, and readily attracted to fermenting baits. It is brightly colored black and red, regarded as an "incipient mimic" by the Browers (1964; Brower, 1969; Brower et al., 1971). Its larva feeds upon *Tragia*, an urticant euphorbiaceous vine, and the adult possesses dorsal scent glands in the abdomen that it displays in evident defensive

behavior when handled. Nevertheless, it is readily eaten by *Nephila* and thus is an ideal substrate for the bioassay of chemical fractions. Alternative organisms used with similar success in the bioassay include some pierids (*Eurema*) and *Heliconius erato*, also eaten by the spider (Vasconcellos-Neto & Lewinsohn, 1982, 1984).

CHEMICAL COMMUNICATION AND COEVOLUTION AT THE SOLANACEAE/ITHOMIINAE INTERFACE

HOST PLANT UTILIZATION PATTERNS

In the principal field sites around Campinas, SP, at least 42 species of Solanaceae are used (or potentially should be used, by analogy with other regions) by larval Ithomiinae (Figs. 5–8). A further four species have been inspected frequently but show no signs of usage: *Capsicum praetermissum* Heiser & P. G. Smith, *Lycianthes rantonnetii* Carr. ex Lescuyer, *Solanum wendlandii* Hook. f. and *Solanum americanum* Mill. Many additional species occur in nearby field sites (at least 15 more species of *Solanum*) or in gardens.

Six of the 25 species (in 17 genera and 11 of the 13 neotropical tribes) of Ithomiinae known from the Campinas region (Figs. 5, 9) can be regarded as only occasional visitors (the two *Melinaea, Episcada philoclea, Pseudoscada quadrifasciata*, and *Hypoleria adasa* and *goiana*); these possibly do not find enough adequate host plants available to establish permanent populations. At least 15 additional species in as many genera occur in healthy populations within 200 km of Campinas, often in different forest types, and may eventually be recorded in the region. Two of the 19 species regularly present feed only on Apocynaceae-Parsonsieae (Fig. 5). Thirty-six species of solanaceous food plants have been recorded for 14 of the remaining 17 species and may be confidently predicted for the rest (Fig. 5); no species is monophagous but only *Mechanitis* (and possibly *Pteronymia*) could be called polyphagous (the rest are best regarded as quite narrowly oligophagous, especially in the chemical sense, as will be shown below).

This pattern is a microcosm of the general picture of food plant usage by the Ithomiinae (see Drummond & Brown, 1987), comprising about 400 species × species interactions. Monophagy or narrow oligophagy is the rule at the present level of information, not only for local populations but also for whole genera. A summary of all these data (Table 2) with chemical information added from many sources in the literature (with preliminary verification in this work, see Table 3) shows a reasonable specificity in the interactions between Ithomiinae and Solanaceae, at least at the level of genera of ovipositing females and host plants. Most of the ithomiine genera are known at present from only one or two Solanaceae genera or subgenera of *Solanum*; even generalists like the common and ubiquitous *Mechanitis* show a strong preference for only two subgenera of *Solanum* in most sites.

The records are still incomplete, however. Ithomiine ovipositions are not commonly observed, and 23 of the 52 genera are still represented by three or fewer confirmed food plant records. The possibility cannot be discounted that the specificity patterns shown in Table 2 are primarily due to lack of adequate information. Confident patterns of food plant specificity are seen only in the well-documented interactions of the

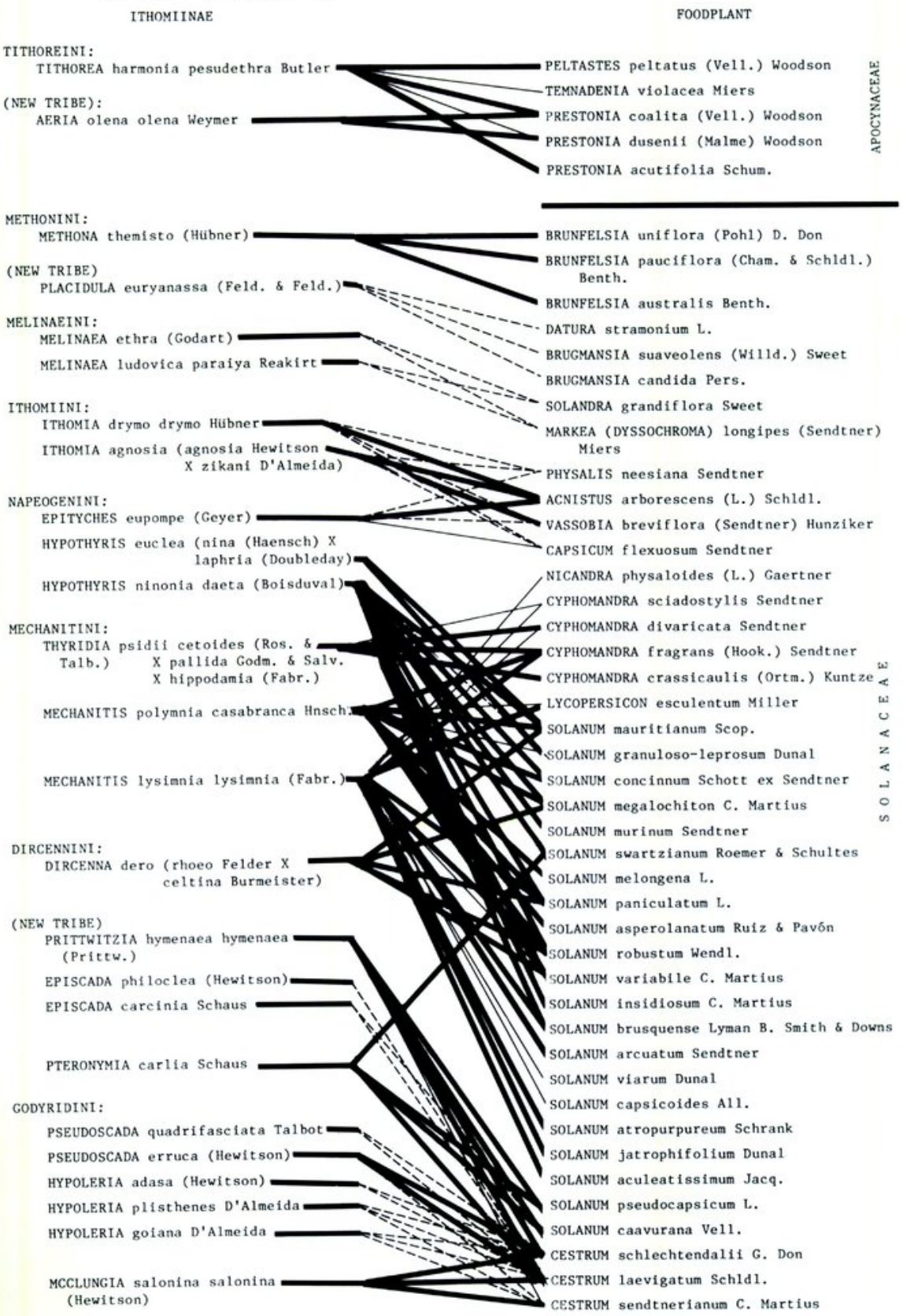
→

FIGURE 5. Known and expected food plant relationships of Ithomiinae in the Campinas region, São Paulo. Heavier lines have all been verified in interbreeding populations occupying various habitats within a 5-km diameter circle between Campinas and Barão Geraldo; note prevalence of oligophagy and some polyphagy (*Mechanitis, Prittwitzia, Pteronymia*). Neither Ithomiine tribes nor Solanaceae genera are in evolutionary order (see Table 2 for this), but have been arranged so as to simplify the figure.

FIGURE 6. Solanaceae of southeastern Brazil (photographed in Campinas, São Paulo unless otherwise indicated): *Solanum*.—A. *S. mauritianum*.—B. *S. swartzianum*.—C. *S. capsicoides*.—D. *S. aculeatissimum*.—E. *S. robustum*.—F. *S. atropurpureum*.—G. *S. paniculatum*.—H. *S. megalochiton*.—I. *S. pseudoquina* St. Hil. For authors' names, see Figure 5.

FIGURE 7. Solanaceae of southeastern Brazil (continued).—J. *Solandra grandiflora* (cultivated).—K. *Brugmansia candida* (cultivated; Jardim Botânico, Rio de Janeiro).—L. *Markea* (*Dyssochroma*) *viridiflora* (Sims.) Miers (Jardim Botânico, Rio de Janeiro).—M. *Brugmansia suaveolens*.—N. *Brunfelsia australis*.—O. *Vassobia breviflora*.—P. *Acnistus arborescens*.—Q. *Cestrum laevigatum*.—R. *Cestrum intermedium* (Joinvile, Santa Catarina).

Known (═══) and presumed (- - - - - -) food-plant records for Ithomiinae in the region of Campinas, São Paulo, southeast Brazil (23° S, 48° W, 600-1,000 m)

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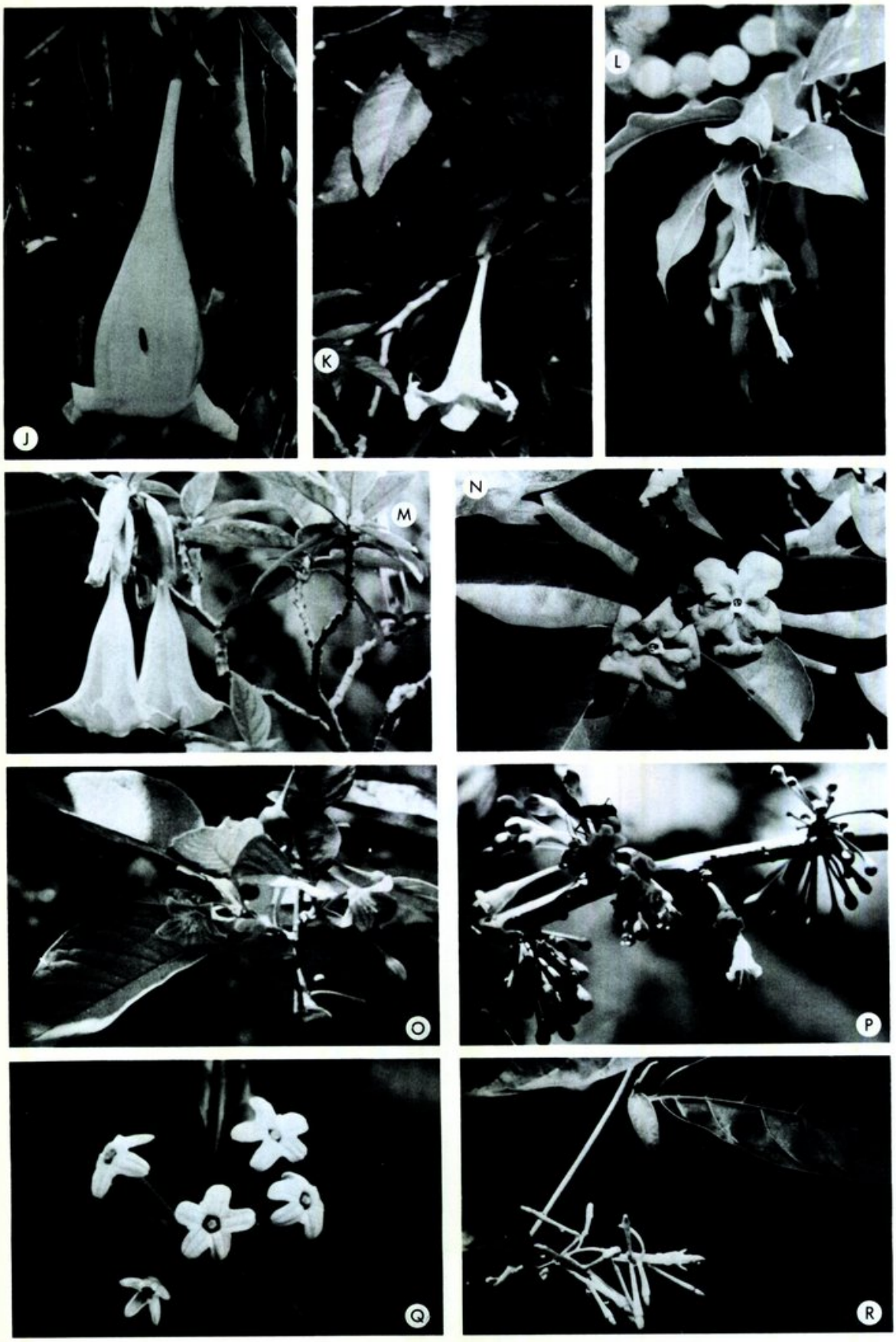
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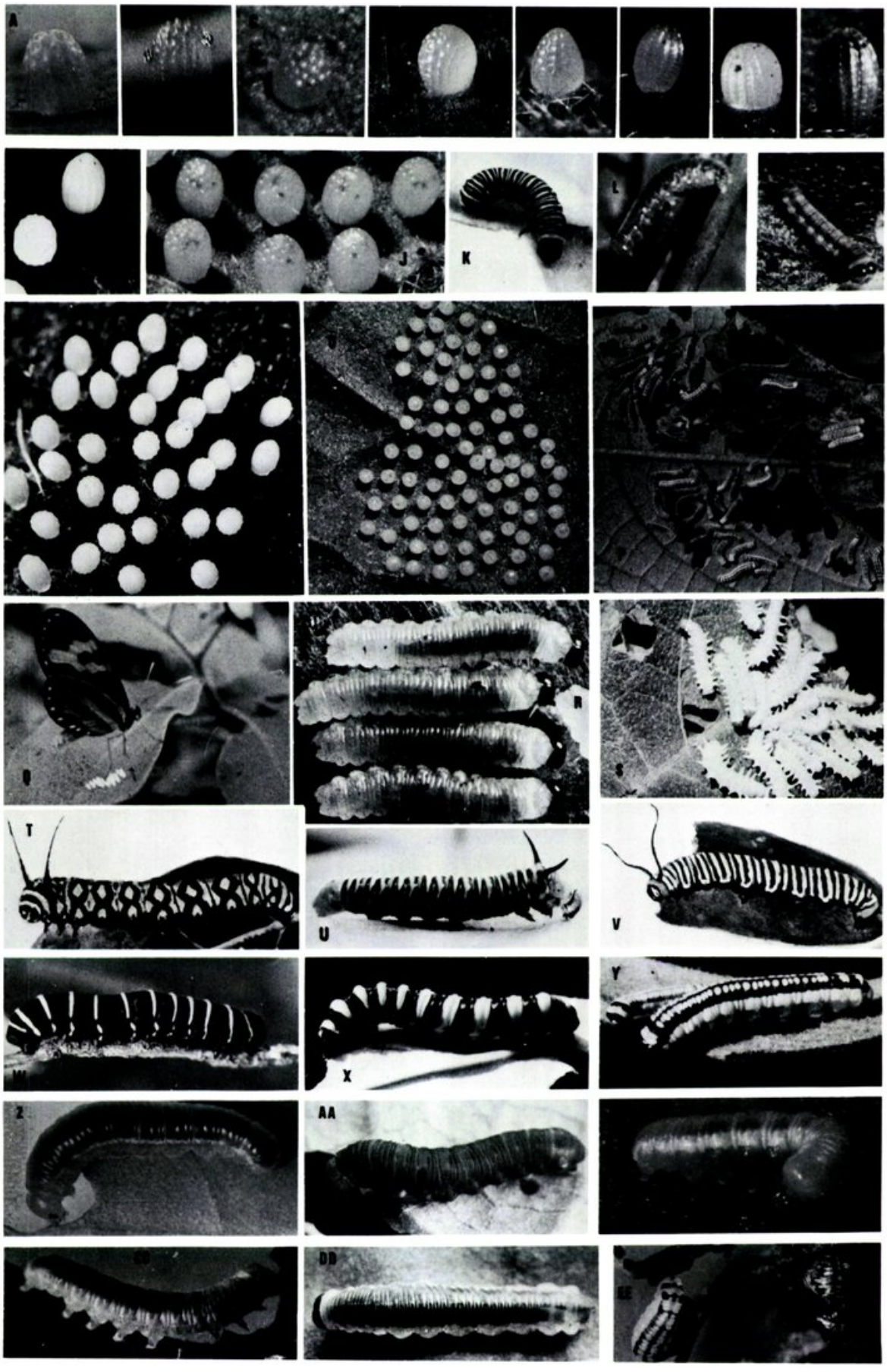
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genera *Tithorea, Elzunia,* and *Aeria* (with Apocynaceae: Parsonsieae), *Methona, Melinaea, Thyridia, Mechanitis, Hypothyris, Ithomia, Dircenna, Pteronymia,* and *Greta.* At a higher level, almost all members of the last two tribes show collectively a strong specificity to *Cestrum* and surprisingly also to the distant section *Geminata* of *Solanum.* It is probable that more work will provide more evidence of the restriction of *Napeogenes* to *Lycianthes* and confirm the nascent patterns in *Placidula, Scada, Epityches, Oleria,* and *Callithomia,* but this is mere speculation at this time.

At the species level, *Mechanitis* females tend to divide up the local *Solanum,* resulting in minimal overlapping of oviposition (see Drummond, 1976; Haber, 1978; Vasconcellos-Neto, 1980, 1986), but the divisions do not coincide with simple taxonomic lines in the plants. They also vary from area to area and are not correlated with larval choice or survivorship in experiments, indicating an unstable ecological determination of the partition.

Though the butterflies may be able to recognize their food plants, biologists have greater difficulty in Solanaceae identification, even to the genus level, which may result in some spurious patterns in Table 2. In 1969, a top Solanaceae taxonomist identified for me a specimen from near Rio de Janeiro as a *Capsicum* on the basis of its cleft anthers, but both S. Knapp (pers. comm.) and I regard this plant as *Solanum* (sect. *Geminata*) *caavurana.* The "diagnostic" trait in this case may have been an artifact of the drying method used for the specimen. If such evolutionarily distant and chemically distinct taxa could be potentially confusing to an experienced expert, how will they appear to the average field biologist who, having just observed a female ithomiine oviposit on a (presumably solanaceous) bush with glabrous entire leaves and no flowers, faces the maze of complex and confusing taxonomy in the family? This plant could be placed preliminarily in over 30 genera, five with over a hundred species and one with over a thousand. Thus the "solid" data base for host plant usage in Table 2, from which a number of completely unlikely records have already been purged, may yet suffer fundamental modifications with further work.

COEVOLUTION AND PHYLOGENETIC DIVERSIFICATION

An attempt to relate the phylogeny of the Ithomiinae with that of their larval food plants, such as was done with reasonable confidence and results for the nymphaline tribe Heliconiini (Benson et al., 1976), has met with very little success (Drummond, 1986; Brown & Drummond, in prep.; Drummond & Brown, 1987). Only the broadest pattern can be seen in the progression from Apocynaceae to Solanaceae (with Gesneriaceae in the middle to provoke the imagination). The most primitive ithomiine genera to use Solanaceae (*Athesis, Methona, Olyras,* and *Melinaea*) feed on the genera regarded as highly advanced in this family. Two large tribes (Mechanitini and Napeogenini) run their food plant

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FIGURE 8. Juveniles of Ithomiinae with food plant and locality. A–J, N–O. Eggs, about 0.6–1.0 mm high.—K–M, P, R. Second instar larvae, about 4–7 mm long.—Q. Adult ovipositing (photo J. Vasconcellos-Neto).—S-DD. Fourth or fifth instar larvae, about 20–35 mm long.—EE. Prepupa and pupa. A. *Tithorea harmonia pseudethra* (*Prestonia*? sp.), Piracanjuba, Goiás.—B, U. *Aeria olena* (*Prestonia coalita*), Campinas, SP.—C, AA. *Napeogenes sulphurina* Bates (*Lycianthes* sp., a vine), Ipojuca, Pernambuco.—D, CC. *Garsauritis xanthostola* Bates (*Solanum* sp., note simple hairs), Faro, Pará.—E, BB. *Hypothyris daphnis amapaensis* Brown (*Solanum asperum*), Lourenço, Amapá.—F, DD. *Oleria astrea thiemei* (Oberthür) (*Solanum stagnale* Moricand), Catu, Bahia.—G, Z. *Ceratiscada canaria* Brown & D'Almeida (*Solanum laxiflorum* Sendt.), Santa Teresa, Espírito Santo.—H. *Pteronymia hemixanthe* (Feld. & Feld.) (*Solanum* subg. *Brevantherum*?), Ubatã, Bahia.—I, R. *Mechanitis lysimnia* (*Solanum* subg. *Leptostemonum*), Brasília, DF.—J, M, O, P, Y, EE. *Hypothyris euclea laphria* (*Solanum asperum*), Linhares, Espírito Santo.—K. *Melinaea menophilus* ssp. (*Markea ulei* (Damm.) Cuatr., originally reported as "*Forsteronia* sp."), Jaru, Rondônia.—L. *Mcclungia salonina* (*Cestrum sendtnerianum*), Sumaré, SP.—N, Q. *Mechanitis polymnia casabranca* (*Solanum* nr. *rugosum, Solanum variabile*), Brasília, DF and Sumaré, SP.—S. *Mechanitis polymnia angustifascia* Talbot (*S. goodspeedii*), Caranavi, Bolívia.—T. *Tithorea harmonia moppa* Bryk (*Prestonia*? sp.), Belém, Pará.—V. *Melinaea ludovica* (Cr.) (*Markea* sp.?), Belém, Pará.—W. *Methona themisto* (*Brunfelsia australis*), Campinas, SP.—X. *Methona confusa psamathe* Godm. & Salv. (*Brunfelsia grandiflora* D. Don), Barinitas, Venezuela. T and V are from watercolors by Emily Fountaine, preserved in the British Museum (Natural History). Solanologists are requested to photograph and rear through to the adult or to preserve in alcohol any eggs (with ridges as in A–J) or larvae of these types found on identifiable plants and send them to B. Drummond or K. Brown for identification and registry in the data bank for Table 2.

TABLE 2. Approximate number of known interactions (species × species) between Ithomiinae and their larval food plants, with data on plant chemistry (Figs. 1, 2) and on attraction to pyrrolizidine alkaloid sources.

Ithomiinae: Tribes, Genera[a,c] (no. species)	Attraction to:[c] Boraginaceae (withered plants)	Attraction to:[c] Eupatorieae (flowers & plants)	Chemical Constituents	Plant Families: Genera[a,b] (no. species) — Apocynaceae: *Prestonia, Fernaldia, Mesechites*, etc.	Gesneriaceae: *Drymonia, Columnea*	Solanaceae: *Solanum* (1000): *Solanum* (incl. *Geminata, Pseudocapsica*)	*Solanum*: *Brevantherum*	*Solanum*: *Leptostemonum*	*Solanum*: *Potatoe, Bassovia*	*Lycianthes* (150)	*Cyphomandra* (40)	*Lycopersicon* (6)	*Capsicum* (25)	*Cuatresia* (6), *Witheringia* (16)	*Physalis* (95)	*Vassobia* (4), *Dunalia* (6), *Acnistus* (2)	*Datura* (10), *Brugmansia* (5)	*Nicandra* (1)	*Solandra* (10)	*Markea* (24)[y], *Juanulloa* (11)[y]	*Cestrum* (250)	*Brunfelsia* (40)
			Alkaloids (Fig. 1)																			
			Indoles, Steroids, PAs (3E–I)	?	·····	ABFLH	·····	BL	ABCL	BL	BC?L	C?	ABC	BC	BCP	C	BC	C?	LC	BCE[j]
			Other Chemical Constituents (Fig. 2)																			
			Cardiac glycosides, XYZ	WY	···	RTUWXYZ	···	R?	Z	RWXY	YQ?	Q	QSY	QY	QYZ	QR	XY	XY	RWXYZ	XY
A. Tellervini[a]																						
Tellervo (1)[a]	1	1	**5**																			
B. Tithoreini																						
Elzunia (2)	1	?	**4**																			
Tithorea (2)[b]	1	1	**16**															1?				
C. (New Tribe)																						
Aeria (3)[b]	2	3	**8**																			
D. (New Tribe)																						
Athesis (1)	1	1									**1**											
E. Methonini																						
Methona (7)[b]	1	1																				**12**
F. (New Tribe)																						
Placidula (1)	2	3							1?						**6**							
G. Melinaeini																						
Olyras (2)	2	2																1?	1			
Eutresis (2)	2	2																1?	**1**			
Melinaea (10)[b]	2	2																1	**9**			
H. Mechanitini																						
Thyridia (1)[b]	2	3							**6**													
Sais (2)	1	3						1														
Scada (6)	1	3				1 **3**																
Forbestra (3)	1	3					1															
Mechanitis (5)[b,c]	1	3			3	15 **56**	1		**7**	2					1?	1						
I. Oleriini																						
Hyposcada (8)	2	3		**3**														**1**				
New genus (3)	1	1						1														
Oleria (30)[b]	2	3				1 **4**	**8**	3														
J. Napeogenini																						
Epityches (1)	2	3									**2**		1	**2**								
Rhodussa (1)	2	3					1?	**1**														
Napeogenes (18)[b]	2	3					1?	**8**														
Garsauritis (1)	2	3					**4**															
Hyalyris (16)	2	3				**2**	**5**															
Hypothyris (16)[b]	2	3			**1**	**24**	**13**	1														
K. Ithomiini																						
Miraleria (1)	2	3										1		**2**	**1**							
Ithomia (21)[b]	2	3						2			**2**	**11**	3	**5**								
L. Dircennini																						
Callithomia (3)[b]	3	3					1 **3**	1														
Velamysta (3)	3	3						1														
New Genus (1)	2	?							1													
Dircenna (7)[b]	1	3				**14** **17**																
Hyalenna (5)	3	3				**1**																
M. (New Tribe)																						
Ceratinia (5)	3	3			**3**		**3**															
Ceratiscada (3)	2	3			**2**																	
Prittwitzia (1)	2	3			**3**		1?														**5**	
Episcada (15)[b]	2	3			**4**																	
Pteronymia (41)[b]	3	3			**25**	**3**		3													2	

TABLE 2. Continued.

Ithomiinae: Tribes, Genera[a,c] (no. species)	Attraction to:[c] Boraginaceae (withered plants)	Attraction to:[c] Eupatorieae (flowers & plants)	Plant Families: Genera[a,b] (no. species)																					
						Solanaceae																		
						Solanum (1000)																		
			Apocynaceae *Prestonia, Fernaldia, Mesechites*, etc.	Gesneriaceae *Drymonia, Columnea*		*Solanum* (incl. *Geminata, Pseudocapsica*)	*Brevantherum*	*Leptostemonum*	*Potatoe, Bassovia*	*Lycianthes* (150)	*Cyphomandra* (40)	*Lycopersicon* (6)	*Capsicum* (25)	*Cuatresia* (6), *Witheringia* (16)	*Physalis* (95)	*Vassobia* (4), *Dunalia* (6), *Acnistus* (2)	*Datura* (10), *Brugmansia* (5)	*Nicandra* (1)	*Solandra* (10)	*Markia* (24)[f], *Juanulloa* (11)[f]	*Cestrum* (250)	*Brunfelsia* (40)
N. Godyridini																								
Godyris (10)[h]	3	3				3															5			
Pseudoscada (6)[h]	2	3																			5			
Hypomenitis (6)	2	3																			2			
Greta (10)	2	3				1	1														16			
Hypoleria (11)[h]	2	3				1															6			
Mcclungia (1)	2	3																			3			
Heterosais (3)[h]	3	3																			3			

[a] Families are in approximate order of advancement and genera of Solanaceae in order according to Hunziker (1979), from primitive to advanced.

[b] In addition to *Nicotiana* (see note i, below), important Solanaceae genera available to Ithomiinae in tropical America for which no food plant records are yet known include *Jaltomata, Athenaea* (but probably used by *Epityches* and *Ithomia*), *Larnax, Saracha, Iochroma, Salpichroa, Jaborosa, Lycium, Grabowskia, Trianaea, Sessea, Metternichia, Petunia, Fabiana, Nierembergia, Bouchetia, Protoschwenkia, Schwenkia, Melananthus, Parabouchetia, Leptoglossis, Browallia, Streptosolen*, and *Heteranthia*.

[c] 1—weak attraction usually including both sexes, 2—males strongly attracted, 3—males strongly and females regularly attracted.

[d] Tribes and genera follow the order of Mielke and Brown (1979) as modified by Brown (in prep.), from primitive to advanced (including genera within each tribe).

[e] No food plants have yet been recorded for the following genera of Ithomiinae (number of species in parentheses). * = Andean genera, usually of high-altitude cloud forests, though *Roswellia* extends out into the foothills.—Tribe D. **Roswellia* (1), **Patricia* (2).—Tribe G. *Athyrtis* (1).—Tribe H. *Paititia* (1).—Tribe I. *a new genus near *Hyposcada* (1).—Tribe J. **Aremfoxia* (1).—Tribe K. **Pagyris* (1).—Tribe N. **Dygoris* (1) and **Veladyris* (1). Total, 9 genera with 10 species.

[f] The recent divisions of these two genera into a number of smaller genera (Hunziker, 1979), most of them used by the same Ithomiinae, are here included under the collective older names.

[g] Restricted to the Old World tropics, regarded by some as closer to the Danainae than the Ithomiinae (see Ackery & Vane-Wright, 1984).

[h] Food plant patterns are constant from the northern to the southern extremes of the range of the genus, usually from Central America to southern Brazil (total of 18 genera).

[i] Ovipositions of *Mechanitis polymnia casabranca* were observed in Campinas, São Paulo, on cultivated *Capsicum annuum* L. and feral *Nicotiana* (rosette only), but the larvae did not grow on these plants.

[j] The published structure for "hopeanine" (1E) is biogenetically and chemically implausible and does not agree with the data presented in the original study (Iyer, 1978); the alkaloids of *Brunfelsia* are presently under investigation.

phylogeny backwards (more advanced butterflies use increasingly primitive plants). And although the most morphologically advanced ithomiine genera concentrate on the relatively advanced *Cestrum*, they use equally well the supposedly primitive section *Geminata* of *Solanum* (Table 2). That the neotropical Ithomiinae represent a widely diversified butterfly group (51 genera in 13 tribes) could help explain why a comparison of their host plant utilization patterns with those of the 65 rather homogeneous species in no more than ten genera of the single tribe Heliconiini does not lead to a fruitful parallel. The patterns for the Ithomiinae are complex, and the arrangement of the plant groups in Figure 5 so as to produce a maximum parsimony scheme (lowest number of crossing lines in the middle) has produced some strange and thought-provoking proximities between genera normally widely separated; note especially the positions of *Brunfelsia* and *Cestrum*.

While the Solanaceae indeed may have represented a new field for adaptive radiation of the Ithomiinae in the New World, it is probable that

the plant family was already very diversified chemically and taxonomically before the butterfly subfamily began to move onto it. A large number of available genera (at least 24) are still not known to be attacked by Ithomiinae (see Table 2). Furthermore, the genus *Brunfelsia*, regarded as a relatively advanced member of the Solanaceae, apparently entered the Caribbean area and diversified greatly (as section *Brunfelsia*) before *Methona*, a relatively primitive ithomiine genus in a monotypic tribe, could evolve as a specialist herbivore on it (Plowman, 1979, p. 489). It is possible, however, that the extensive speciation verified in *Solanum* and *Cestrum* eventually may be related to their heavy use by a variety of Ithomiinae (Table 2). This can be investigated only by careful chemical analysis and bioassay with larvae, following in parallel two independently developing phylogenetic lines of species or populations that show specific interactions.

Further considerations of the incongruities between phylogenies of genera of Solanaceae and Ithomiinae are presented in Brown & Drummond (in prep.).

BIOCHEMICAL COEVOLUTION

The taxonomic diversification of the Solanaceae has been accompanied by an impressive diversification in secondary chemicals, leading some to suggest polyphyly for the family as presently constituted. Indeed, no other plant family can challenge the Solanaceae in having alkaloids representing all four major biosynthetic pathways (lysine-ornithine, phenylalanine-tyrosine, tryptophan, and steroid), an equal number of less important pathways (nicotinic acid, anthranilic acid, glycine, acetate), and a further set of aberrant or combined sources unique to the family (leading to capsaicins, solanines, withasomnine, and hopeanine) (Fig. 1), not to mention more common amines such as choline, noradrenaline, and hypoxanthine; some species also produce very toxic peptides. As is often the case, these alkaloid-rich plants are singularly poor in lower (volatile) terpenes, but some *Datura*, *Cyphomandra*, and especially *Cestrum* and *Solanum* section *Geminata* share a similar and abundant pungent oil that may include terpenes. Diterpenes and triterpenes abound in some Solanaceae, and nonalkaloidal steroids are represented as saponins (such as diosgenin glycosides) and withanolides/physalins, unusual steroidal lactones with marked biological activity (Fig. 2). Flavonoids and coumarins also are documented amply in diverse members of the family (Fig. 2; Harborne & Swain, 1979), and further phenolics and their glycosides (including tannins) are almost always found when sought in polar extracts. The C_{45} alcohol solanesol is an unusual unsaturated linear nonaprenol (Fig. 2). Lacking are reports of cyanogenic glycosides, cardenolides, glucosinolates, nonprotein amino acids, and iridoid glycosides, but their absence cannot be definitively affirmed because few phytochemists have specifically sought them in the Solanaceae.

Thus the plants in this family are well defended chemically and attacked by rather few insect herbivores other than Ithomiinae (Table 1). The only ones that seem to promote similar damage in natural systems are generalist grasshoppers and meloid beetles, perhaps molluscs in more calcium-rich areas, and specialist chrysomelid beetles. The plants also at times are defended admirably against vertebrate chewers by prickles (Symon, 1986) but these have very little effect on smaller invertebrates, who walk and chew around them or build silken pads over them. Other defenses (tough leaves, glandular trichomes) are more effective against invertebrates (Vasconcellos-Neto, 1980, 1986), as may be further quantitative defenses (tannins, amino-acids, and resins) and a host of ecological strategies such as phenology (see Vasconcellos-Neto, 1986) habitat, growth form, seed dispersal by chiropterochory, and encouragement of spiders and predatory pentatomids on the leaves, among others observed in Brazil.

When the generic preferences of the butterflies are analyzed in terms of plant chemicals (Table 2), the associations seem to show patterns, though the data are still chemically and biologically very incomplete. There appears to be a certain tendency toward chemical specificity, at times without reference to taxonomic relationship (as in the similarly potent-smelling *Solanum* sect. *Geminata* and *Cestrum*). It seems probable that female Ithomiinae search for and find specific chemical cues for oviposition on certain genera of Solanaceae and may be led to place eggs on unrelated plants with similar chemicals. What else would permit a *Mechanitis polymnia* female in Campinas in May 1981 to recognize a small *Nicotiana* plant in Monjolinho as a potential Solanaceae host, or to oviposit on *Capsicum annuum* in my garden, especially when the larvae survived on neither? (The first shares nicotine and the second

steroidal glycoalkaloids with a usual host, *Solanum mauritianum*.) Based on the patterns in Table 2, some still unknown chemical may be predicted to set *Solanum* subgenera *Bassovia, Potatoe,* and *Lycianthes* apart from all other Solanaceae (see *Napeogenes, Oleria,* and *Callithomia*) and possibly associate *Lycianthes* with the withanolide-elaborating genera (see *Ithomia*) and even with *Solanum* sect. *Geminata* (*Pteronymia* uses both).

Further support for some degree of chemical mediation at the oviposition sector of the interface comes from the broad geographical consistency of the more specific relationships, at least from Mexico through Costa Rica and Ecuador to south Brazil for the 18 genera for which food plants are known over this range (Table 2; Drummond & Brown, 1987). This surely must have been established over long evolutionary time in developing phyletic lines of the butterflies. The chemical proximity of disparate taxonomic groups of plants, treated as interchangeable by ovipositing females, also supports a chemical mediation at the interface. In order to separate this possible evolutionary component in food plant usage from the ecological noise in the system, careful comparative tests with free-flying females must be performed under controlled conditions, coupled with larval feeding preferences in multiple-choice tests, and thorough chemical analysis of all potentially active compounds in the food plants chosen or rejected.

The patterns broadly reflected in Table 2 and supported on a local scale by Figure 5, however, do not support any easily envisioned hypothesis of mutual interaction and fine coevolutionary adjustment between the two groups at a taxonomic level. Although some regularity in chemical cues for oviposition and chemical specificity is suggested, the data are still very limited and give little support for parallel diversification in the two groups. Much more complete biological and chemical information will be necessary before any claims of biochemical coevolution can be advanced at this plant/herbivore interface. The present information already seems to falsify many attractive and conventional hypotheses.

In view of the fact that Ithomiinae populations probably are controlled principally by certain limiting adult resources (suitable humid open-understory "pockets" and sources of PAs, see below), they may exert very little, if any, selective pressure for chemical and phylogenetic diversification on their host plants. In any case, the specific choices seem to vary appreciably with local ecological conditions. Under a moderate degree of Ithomiinae attack, some Solanaceae even respond by vigorous new growth and flowering (especially in *Brunfelsia* and in section *Brevantherum* of *Solanum*). Under these conditions, it is difficult to establish the most basic prerequisites for "classical" coevolution between herbivores and host plants.

An Alternative Hypothesis: Sequential Colonization by Chemical Adaptation (Table 4)

The results of chemical extraction, fractionation, and *Biblis/Nephila* bioassay of 48 ithomiine host plants are presented in Table 3. In the cases investigated so far the Solanaceae have shown in their leaves the compounds already reported or presumed for each species, in good quantities.

If these chemical characteristics of the host plants are combined with the utilization patterns in Table 2, a most suggestive picture emerges, as summarized in Table 4. Nothing is known of the paleophytochemistry of these plants beyond the global variation in secondary compounds seen in widespread geographical populations of each genus today, but it may be presumed that they were diversified generically before Ithomiinae began attacking them in the New World (since the most advanced genera were attacked first) and probably similarly protected chemically to modern species.

It then becomes possible to trace a series of small hypothetical advances in larval toleration of food plant chemicals, each facilitated by preadaptations on the existing host plants, which lead to a complete picture of chemical specificity as observed today (Tables 2, 4). The scheme is clearly oversimplified but is attractive as a hypothesis for the sequential colonization of plant hosts, already diversified taxonomically and chemically, by progressively adapting herbivores (Futuyma, 1983). It is in excellent agreement with the great diversity and apparent nonstorage by herbivores of the Solanaceae chemicals and the initial dependence of the Ithomiinae stock on PAs (see below).

Thus, Apocynaceae-feeding Ithomiinae dependent on PAs probably did not adapt sufficiently to the great chemical variability in the Parsonsieae (seen today also), which in some cases repelled or poisoned larvae, in others left adults unprotected against predators and deficient in

TABLE 3. Summary of chemical fractionation of Ithomiine larval food plants (leaves).

Larval Food Plant[a]	Source[b]	Weight Fresh	Weight Dry[c]	% H_2O	Fractions (as % of dry wt.)[d] A	B (% N-Ox.)[e]	C	F	Fibre	Probable Compounds[f]	Fractions Tested[d]	Bio-assay Results[g]
SOLANACEAE												
1. *Solanum* (Sect. *Geminata*) *caavurana*	C	64	14.4	78	8.3	1.3 (0)	7.0	0.7	82.7	ABL_1L_2RWYZ	Ex, 2	–
2. *S.* (Sect. *Geminata*) *pseudoquina* St. Hil.	C	46	14.1	65	5.0	4.2 (0)	18.9	6.7	65.2	ABL_1L_2RWY		NT
3. *S.* (Sect. *Geminata*) nr. *ripense* Dunal	V	14.5	2.4	84	2.8	2.2 (0)	9.4	1.7	83.9	ABLWYZ	Ex	–
4. *S.* (Sect. *Pseudocapsica*) *pseudocapsicum*	C	40	9.5	76	4.9	3.5 (0)	7.7	6.7	77.2	ABL_2RWY	Ex, 2	–
5. *S.* (Sect. *Lepidota*) *murinum*	C	72	20.5	72	3.0	0.67 (0)	10.9	5.1	80.3	ABL_1RTWY	Ex	–
6. *S.* (Sect. *Indubitaria*) *megalochiton*	C	131	38.2	71	3.4	0.5 (0)	7.2	6.2	82.7	ABL_1L_2RWYZ	Ex	–
7. *S.* (Sect. *Anthoresis*) nr. *schwackeanum*	J	50	12.1	76	5.9	1.3 (0)	2.0	3.1	87.7	ABL_1RWY		NT
8. *S.* (Sect. *Anthoresis*) *concinnum*	C	107	33.4	69	4.8	0.99 (0)	4.9	4.6	84.6	ABL_1L_2RWY	Ex	–
9. *S.* (Sect. *Brevantherum*) *mauritianum*	C	100	28.3	72	5.9	0.2 (25)	5.6	4.5	83.8	$ABFL_1L_2RTWY$	Ex	–
10. *S.* (Sect. *Brevantherum*) *asperum* Rich.	M	–	9.8	–	3.5	2.2 (0)	9.7	1.3	83.3	$ABFL_1L_2RTWY$		NT
11. *S.* (Sect. *Melongena*) *melongena*	C	34	8.4	75	4.0	0.37 (0)	10.8	5.9	78.9	ABL_1RWY	Ex	–*
12. *S.* (*Leptostemonum*) *robustum*	C	60	10.4	83	5.6	0.4 (0)	7.3	2.9	83.8	AL_1RWY	Ex, A, B, F	–
13. *S.* (*Leptostemonum*) *variabile*	C	44	12.0	73	4.5	1.3 (0)	8.1	2.8	83.3	ABL_1RWY	Ex, 2	–
14. *S.* (*Leptostemonum*) *brusquense*	C	30	6.6	78	11.2	0.33 (0)	3.8	6.2	78.2	ABL_1L_2RWY	Ex	–*
15. *S.* (Sect. *Acanthophora*) *atropurpureum*	C	39	7.4	81	4.8	0.46 (0)	11.8	6.1	76.8	ABL_1L_2RWY	Ex, A, B, C	–*
16. *S.* (Sect. *Jasminosolanum*) *flaccidum*	MP	93	16.8	82	5.8	0.35 (0)	11.8	4.0	78.0	$ABC?L_1L_2RWY$		NT
17. *S.* (Sect. *Aculeigerum*) *wendlandii*	C	18	2.2	88	1.5	0.28 (35)	5.3	9.2	83.7	$BC?L_1L_2WY$	Ex	–
18. *Lycianthes rantonnetii* Carr. ex Lesc.	C	30	5.2	83	5.3	0.98 (0)	17.8	6.8	69.1	$BC?L_1L_2WY$		NT
19. *L.* sp. (Goiânia)	G	18	6.3	65	1.1	0.13 (0)	3.7	1.2	93.8	$BC?L_1L_2WY$	A	–
20. *Cyphomandra sciadostylis*	SN	60	15.5	74	5.2	0.17 (15)	5.6	3.9	85.1	$ABCL_1WYZ$	Ex	–
21. *C. fragrans*	C	55	9.8	82	6.4	0.84 (0)	10.2	4.0	78.6	ABL_1WY		NT
22. *Lycopersicon esculentum*	C	42	5.5	87	7.0	1.8 (0)	6.4	4.2	80.6	BL_1L_2WYZ	Ex	–
23. *Capsicum ciliatum* (Kunth) O. Kuntze	V	183	42.5	77	2.9	0.4 (10)	17.7	1.6	77.4	BCL_1QRWY		NT
24. *C. villosum* (Sendtner) (?)	J	10	1.4	86	10.0	1.4 (0)	16.8	2.3	69.5	BL_1QWY		NT
25. *C. flexuosum*	J	28	6.9	71	7.6	2.5 (0)	11.7	0.3	78.0	BL_1QRWY	Ex	–
26. *C. lucidum* (Moricand) O. Kuntze	RJ	40	10.0	75	11.2	1.7 (11)	16.8	4.1	66.2	BL_1QRWY	Ex	–
27. *C. mirabile* Sendtner	SL	120	26.3	78	7.6	0.95 (0)	13.9	2.2	75.4	BL_1RWY		NT
28. *Physalis neesiana*	C	65	9.3	86	5.3	1.05 (0)	4.2	4.2	85.2	ABCQRSWY	Ex, A, B	–*
29. *P.* sp. nr. *neesiana*	C	20	2.6	87	8.8	2.8 (0)	11.4	2.9	74.1	ABCQRSWY	Ex	–*
30. *Vassobia breviflora*	C	90	12.8	86	8.7	0.84 (0)	7.1	4.4	78.9	BCQRWY	Ex, A	–*
31. *Acnistus arborescens*	C	227	32.6	86	5.0	0.64 (0)	9.6	5.6	79.1	BCQRWY	Ex, A, B, C	–*
32. *Datura stramonium*	C	66	7.8	88	6.9	1.4 (0)	7.4	4.2	80.1	BCQWYZ	Ex, B	–

TABLE 3. Continued.

Larval Food Plant[a]	Source[b]	Weight Fresh	Weight Dry[c]	% H_2O	Fractions (as % of dry wt.)[d] A	B (% N-Ox.)[e]	C	F	Fibre	Probable Compounds[f]	Fractions Tested[d]	Bioassay Results[g]
33. *Brugmansia suaveolens*	C	50	6.7	87	5.2	1.8 (0)	9.2	3.5	80.3	BCQWY	Ex	−
34. *Nicandra physaloides*	PC	115	13.4	88	7.8	0.63 (0)	5.9	3.5	82.2	BCQRWY	Ex, A, B, C	−*
35. *Solandra grandiflora*	C	30	5.2	83	4.2	0.73 (0)	7.5	7.0	80.5	BCWY	Ex	−
36. *Markea* (*Dyssochroma*) *viridiflora* (Sims.) Miers	RJ	41	6.6	84	1.4	0.24 (40)	3.9	3.9	90.6	BCWY	B	−
37. *Juanulloa mexicana* (Schldl.) Miers	LG	17	2.7	84	2.4	0.33 (50)	2.6	6.7	88.1	BCWY	Ex	−*
38. *Cestrum schlechtendalii*	C	40	6.8	83	4.3	0.38 (15)	8.9	5.4	81.0	BC?L_1RWYZ	Ex, A, B	−
39. *C. laevigatum*	C	50	11.1	78	6.5	1.3 (0)	6.8	6.3	79.1	BC?L_1RWYZ	Ex	−
40. *C.* nr. *amictum*	SL	42	7.3	83	4.4	0.18 (0)	10.7	4.0	80.7	BC?L_1RWY		NT
41. *Brunfelsia australis*	C	40	10.4	74	3.4	0.21 (0)	5.2	3.3	87.9	BC?EWXY	Ex	−
42. *B. pauciflora*	C	45	9.1	80	3.5	0.36 (0)	5.6	2.9	87.6	BC?EWXY	Ex	−
APOCYNACEAE: PARSONSIEAE												
43. *Prestonia coalita*	C	36	5.3	85	1.7	0.45 (0)	5.2	6.0	86.6	3B?O?, YZ	Ex, A, B	−*
44. *P. dusenii*	SN	14	3.3	62	4.9	0.24 (0)	0.96	1.8	92.0	3B?O?, YZ	Ex	−
45. *P. acutifolia*	MP	59	12.7	78	5.8	0.49 (43)	16.4	3.2	74.1	3B?EN, Y		NT
46. *Peltastes peltatus*	C	54	9.0	83	4.3	0.17 (0)	5.9	3.1	86.6	3B?O?, Y	Ex	−*
47. *Temnadenia violacea*	C	128	28.1	79	2.4	0.27 (0)	12.2	6.4	78.7	3B?, WY		NT
GESNERIACEAE												
48. *Nematanthus fritschii* Hoehne	SL	175	19.4	89	3.6	3.8 (20)	19.8	2.0	70.8	C?, WY		NT

[a] Leaves extracted with MeOH-H_2O 4:1 as in Figure 4. Authors' names not given are in Figure 5. Additional genera of food plants used by Ithomiinae not yet investigated are *Witheringia, Dunalia, Cuatresia* (Solanaceae) and *Drymonia, Columnea* (Gesneriaceae, both quite close to *Nematanthus*).

[b] Codes for source localities (also applying to Tables 5 and 6): C = Campinas, São Paulo and vicinity. G = Goiânia, Goiás. J = Serra do Japi, Jundiaí, São Paulo. LG = *ex* Larry Gilbert, Austin, Texas (from Costa Rica). M = Manaus, Amazonas, and region to north. MP = Martinho Prado, São Paulo. PC = Poços de Caldas, Minas Gerais (with help of Renata S.C. Dias). RJ = region of Rio de Janeiro, including eastwards along coast. SL = coastal São Paulo (Bertioga, São Vicente, Mongaguá). SN = Serra Negra, São Paulo. V = northern Venezuela (with help from S. S. Tillett, Francisco Romero, and Roberto and Renato Mattei).

[c] Dry weight calculated as the sum of the fractions and fiber. The water content varies with collecting and transporting conditions.

[d] See Figure 4 for letters of fractions. Ex = total extract, freed of methanol. Most fractionations were performed by Silvana Aparecida Henriques.

[e] No dehydropyrrolizidine alkaloids were detected in any Solanaceae and only one Apocynaceae investigated showed appreciable quantities. *Brunfelsia, Brugmansia,* and *Juanulloa* may contain similar compounds acting as precursors or analogs. Presence of N-oxides may indicate tropanes or nicotine analogs.

[f] Based on literature references, plus preliminary investigation in this work. Much variation was often seen in the secondary compounds encountered in a plant species; detailed isolation and identification of principal chemical constituents is underway. The letters indicate structure types (not necessarily individual compounds) in Figures 1 and 2, unless otherwise indicated (Fig. 3 in Apocynaceae).

[g] + indicates positive (*Biblis* cut out), − negative, −* positive at high concentrations, becoming negative upon dilution. NT indicates not yet tested in the *Nephila* bioassay. A positive test at normal concentrations (at least 300 μg on the *Biblis*) sometimes became negative with less than 100 μg; this is indicated by the symbol +*.

PA-derived sex attractants and other pheromones (Fig. 3D). Adults increasingly exploited PA sources (Boraginaceae leaves, Compositae flowers) and may even have found some substitutes for PA precursors in Solanaceae (*Brunfelsia*?). Since they were preadapted to bicyclic 8-carbon monoester alkaloids and their N-oxides (both PAs and tropanes fall under this classification), the generally well-protected, tropane-rich Solanaceae represented an open niche. At least two separate radiations could have moved onto these plants (discussion of the move over onto the Gesneriaceae must await further biological and chemical study of *Hyposcada* and its food plants; the move onto *Brunfelsia*, whose alkaloids are still enigmatical, was probably a sidetrack of one of the radiations). The line that colonized the tropane-containing *Brugmansia* (represented by *Placidula* and *Miraleria* today) also encountered in these plants the steroidal bitter principles, to which they were also preadapted through their experience with cardenolides in the Apocynaceae. Tolerance of these bitter steroids permitted further colonization of the many genera defended by them, most of which also contain tropane alkaloids, sometimes with N-oxides. Utilization of *Capsicum* by this line also gave a gateway, through its steroidal glycoalkaloids and saponins (Table 2), to the immense and underutilized resource represented by *Lycianthes* and *Solanum*, on which the more advanced genera persist today.

The second radiation found tropanes initially in *Solandra* and relatives, and thence in *Cyphomandra*, on which it adapted to tolerate the steroidal glycoalkaloids also present there and initiated tolerance of pungent oils. This permitted a similar colonization of *Lycianthes* and *Solanum* (*Sais*, *Scada*). As a final step, adaptation to saponins and strong-smelling oils present in some *Solanum* (especially section *Geminata* and relatives) permitted exploitation of the abundant *Cestrum* in the forest understory. In all cases, tolerance of toxins encountered previously in the evolutionary history of each line could be maintained as new enzymes were developed to detoxify progressively more effective classes of plant defensive chemicals, in many cases not correlated strictly with taxonomy and evolution of the plants. Indeed, some of the more primitive genera—possibly with a broader range of biosynthetic capabilities, according to present concepts of biochemical evolution in plants—seem to have been the last to be colonized by the most advanced Ithomiinae.

Persuasive evidence for this hypothetical scenario (Table 4) can come only from extensive studies of female oviposition preferences and larval tolerance of food plants or chemical compounds. Preliminary data (Table 2) and some initial experiments are encouraging: *Placidula* cannot eat *Solanum*, nor does *Aeria* accept any Solanaceae, but *Mechanitis* can use tropane- and withanolide-containing plants (*Datura*, *Nicandra*), and some *Cestrum*-feeders continue to use *Solanum* and even *Lycianthes*. Experiments in this area inevitably will encounter problems related to individual and local adaptation, lateral radiation (as onto *Brunfelsia*), and loss of ancestral genes but should in the long run help to define the viability of this proposed sequential expansion of Ithomiinae through progressive chemical tolerance to Solanaceae food plants.

HERBIVORES AS PHYTOCHEMISTS

The chemosensory apparatus of the average insect is capable of detecting very small amounts of specific chemicals, either through smell (antennae) or taste (tarsal or buccal chemoreceptors). This highly specialized analytical instrument transforms chemical structures into recognizable electrical potentials through mechanical contact between compound and receptor and uses these potentials to stimulate a variety of response behavior sequences. It can be fooled at the receptor end by substances of different chemical composition but similar conformation and spatial relations among active binding sites. It is far more sensitive and accurate, however, than the majority of the instruments in the modern phytochemist's laboratory. Its specialization is selected over many generations of advantages accrued by those who could recognize a few chemical compounds and thereby compete better for higher quality food, defense, or mates.

To the extent that ovipositing female Ithomiinae seem to demonstrate an appreciable specificity in host plant recognition (Table 2), which involves use of chemoreceptors on the forelegs ("drumming" on leaves) and antennae, they can serve as precise analytical tools for the phytochemist. If some chemical patterns are already evident on a very rough scale in the food plant choices of the Ithomiinae (Table 2), these could be used to suggest analytical methods for still-uninvestigated Solanaceae and predict chemicals in others that have undergone only preliminary analysis.

TABLE 4. Possible scheme for sequential colonization of Solanaceae by Ithomiinae through progressive preadaptive tolerance of secondary chemical classes.

Step	Ithomiines (genera)	Plants Colonized	Substances Tolerated
1	*Tellervo, Elzunia, Tithorea, Aeria*	Apocynaceae: Parsonsieae (*Parsonsia, Prestonia,* etc.)	3E–K: PAs and N-oxides 3A–B: Cardenolides
2a	*Athesis, Placidula, Miraleria*	Solanaceae: *Brugmansia, Capsicum ciliatum*	1C: Tropane alkaloids and N-oxides
2b	*Olyras, Melinaea, Hyposcada*	*Juanulloa, Markea, Solandra*	2Q: Bitter steroids PA precursors?
2c	*Methona*	*Brunfelsia*	
3a	*Epityches, Ithomia*	*Acnistus, Physalis,* etc. further *Capsicum*	1C + much 2Q (varieties) Some 1L: steroid alkaloids
3b	*Thyridia*; New genus	*Cyphomandra*	1C, 1L, 2R: saponins
4a	*Napeogenes, Rhodussa, Oleria*	*Lycianthes*; *Solanum* subgen. *Bassovia, Potatoe* (sect. *Jasminosolanum*)	1L: glycoalkaloids
4b	*Scada, Sais, Forbestra*; *Callithomia*		2R; 1A; possible 1C
5a	*Garsauritis, Hypothyris, Hyalyris*	*Solanum* (subgenera *Leptostemonum* and *Brevantherum*)	1L, 2R, 1A; occasional nicotine, pungent oils
5b	*Mechanitis*; *Dircenna, Pteronymia*		
6b	*Ceratinia, Pteronymia, Godyris, Hypoleria, Pseudoscada, Greta*	*Solanum* (sect. *Geminata*), *Cestrum*	IL, 2R; 2Z: pungent oils

For example, it could be predicted that the *Solandra-Markea-Juanulloa* group of genera, which support the danaioid larvae of *Melinaea* (Fig. 8K, V), might have some protective chemicals similar to those of Apocynaceae-Parsonsieae; and these chemicals should be more storable by larval insects than the usual run of Solanaceae chemical compounds. These have yet to be identified (cardenolides are strongly suspected) and should be investigated. Similarly, the strong-smelling essential oil of *Solanum* sect. *Geminata* should be chemically very similar to the like-smelling oil of *Cestrum*; this also seems to have escaped chemical identification. *Witheringia* could have been predicted to show physalins by *Ithomia*, which also recognized these chemical components with withanolide-structures in *Physalis* and divided its specificity among *Acnistus* and these genera. Since both *Ithomia* and *Epityches* use wild *Capsicum* species, these should surely contain a withanolide-like compound; at least one major substance, possibly with this type of structure, is present in the neutral fraction (A) of two *Capsicum* species investigated (Table 3).

Do the genera *Deprea* and *Athenaea* contain withanolides also? Start by testing some cooperative females of *Ithomia* and *Epityches*. Are any other Solanaceae besides *Datura/Brugmansia* especially rich in scopolamine? One could begin with some *Placidula* females or perhaps even larvae. If the latter were found on a *Solanum* or *Cestrum*, I would bet on the presence of tropanes in the leaves. Are there glyco-alkaloids in *Dunalia*? I might start by asking *Hypothyris*, known only from *Solanum*, or perhaps even *Pteronymia*, which specializes on the high-alkaloid (Bradley et al., 1979; Table 3) *Geminata* section of *Solanum*. This analysis could be extended even to individual constituents once appropriately specialized populations of Ithomiinae have been identified. It may also give some false results, but these will hold even more interest than the expected ones, pointing out new types of plant components. As a preliminary survey, it should be strongly suggestive of certain types of chemicals.

CHEMICAL CONTRIBUTIONS OF THE SOLANACEAE TO PREDATOR DEFENSE IN THE ITHOMIINAE

ITHOMIINE CHEMISTRY

Chemical investigation of the passage of Solanaceae toxins to adult Ithomiinae started with

TABLE 5. Summary of PA content and distribution, and bioassay of adult Ithomiinae and Danainae.

Tribe	Genus	No. of spp. In Genus	No. of spp. Analyzed	Typical Species[a]	Source[b]	Host Plants[c]	Number Analyzed
ITHOMIINAE[g]							
B	*Tithorea*[h]	2	1	*harmonia*[ij]	VB	45	21/16
C	*Aeria*[h]	3	3	*olena*[j]	C	43, 44	31/20
				elara	G	*43*	10/15
E	Methona[h]	7	5	*themisto*[ikj]	C	41, 42	36/27
F	*Placidula*[h]	1	1	*euryanassa*[k]	C, J, SL	33	24/17
G	*Olyras*[h]	2	1	*crathis*[k]	V	*37*	4/2
	Melinaea[h]	10	7	*mneme*	RO	*36*	5/4
				ludovica[k]	M	*36*	50/1
				menophilus	RO	*37*	15/15
H	*Thyridia*	1	1	*psidii*[i]	C	20, 21	10/10
	Sais	2	1	*rosalia*[i]	G	19	12/12
	Scada	6	2	*reckia*[k]	RO	*16*	35/13
				karschina	ES	*16*	15/4
	Mechanitis	5	5	*polymnia*[ikj]	C	6–13	44/37
					M	*14*	12/12
				lysimnia[ikj]	C	13–15	28/14
I	*Hyposcada*	8	4	*egra*[k]	M	*36*	11/1
	Oleria	30	13	*astrea*	BJ	*12*	4/12
					RO	*12*	13/7
J	*Epityches*	1	1	*eupompe*[k]	C	25–31	30/33
	Rhodussa	1	1	*cantobrica*	RO	*18*	17/9
	Napeogenes	18	8	*cyrianassa*	ES	*18*	10/8
				inachia	RO	*18*	10/11
	Garsauritis	1	1	*xanthostola*	M	*13–14*	48/20
	Hypothyris	16	13	*ninonia daeta*[k]	C	5–14	51/40
				euclea[hikj]	C	9	22/22
				(males attracted)	M	10	16/12
					V	*10*	17/14
K	*Ithomia*	21	7	*agnosia*[ik]	C	30, 31	29/38
L	*Callithomia*[h]	3	2	*lenea xantho*[k]	MP	16	18/21
	Dircenna	7	4	*dero*[k]	C	6–13	29/18
M	*Ceratinia*	5	4	*neso*[h]	BJ, RO	*13*	24/24
	Ceratiscada	3	2	*doto*	BJ	1?	6/7
	Prittwitzia	1	1	*hymenaea*[k]	C	1	10/12
	Episcada	15	5	*clausina*	ES	1	17/14
				carcinia	C	1?	25/29
	Hyalenna	5	1	*pascua*[k]	J	7	1/9
	Pteronymia[h]	41	10	*carlia*[k]	C	1	31/17
N	*Godyris*	10	3	*zavaleta*[k]	RO	*38*	10/9
	Pseudoscada	6	4	*erruca*[k]	C	39	31/28
	Greta	10	1	*andromica*[h]	V	*38*	4/4
	Mcclungia	1	1	*salonina*[k]	C	39	11/11
	Hypoleria	11	10	*arzalia*	G, VB	*39*	8/4
	Heterosais	3	3	*nephele*	VB, RO	*40*	16/11
				giulia	V	*40*	7/4
DANAINAE							
	Danaus (*Anosia*)[h]	2	2	*gilippus*[i]	C	*Oxypetalum*	25/16
	Danaus (*Danaus*)[h]	1 (S.A.)	1	*plexippus*	C	*Asclepias*	22/14
	Ituna[h]	1	1	*ilione*[e]	C	*Ficus*	6/5
	Lycorea[h]	2	2	*cleobaea*	C	*Ficus*	12/11

TABLE 5. Continued.

Average Dry wt. (mg)	Individual PA Analysis (♂/♀)[d] Average PA conc. (% N-Ox.)	Maximum (% av. dry wt.)	Other Fractions[e] (% average dry wt., ♂/♀) A (neut.)	C (polar)	F (fat)	Fract. Tested	Bioas. Results[f]
60/75	0.3/0.3 (48/45)	0.9/0.6	8.4	8.4	13.9	Ex	+
9/13	2.5/1.6 (55/55)	7.1/4.0	3.9/5.2	14.7/15.8	13.9/16.3	Ex	+
9/13	1.3/1.1 (61/57)	1.9/1.9					NT
100/120	0.06/0.1 (41/48)	1.6/0.9	3.2/1.9	7.2/7.0	10.8/26.6	Ex	+
54/65	0.2/0.2 (48/48)	1.6/0.9				Ex	+*
110/110	1.2/0.3 (54/49)	1.8/0.5					NT
55/60	6.3/1.2 (54/49)	10.5/1.5					NT
55/60	0.6/1.3 (57/59)	2.5/—	5.9/3.7	7.2/9.6	15.7/13.9	Ex	+
55/60	3.6/1.3 (50/49)	6.5/4.4					NT
64/70	2.7/2.2 (70/68)	5.1/3.9	3.5/4.3	9.3/7.1	12.3/20.7	Ex	+
25/27	3.6/1.6 (59/58)	6.8/3.6	7.0/7.2	12.7/7.4	20.7/21.6	Ex	+
6/7	9.7/3.2 (47/54)	20.6/5.3					NT
8/9	13.1/6.7 (61/65)	20.0/10.4					NT
33/45	1.8/2.4 (60/60)	5.8/4.0	8.9/5.8	6.4/6.7	14.1/23.8	Ex, B, C	+
		September:	5.6/6.1	5.4/3.0	9.7/18.9	A, F	−
35/45	4.4/2.9 (58/57)	7.6/4.7	6.2	8.3	10.7		NT
25/35	3.8/1.8 (50/55)	8.8/6.4				Ex	+
20/22	0.7/0.2 (51/48)	3.8/—					NT
13/15	2.0/1.9 (53/58)	4.5/4.0					NT
13/15	4.0/1.8 (50/43)	8.2/3.4					NT
24/26	6.9/3.2 (56/58)	11.4/4.3	2.3	11.4	11.9	Ex	+
18/20	3.1/3.3 (48/52)	8.6/6.1					NT
18/20	7.7/3.5 (55/56)	12.9/5.9					NT
13/14	5.4/3.6 (56/54)	11.7/6.2					NT
20/23	0.9/1.0 (53/53)	4.9/3.2					NT
25/27	3.3/2.8 (51/57)	9.2/6.5	3.5	6.3	21.8	Ex	+
22/25	2.2/1.5 (48/44)	9.3/5.4	4.2	10.3	12.2	Ex	+
20/23	0.8/1.5 (55/46)	2.9/4.5	5.3	10.8	10.4		NT
20/22	5.1/2.8 (58/60)	9.0/5.7					NT
14/16	5.4/2.0 (56/58)	12.9/7.1	1.9	10.6	23.2	Ex	+
24/28	0.5/0.4 (52/53)	2.6/1.5					NT
40/45	1.7/0.9 (65/69)	4.3/2.4	4.0	9.9	12.3	Ex	+
15/17	2.0/1.0 (55/56)	5.7/3.3				Ex	+
14/15	1.1/0.5 (52/48)	1.9/1.2					NT
14/16	2.8/1.7 (57/60)	5.8/3.5	4.6	7.4	19.5	Ex, B, C	+
16/17	5.7/5.3 (53/58)	10.0/7.0				A, F	NT
17/18	1.6/2.0 (51/58)	6.0/5.2				Ex	+
18/20	1.1/2.0 (47/52)	—/3.4					NT
14/17	7.8/3.6 (58/62)	13.3/5.8				Ex	+
33/33	1.5/1.4 (43/51)	2.9/3.1					NT
15/17	4.9/2.7 (57/54)	9.7/5.8	4.9	14.0	9.6	Ex	+
15/17	7.2/5.0 (56/52)	10.6/6.0					NT
16/17	1.7/1.1 (70/82)	3.3/2.2	5.2	7.5	15.5	Ex	+
14/15	1.1/0.5 (56/59)	2.3/0.9					NT
21/26	1.6/0.6 (55/53)	4.4/1.0					NT
20/24	2.7/0.8 (53/43)	5.3/1.7	4.8/—	9.6/—	8.2/—	Ex	+
80/75	2.1/1.8 (57/52)	4.5/4.5					NT
180/170	0.1/0.1 (50/51)	0.4/0.2					NT
160/140	1.1/1.2 (54/53)	2.5/2.1					NT
130/110	2.7/1.2 (58/55)	4.8/3.2					NT

TABLE 5. Continued.

ANALYSIS OF PARTS:[m] Species & Sex	WINGS Basal	WINGS Hairpencil	WINGS Apical	Appendages (Legs, Anten.)	Rest of Head
Mechanitis polymnia casabranca (males)	1.13/88	**4.15**/64	0.14/100	0.26/77	0.60/70
	1.70/100	1.10/100	1.03/65	0.52/92	1.03/72
	1.03/58	**1.70**/58	0.83/51	0.98/97	1.43/75
	2.60/85	**4.40**/100	2.10/48	4.20/77	3.40/75
Ithomia agnosia agnosia (females)	4.50/81	—	1.38/78	7.50/68	**12.5**/69
	0.69/42	—	0.63/45	**1.60**/55	**2.00**/68
	0.70/67	—	0.75/48	0.72/100	**1.05**/44
		"pockets"			
Danaus gilippus (male)	3.10/63	1.00/55	0.73/49	2.00/57	1.75/55
(female)	1.45/50	—	0.47/42	1.20/46	1.50/51

	Displaying Male	
	Rest of Insect	Spermatophore
Mechanitis lysimnia	3.09/61	8.50/54
	3.32/59	8.85/55
	3.06/52	9.75/49
	0.78/54	2.40/64
	4.20/52	**25.3**/55
	6.40/56	**17.4**/54
Mechanitis polymnia	2.23/52	**8.60**/62
	0.26/46	1.05/54
	0.92/62	3.92/55
Aeria olena	3.00/50	**12.3**/67
	3.60/69	**10.0**/72

[a] Authors' and subspecies names are in Figure 5 or Brown (1985).

[b] See Table 3, footnote b. Additional localities: BJ = Bujaru, Pará. ES = various parts of Espírito Santo. RO = various parts of Rondônia. VB = Vila Bela, western Mato Grosso.

[c] Numbers refer to the plants listed in Table 3. Italicized numbers indicate probable food plants or species near to that number.

[d] Just-captured butterflies were cut up under 2 ml absolute methanol (3 ml for heavier species) and after at least one day's standing aliquots (usually 1/20, or 1/4 for component parts) were assayed directly for total PA and N-oxide, following Mattocks (1967, 1968) and Bingley (1968); see text for details.

[e] See fractionation scheme in Figure 4 for letters.

[f] See footnote g of Table 3 for bioassay codes.

[g] No secondary compounds from larval food plants have been found stored in adult Ithomiinae. Additional genera examined (few specimens) were (Tribe letter, genus and species, host plant): A *Tellervo zoilus, 45*; B *Elzunia humboldt, 45*; D *Athesis clearista,* 23 and *Patricia dercyllidas, 30*?; G *Eutresis hypereia, 37* and *Athyrtis mechanitis, 37*?; H *Forbestra equicola, 16* and *F. truncata, 16*; I (New genus) *canilla, 18*; J *Hyalyris coeno, 13* and *Hyalyris oulita metella, 13*; K *Miraleria cymothoe,* 31 + 33; L *Velamysta pupilla, 17*; N *Dygoris dircenna, 39* and *Hypomenitis dercetis, 39* and *H. libethris, 39.*

Rothschild (1973), who obtained only negative results. In August 1978, 3,200 dry bodies of *Mechanitis polymnia* (75 g, representing over 300 g fresh weight of insects) were mailed to Dr. Desiré Daloze of the Collectif de Chimie Bio-Organique in Brussels, where ant bioassays were used to follow repellent activity in the fractionation. No alkaloids, cardiac glycosides, or other interesting active compounds, or even their degradation products, could be found (D. Daloze, pers. comm., 1980). This along with the previous results suggested that the protective compounds of adult Ithomiinae might be labile, degraded after death or upon storage, and possibly unrelated to the larval food plant poisons.

Total MeOH-H_2O extracts of fresh ithomiines

TABLE 5. Continued.

THORAX		ABDOMEN				
Exo-skel-ton	Muscles	Exoskeleton	Fat	Reprod. Organs	Intestine	
0.29/75	0.21/100	0.21/92	0.49/65	0.12/91	0.02/100	NR
1.18/42	0.92/48	1.82/91	0.81/66	**2.54**/99	0.63/89	
1.02/66	0.63/65	0.50/100	0.19/69	0.60/100	0.14/56	
4.10/67	4.40/68	**6.60**/72	3.20/100	4.10/92	4.00/97	
						Eggs
1.06/79	3.56/50	4.45/65	1.76/65	**52.0**/45	0.13/91	**8.85**/78
0.35/45	0.47/20	0.47/38	0.42/54	**1.67**/40	0.28/14	1.00/30
0.62/48	0.40/33	**0.87**/100	0.26/18	0.34/100	0.15/60	NR
1.90/57	1.20/50	2.20/56	0.73/65	**4.20**/58	Hairpencil	3.50/55
1.60/52	1.10/48	1.40/62	**3.30**/59	0.90/62	Eggs	0.90/12

Pairs captured in copula			
Male: Rest of Insect	Spermato-phore	Female: Rest of Insect	Abdomen Ducts
2.12/52	**12.8**/65	6.26/52	3.50/59
2.23/64	**11.9**/54	0.40/74	0.35/57
3.71/54	**12.7**/66	2.26/62	0.83/100
0.44/56	**1.31**/78	3.00/62	3.30/60
1.29/50	**22.5**/60	3.30/51	3.51/65
0.25/54	4.70/48	3.58/63	5.10/62
0.87/52	9.10/61	2.52/48	3.26/58
0.38/48	**14.6**/72	2.06/72	7.20/88
2.96/59	**12.6**/72	5.06/61	**13.6**/63
2.26/51	2.32/57	3.90/58	**40.5**/64
8.90/62	**18.7**/77	4.10/54	5.40/59

[h] Larvae of these species may be considered as aposematic in color-pattern and behavior.

[i] Eggs of these subspecies contained up to 9% of dry weight as PAs.

[j] Larvae of these species feeding on the indicated plants did not contain any PAs nor did their extracts protect *Biblis* against predation by *Nephila*; possible exceptions are *Methona themisto, Aeria olena* and *Tithorea harmonia*.

[k] Recently emerged adults of both sexes of these species reared from larvae on the indicated food plants, were consumed without hesitation by *Nephila*, which in most cases had just cut out an adult of the same species and sex captured in the field. Notice that almost all Solanaceae genera and chemicals are included in the food plants.

[m] Data are given as percent of dry weight of part/% of PA as N-oxide. When the N-oxide value is high, it usually indicates appreciable PA present as dihydropyrrolizines (types 3MNO), usually confirmed. Especially noteworthy values are printed in boldface type.

(prepared as in Fig. 4), when applied to the edible *Biblis hyperia*, were at least as repellent to *Nephila* spiders as were the live butterflies. The repellency was then located in the alkaloid fraction B (Fig. 4) and also in the polar fraction C. When these fractions were compared with the corresponding fractions of the larval food plants, there appeared to be no compounds in common. Furthermore, all food plant extracts and fractions tested in the spider bioassay were negative (Table 3). To put the final nail in the coffin, both sexes of 30 species in 26 genera of Solanaceae-feeding Ithomiinae, reared from the larvae in the laboratory on fresh leaves of natural food plant, upon emergence from the pupa and introduction into *Nephila* webs were promptly and enthusiastically eaten (Table 5). In most cases, the *Nephila* had just as efficiently rejected a field-captured adult ithomiine of the same species and sex.

In all, 142 species of Ithomiinae in 45 genera and all 14 tribes were examined in parallel with 48 host plants in 16 genera of Solanaceae, three genera of Apocynaceae, and one of Gesneriaceae (see Tables 2, 3, 5), both in chemical analysis

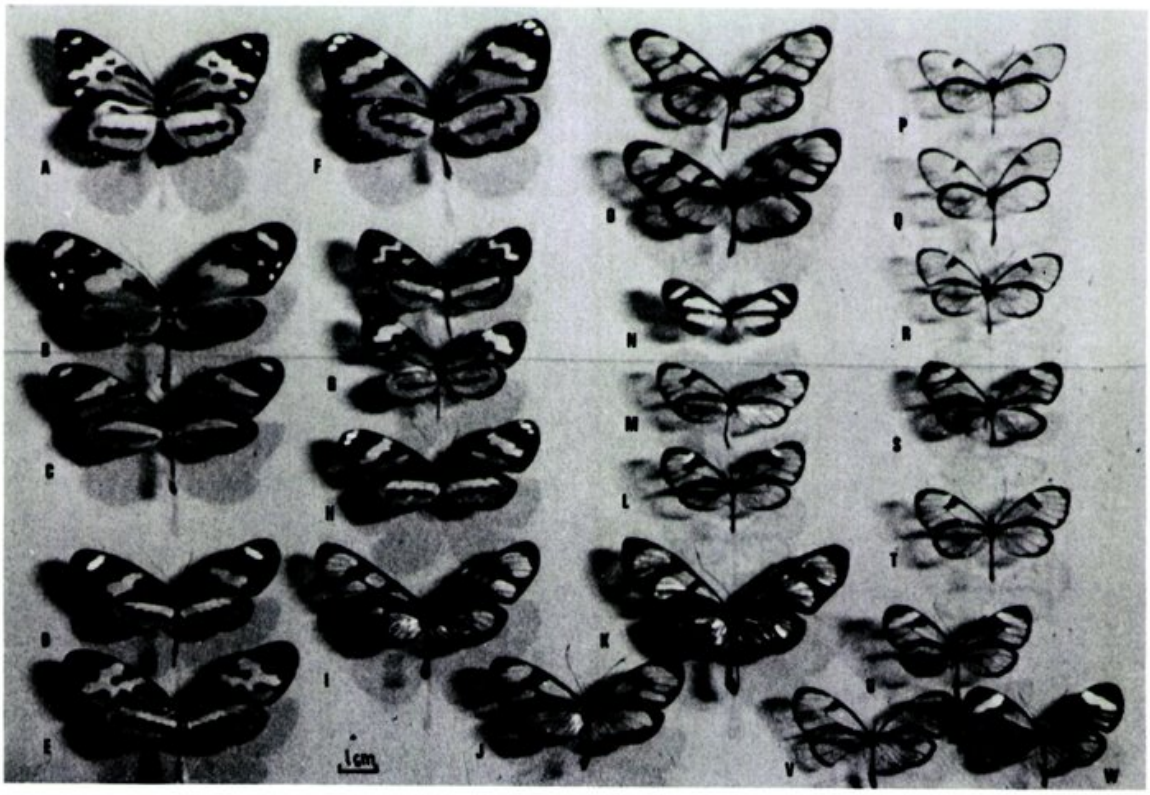

FIGURE 9. Ithomiinae found in the Campinas region, interior of São Paulo state, SE Brazil. Missing are **Episcada philoclea* (Hewitson) and †*Pseudoscada quadrifasciata*, very similar to U. * indicates rare visitors from the coastal mountains or plain, † rare visitors from the tropical Paraná valley to the west. Names follow in order of natural abundance in dry season assemblages.—E. *Mechanitis polymnia casabranca* (about 50% of all Ithomiinae, can increase to 75% in some seasons).—D. *Mechanitis lysimnia* (17%).—S. *Mcclungia salonina* (13%).—H. *Hypothyris ninonia daeta* (8%).—L. *Ithomia agnosia* (5.5%).—N. *Aeria olena* (2.5%).—P. *Prittwitzia hymenaea* (1.1%).—O. *Dircenna dero* (upper *D. celtina*, lower *D. rhoeo*) (0.8%).—G. *Hypothyris euclea* (*laphria* × *nina*, mixed population) (0.4% but can be common in some years).—A. *Tithorea harmonia pseudethra* (0.4%).—J. *Epityches eupompe* (sometimes common in September).—T. *Pseudoscada erruca* (common in some pockets, not in others).—U. *Hypoleria plisthenes*.—I. *Thyridia psidii cetoides*.—Q. *Episcada carcinia*.—K. *Methona themisto* (common on tree-lined city streets and in gardens).—R. *Pteronymia carlia*.—W. †*Hypoleria goiana*.—M. **Ithomia drymo* (sometimes moves through in numbers in September).—F. **Placidula euryanassa*.—V. **Hypoleria adasa*.—B. **Melinaea ludovica paraiya*.—C. **Melinaea ethra*. The ranking is based on over 20,000 captures, mostly for marking and recapture population studies. For systematic order of genera and species (primitive to advanced), see Tables 2 and 5; for authors' names, see Figure 5.

and bioassay. In terms of the hypothesis of sequestration of defensive chemicals by ithomiine larvae, these results were thoroughly disappointing; no important compounds were detected that were shared by butterflies and their larval food plants. All butterfly extracts were positive and all plant total extracts and fractions were negative in the *Nephila* tests. However, all butterflies showed a strong Mayer's test on the acidified total aqueous extract, suggesting the presence of some kind of alkaloids.

Since adult ithomiines, like danaines, are known to be strongly attracted to sources of dehydropyrrolizidine alkaloids (Pliske, 1975a, 1975b) and use these at least for pheromone synthesis (Edgar et al., 1976), the extracts were examined for these compounds, using on TLC plates the iodine/Ehrlich test (dehydrogenation followed by *p*-dimethylaminobenzaldehyde in conc. HCl/acetone). All butterfly species showed the presence of similar moderately polar PAs as the major components of the alkaloid fraction B; in all cases, this fraction was greatly augmented and often became over 90% of a single compound after the zinc-reduction loop in Figure 4, indicating that much of the PAs were present as a single alkaloid and N-oxide in the butterflies, the latter probably responsible for the activity seen in fraction C. Pure pyrrolizidine alkaloid fractions accounted for essentially all the activity

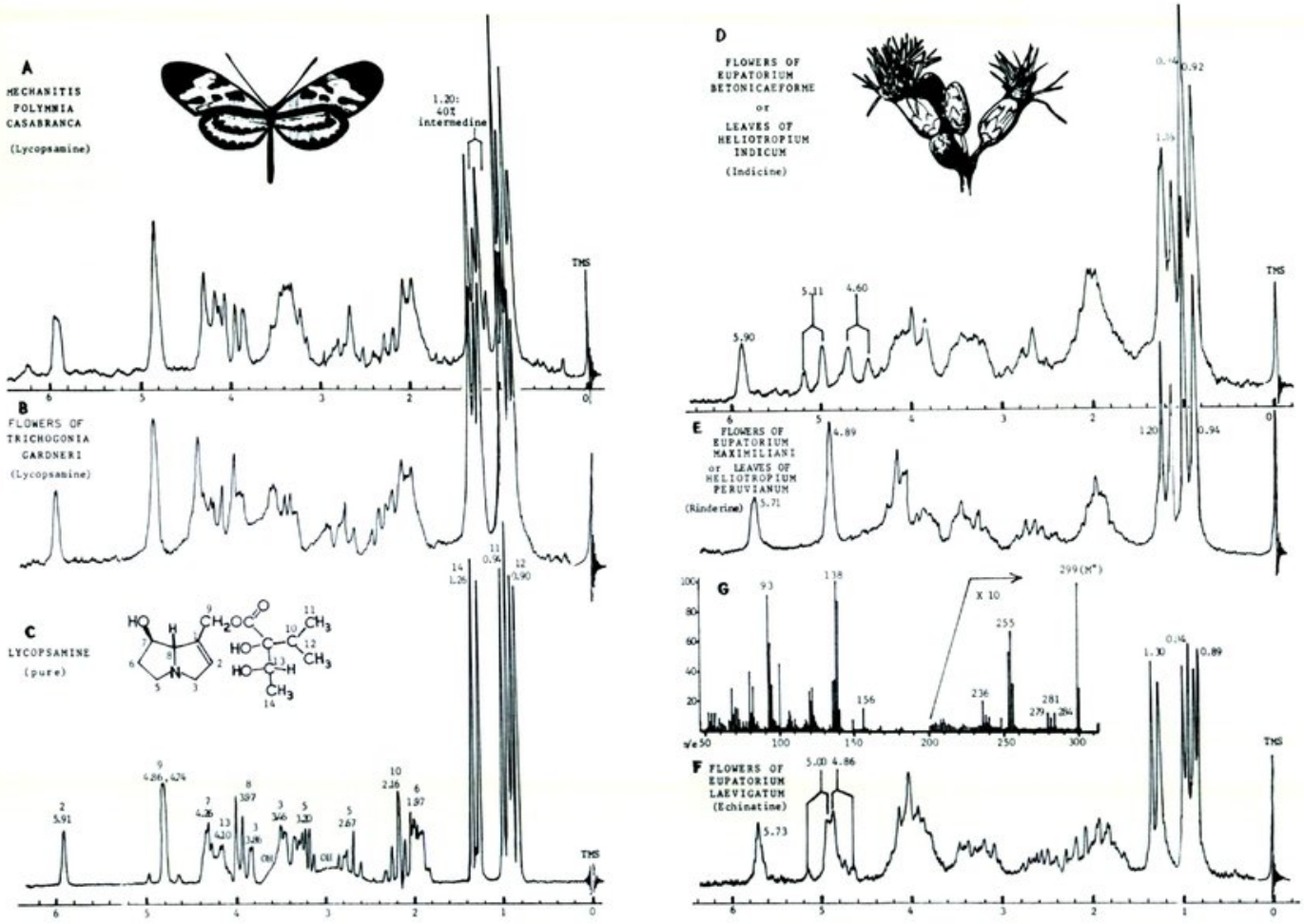

FIGURE 10. NMR spectra at 60 MHz in $CDCl_3$.—A. Crude total alkaloid from *Mechanitis polymnia* (lycopsamine/intermedine 6:4).—B. Crude total alkaloid from *Trichogonia gardneri* flowers, Amarais (same).—C. Pure lycopsamine (100 MHz, spectrum sent by J. A. Edgar).—D. Crude total alkaloid from *Eupatorium betonicaeforme* flowers (Bertioga) or *Heliotropium indicum* leaves (indicine).—E. Crude total alkaloid from *Eupatorium maximiliani* flowers or *Heliotropium peruvianum* leaves (rinderine, very easily crystallized).—F. Purified total alkaloid from *Eupatorium laevigatum* buds or flowers (isolated by J. R. Trigo).—G. Mass spectrum of echinatine (F) at 90 eV similar to that of all other isomers.

FIGURE 11. Feeding of Ithomiinae at PA sources.—A. *Mechanitis polymnia casabranca* on *Trichogonia gardneri* flowers, Amarais, Campinas, SP.—B. *Mechanitis lysimnia elisa* (Guérin) on *Eupatorium macrophyllum* flowers, Colorado, Rondônia.

seen in the *Nephila* bioassay, protecting the adults against predation by this spider.

The most common species in the Ithomiinae communities in the Campinas area, *Mechanitis polymnia casabranca* (Brown & Vasconcellos-Neto, 1976; Vasconcellos-Neto, 1980, 1986; Fig. 9), was chosen initially for detailed chemical investigation, using the fractionation scheme shown in Figure 4. The ethyl acetate extract (F) from 455 butterflies captured in Amarais in August 1982 (19.1 g dry weight, 13 of this as insoluble tegument) weighed nearly 5 g and readily solidified at 8°C to an off-white crystalline mass, indicating nearly pure saturated triglyceride; its exact composition is presently under investigation, but it was negative in the *Nephila* bioassay—probably quite nutritive, in fact. This extensive storage of high-energy fat (26% of dry weight at the height of the winter dry season) reflects the long (up to six months) reproductive diapause of these species (May–October; Vasconcellos-Neto, 1980) and also helps to explain the advantages of the learned predation behavior of the tanager (Brown & Vasconcellos-Neto, 1976), which squeezes this fat out of the abdomen of the butterflies. It is interesting that the fat reserves fell to only half of August levels in butterflies extracted in late September; the butterflies are long-lived and have few exogenous resources in September. Tanager predation usually ceases in September, probably from a combination of a lower reward (here confirmed) and more difficulty in capture as the days become warmer (Brown & Vasconcellos-Neto, 1976).

The total alkaloid extract B, isolated after the zinc reduction loop (which increased its weight from 60 to 260 mg equivalent yield from 19 g butterflies = 1.4% of dry weight) showed on TLC a single major spot (90%) that gave a positive I_2/Ehrlich test for PAs. The 60-MHz NMR spectrum of this noncrystallizable fraction (Fig. 10) showed it to be almost exclusively a 60 : 40 mixture of lycopsamine and intermedine (Fig. 3E, F) compared with a spectrum of pure reference lycopsamine contributed by J. A. Edgar (Fig. 10C). Very minor impurities due to other dehydropyrrolizidines could be seen in the NMR spectrum. The alkaloid fraction co-chromatographed with authentic lycopsamine (donated by J. A. Edgar) on TLC in four different systems, giving indications of latent separation of the isomers in some solvent mixtures.

The NMR spectrum of the total alkaloid fraction showed no signs of the "methylene envelope" or quaternary methyl signals typical of steroid alkaloids of *Solanum*, nor of the anomeric protons of glycosides, nor of the N-methyl groups of solanines, nicotine, and tropane alkaloids (Figs. 1, 10). This supports nonsequestration of alkaloids from the larval food plant. The spectrum also indicated that most of the butterflies obtained their alkaloid from a single source; the structure and the mixture strongly support flowers of *Trichogonia gardneri*, common in Amarais and intensively visited by *M. polymnia* (Fig. 11A; Table 6).

When the zinc reduction was not performed, the much diminished alkaloid fraction showed two spots, one corresponding to the lycopsamine/intermedine mixture and the other more mobile (probably the isomers echinatine/rinderine, Fig. 3G, H). The NMR spectrum of the whole fraction was rather complex, indicating several additional components of diverse structures including saturated PAs, but still showed no signs of the presence of other classes of alkaloids.

In expansion of the work on *M. polymnia* preliminary fractionation gave PAs representing various mixtures of the five isomers of a single structure (Figs. 3, 10) in up to 13%, and in *Scada* over 20% of dry weight in some individuals, in all 141 additional Ithomiinae species indicated in Table 5, many captured in the Campinas region but also sought in more distant places for comparison and to verify the generality of the phenomenon. No PAs were seen in the Zn-reduced extracts of any of the food plants tested (summarized in Table 3).

ITHOMIINE DEFENSE

That the protection of adult Ithomiinae against predation by *Nephila* is due to PAs was confirmed by feeding 200–400 μg of echinatine N-oxide (Fig. 3J) in dilute honey solution to newly emerged adults of *Mechanitis lysimnia* and *Pseudoscada erruca* (which normally were eaten by *Nephila*); within an hour, the butterflies were routinely rejected by the spiders (experiments performed together with J. R. Trigo). Reared butterflies kept alive for many days did not biosynthesize any protective chemicals; protection was lost within a day after death of a butterfly rejected by the spider, again indicating the instability of the PAs. Very fresh field-captured Ithomiinae often showed no PA and could be consumed by *Nephila*, but in general the adults seemed to be able to accumulate sufficient defensive compound from different sources in their environment within a very short period after emergence from the pupa.

The strong dependence of adult ithomiines on PA sources, including for their courtship pheromones, perhaps made it inevitable that they should also retain the PAs for their defense. The lack of storage of Solanaceae defensive compounds, already verified in early investigations, increased the probability of alternate defensive compounds in the Ithomiinae—labile compounds not detectable in dead insects. The universal rapid cutting out of Ithomiinae from *Nephila* webs suggested that such alternate defense substances did not derive from the widely variable larval foods but from a more homogeneous adult food source. In the only other reported case of a lepidopteran being cut out from spiders' webs—*Utetheisa ornatrix*, an aposematic day-flying arctiid whose larvae feed on *Crotalaria* and pass sequestered PA diesters on to the adults, where they are also used in pheromone synthesis—it has been shown that PAs are responsible for this deterrent activity (Conner et al., 1981; Eisner, 1982).

The Solanaceae poisons have thus been absolved, at least for the time being, from participation in the unpalatability of adult Ithomiinae to their most dangerous predators. It seems possible that exceptions will be found to this, especially as more predators are incorporated into the bioassays and more butterflies and food plants into the chemical analysis. However, the PAs and especially the biologically active (hepatotoxic, tumor-inhibiting) lycopsamine-group monoesters and their N-oxides should be regarded as the principal, perhaps nearly universal chemical protection for adult Ithomiinae.

PAs are also widespread in Danainae and Arctiidae moths (Ctenuchinae, Pericopinae, and Arctiinae), whose adults sometimes inherit them from the larvae but inevitably seek them out at the same sources visited avidly by the Ithomiinae, and similarly use them for defense and pheromone synthesis. Indeed, lycopsamine and its stereoisomers have been found in wild populations of essentially every species of Danainae and Ithomiinae investigated (Edgar, 1982; Table 5), though their presence is erratic in the moths. These compounds seem to represent a very effective "ancestral predator defense" that has been retained in diverging phyletic lines up to the present (Edgar, 1975) in spite of a variety of habitats, food plants, and behaviors.

Adult Ithomiinae sequester their PAs from a variety of sources: decomposing borages and composite flowers (Eupatorieae) especially, but also orchids, *Crotalaria*, apocynes, and less traditional PA-containing materials. These are inevitably abundant wherever Ithomiinae occur in numbers.

The proponderance of PAs in adult Ithomiinae defense could also help to explain the cryptic coloration and behavior observed in most ithomiine larvae (Fig. 8), notable exceptions being *Methona* on *Brunfelsia* (Fig. 8W, X), the feeders on Apocynaceae (Fig. 8T, U), *Melinaea* with similar larvae (Fig. 8K, V) feeding on *Markea*, *Juanulloa*, and related genera, and a few showy larvae scattered on other plants. Newly emerged ithomiine larvae eat their eggshells (which contain PA derived from their mother; see below) and immediately move to the underside of the same leaves (if not already there), usually acquiring a cryptic coloration. The fact that most ithomiine immatures are cryptic translucent green, closely matching their substrate, and feed

TABLE 6. Summary of PA content and distribution, and bioassay of food sources of adult Ithomiinae.

Adult Food Source[a]	Source[b]	Weight Fresh	Weight Dry[c]	% H_2O	Fractions (as % of dry wt.)[d] A	B (% N-Ox.)[e]	C	F	Fibre	PAs[f]	Fractions Tested[d]	Bioassay Results[g]
COMPOSITAE: SENECIONEAE (flowers)[h]												
Erechtites valerianaefolia (Wolf.) DC.*[h]	SL	6.5	1.7	64	1.9	0.47 (?)	11.2	3.8	82.6	?	Ex	+*
COMPOSITAE: EUPATORIEAE (flowers)[h]												
Mikania cordifolia Willd.	C	26	7.9	68	5.5	0.5 (?)	14.1	5.3	74.6	—	Ex, B, C	+*
Trichogonia gardneri A. Gray*[h]	C	14	3.8	72	5.0	1.4 (60)	15.2	7.3	71.1	E, (F)	Ex, B, C	+
Adenostemma involucratum King. & H. Robinson[i*h]	RJ	6.8	1.4	80	4.6	0.87 (70)	10.9	3.6	79.9	EH?	B	+
Adenostemma brasilianum Cass.[i]	I	17	3.5	79	7.9	3.4 (70)	14.2	3.1	70.8	EH?		NT
Adenostemma sp.[i]	ES	—	0.54	—	4.4	2.0 (?)	17.3	2.4	73.9	E?		NT
Eupatorium itatiayense Hieron.	J	160	54	66	7.4	0.98 (?)	19.2	7.6	64.8	F, (H)		NT
Eupatorium vauthierianum DC.	I	9.1	2.3	74	6.5	1.6 (60)	20.3	2.9	68.7	H?		NT
Eupatorium macrophyllum L.	AP	—	10.7	—	3.2	0.86 (35)	9.2	2.9	83.8	H	Ex	+
	ES	—	1.4	—	3.4	1.7 (46)	13.3	4.2	77.4	H		NT
	V	—	1.2	—	1.8	0.73 (?)	11.3	3.1	83.3	H		NT
Eupatorium odoratum L.	BJ	—	6.4	—	1.9	0.25 (30)	8.2	1.6	88.2	H?	Ex	+
Eupatorium maximiliani Schrad. ex DC.	C	13	4.1	69	1.6	0.73 (60)	10.0	2.1	85.6	H	Ex	+
Eupatorium laevigatum Lam.[j]	C	9.8	1.7	82	12.2	3.9 (60)	17.4	4.6	63.2	G	Ex, B, C	+*
Eupatorium macrocephalum Less.	PA	—	5.9	—	6.3	1.4 (?)	12.7	9.6	70.0	H		NT
Eupatorium betonicaeforme (DC.) Baker*[h]	SL	75	13.8	82	1.4	1.64 (60)	8.4	3.3	79.3	I		NT
	C	360	90	75	7.8	2.5 (60)	8.8	7.0	73.9	I + ??		NT
BORAGINACEAE: HELIOTROPOIDEAE												
Heliotropium indicum L. (aerial parts)	C	82	22.6	72	3.4	3.7 (49)	17.6	1.0	74.3	I	Ex, B	+
Heliotropium peruvianum L. (leaves)[j]	C	64	11.8	82	2.9	10.7 (64)	9.4	2.5	74.5	H	B	+
(stems)	C	35	7.0	80	2.3	6.7 (65)	25.4	1.6	61.7	H	Ex	+
Heliotropium transalpinum Vell. (leaves)	RC	130	26.5	80	5.1	0.44 (42)	12.1	2.6	79.6	HI	Ex, B	+
(whole plant)	C	—	3.5	—	4.4	0.77 (25)	13.2	0.6	81.0	HI		NT
Tournefortia breviflora DC. (flowers)	C	31	8.6	72	1.9	6.7 (55)	31.8	5.0	54.6	E + ??		NT
(leaves)	C	67	21	69	5.3	3.0 (58)	15.2	5.1	71.4	??		NT

TABLE 6. Continued.

ANALYSIS OF PLANT PARTS:[m] Eupatorieae Species (Source[b])		Young Leaves	Stems and Peduncles	Immature Buds		Mature Flowers				Seed Capsules		
				Involucre	Young Flowers	Involucre	Ovaries	Nectaries	Upper Part	Involucre	Seeds	Dry Flowers
Trichogonia gardneri (June)	C	3.50/12	0.24/18	1.70/73	2.20/77	1.16/79	**4.10**/60	**4.10**/32	2.90/49	0.94/87	2.13/49	1.04/46
(September)	C	1.82/19	0.93/13	2.86/38	4.93/48	2.67/47	2.48/76	**7.70**/60	5.03/52	0.60/38	0.80/37	0.38/21
Eupatorium maximiliani (June)	C	0.24/54	0.23/13	0.41/23	1.67/80	0.25/30	0.79/46	**2.20**/17	0.82/70	0.60/17		0.87/57
Eupatorium betonicaeforme (Oct.)	SL	0.59/46	0.07/88	1.67/46	**4.09**/58	0.61/42	**3.30**/40		1.64/47	0.72/49	2.95/50	1.07/61
(older parts[k]) (roots: 0.56/55)		0.00/—	0.004/—	1.01/49	**3.22**/57					0.18/52	0.38/22	0.29/44
(December)	C	0.13/23	0.23/8	1.26/50	2.64/55	0.80/40	1.24/67	**3.24**/56	1.22/54	0.33/33	1.16/47	0.67/42
(older parts[k]) (roots: 0.16/69)		0.02/53	0.005/—	0.78/32	**3.73**/53	0.36/37	1.35/46		0.68/40	0.30/48	0.71/53	0.92/59

[a] Whenever possible, food sources actually being used intensively by Ithomiinae adults were collected for extraction.

[b] See footnoes b of Tables 3 and 5. Additional localities: AP = upper Rio Amapari, Amapá. I = Serra de Itatiaia, Rio de Janeiro. PA = Porto Alegre, Rio Grande do Sul. RC = Rio Claro, São Paulo.

[c,d,e,g] See footnotes c, d, e and g of Table 3.

[f] See structures and stereochemical configurations in Figure 3.

[h] Also examined were *Eupatorium xestolepis* Rob.[i], probably containing 3E, *E. vitalbae* DC.[j], *E. squalidum* DC.[j], and *Ageratum conyzoides* L.[j], all showing PAs in good quantities. When an asterisk is used (*[h]), only the interior of the flowers (flowerlets, without involucres) was extracted.

[i] *Adenostemma* and some *Eupatorium* are fragile plants of the dark, humid forest understory, with isolated, small white flowers, typically and specifically pollinated by Ithomiinae and other PA-seeking Lepidoptera of this habitat (Pliske, 1975a, 1975b; Drummond, 1976; Haber, 1978).

[j] Collected and fractionated by José Roberto Trigo.

[k] The lower line of figures represents corresponding older parts: large leaves, lower stem, older flower bud, open flowers already pollinated, and dry seed capsules.

[m] See footnote m, Table 5.

on the underside of leaves (Fig. 8) may indicate rapid metabolism of the Solanaceae poisons (though cryptic and toxic insects are not rare); at least the poisons are not carried through to the adults, whom they would not protect in any case, and do not seem to be stored in the larvae. Aposematic but nontoxic insects are rare (except for obvious Batesian mimics that diverge from their taxonomic relatives), the main case being automimics in the Browerian sense (Brower et al., 1971; Rothschild, 1979). For this reason the strongly aposematic larvae in a few ithomiine genera may incorporate or synthesize some sorts of unpleasant substances that have yet to be identified.

In previous work reported without details, a lycopsamine/intermedine mixture was found in *Hypomenitis dercetis* and *Oleria makrena* from northern Venezuela (Edgar et al., 1976) and regarded as primarily precursorial to pheromones (only males were analyzed) rather than as defensive. Later work (Edgar, 1982) emphasized the potential defensive role of these compounds, here confirmed.

SELECTIVE PA STORAGE

When male and female *Mechanitis polymnia* were separately extracted in late September 1982, some interesting differences were observed, indicating that careful work on this chemical interaction should always maintain the sexes apart in analysis. At least in September, at the beginning of the reproductive season, the females contained much more fat and PA than males (Table 5). That this difference is closely related to the respective reproductive tasks is supported by the previous pheromone study (Edgar et al., 1976) and by analysis of *Mechanitis* eggs. A raft of 31 *M. polymnia* eggs, weighing 11 mg, was extracted with 2 N H_2SO_4 and directly reduced with zinc, alcalinized, and extracted with $CHCl_3$-MeOH. This showed on TLC the presence of abundant lycopsamine/intermedine mixture, perhaps as much as 1% of the fresh weight or 0.1 mg. All other Ithomiinae eggs analyzed also showed the presence of appreciable PA (Table 5). The bright white *Mechanitis* eggs, laid in rafts of 5–100 on top of the host plant leaves, with which they contrast vividly (Fig. 8I, N, Q), could be described as a true collective display of aposematic insects, just like the adult assemblages in dry season "pockets."

As shown in Table 5, male Ithomiinae generally accumulate more PAs than the females; indeed, in many species they are the principal or only sex found on the sources (Table 2; Pliske, 1975a, 1975b). The females of most species seem to get the majority of their PAs in the spermatophores received from males during mating, which is repetitious (Ehrlich & Ehrlich, 1978); these small sacs often have 20–50× the PA concentration as the rest of the male (Brown, 1984, 1985).

Both males and females are able to selectively distribute the collected PAs to different tissues, including tegument and wings (possibly by regurgitation with fat as a wetting agent) and especially to reproductive parts (pheromone glands, spermatophores, ovaries; Table 5), thence in females to the eggs.

Males attracted to *Heliotropium* baits placed in relatively PA-poor areas and females attracted to displaying males showed lower average and maximum PA content than random samples of the same populations, indicating an "appetite effect" that obviously would be highly adaptive in these organisms.

More complete information on PA storage, use, and distribution in Ithomiinae may be found in Brown (1985).

SOURCES OF DEHYDROPYRROLIZIDINE ALKALOID MONOESTERS

In the same paper in which he predicted that PAs would be used for defense in the Ithomiinae, Edgar (1982) predicted that they would be found in the nectar of Eupatorieae, in view of the heavy dependence of Ithomiinae on these plants and their characteristic occurrence in the Boraginaceae, also frequently visited by Ithomiinae and source of precursor for the pheromone (Fig. 3D) as well as of the most attractant esterifying acids for ithomiine males (Pliske et al., 1976). Indeed, many genera and most females are more strongly attracted to Eupatorieae flowers than to *Heliotropium* (Table 2), the difference being especially pronounced in *Mechanitis* and allies (Fig. 11).

Extraction of the flowers of 16 species in four genera of the Eupatorieae actively visited by Ithomiinae in the field led to the isolation and identification of a variety of PAs but usually only one isomer and structure in each species (Table 6; see Figs. 3, 10). Alkaloids of this structure were also found in *Heliotropium* and *Tournefortia* flowers (Boraginaceae-Heliotropoideae) often visited by Ithomiinae, as well as in the leaves of

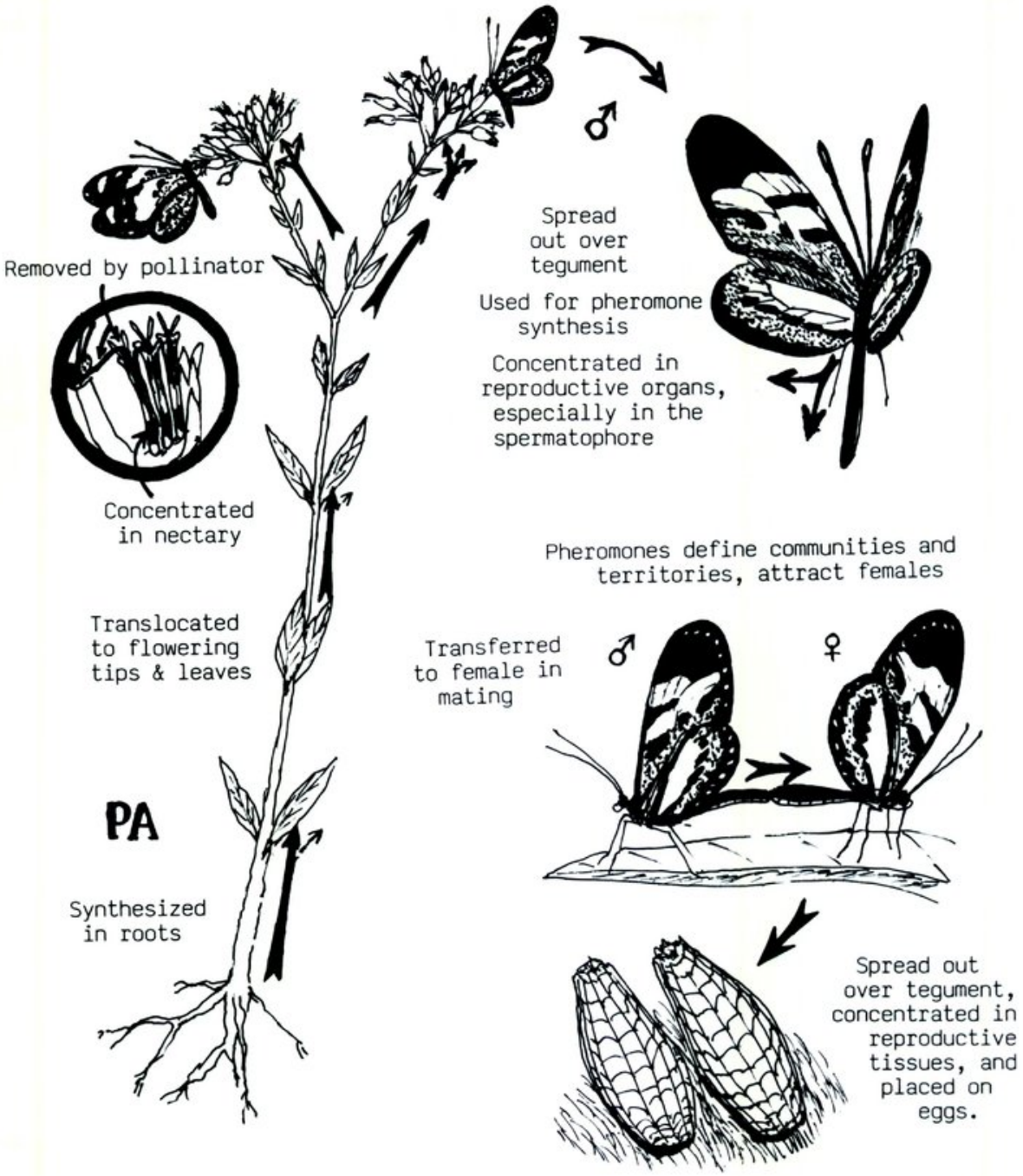

FIGURE 12. Flow of dehydropyrrolizidine alkaloid monoesters in natural ecosystems.

various *Heliotropium* species (Table 6), but not in *Mikania* (Eupatorieae), *Cordia* (Boraginaceae), or several other flowers sporadically visited by Ithomiinae. When the plants were analyzed for PAs by parts (Table 6), the highest concentrations always appeared in the nectaries of still unopened flowers, suggesting that they might be a reward to specific PA-seeking pollinators; the concentration was often 2–4% of dry weight of the whole flower.

In open vegetation or poor soil areas with few Ithomiinae present, Eupatorieae gave lower yields of less pure isomers in the flowers and showed dramatically reduced seed set, even though pollination was effected by other groups that also depend on PAs (Danainae and Arctiidae). Areas

with few Eupatorieae or only very seasonal species showed scattered and transient Ithomiinae populations, whereas in areas with abundant Ithomiinae there were always common Eupatorieae in flower throughout the year, especially *Trichogonia* (Fig. 11A; see also Pliske, 1975a). It is evident that this mutualistic relationship, profoundly affecting the reproduction and abundance of both butterflies and plants, is of fundamental importance to both groups and has determined many adaptations on both sides of the interaction, in a coevolutionary picture probably much stronger and more stable than that of the Ithomiinae with the Solanaceae.

Flow of Dehydropyrrolizidine Alkaloids in Nature (Fig. 12)

The analysis of PA monoesters in different parts of plants and butterflies in various physiological and reproductive states (Tables 5, 6; Brown, 1984, 1985), made possible by the selectivity and sensitivity of the Mattocks-Bingley assay (Fig. 3), permits a diagram to be drawn of the synthesis, flux, use, and eventual dissipation of these highly active compounds in ecological systems (Fig. 12). Thus the PAs are probably synthesized in the roots of Apocynaceae, Boraginaceae, and Compositae-Eupatorieae (diesters are also elaborated by Compositae-Senecioneae and Leguminosae: *Crotalaria*); young plants show highest concentrations in the roots and even mature plants show a bimodal concentration distribution between flower heads and roots. The compounds also may be concentrated in the leaves when this will give important protection against herbivores; in a few cases, these leaves are attacked by specific insects, including larvae of some Danainae, Ithomiinae, Ctenuchinae, Pericopinae, and Arctiinae among the Lepidoptera, and a variety of Hemiptera and Coleoptera. Many of these specific herbivores store and use the compounds directly for defense or adult reproduction, whereas others excrete them.

As the plant comes into flower the alkaloids are translocated to the flowering tips and then into the nectaries, where they guarantee attraction and relatively long visits of specific Lepidopteran pollinators in the same five subfamilies, the first three pantropical (with scattered species in north temperate areas), the fourth neotropical and the last cosmopolitan. The alkaloids are also retained in the seeds to deter predation.

In the frequent case of selective attraction of male butterflies to the PA sources, these spread the PAs over their tegument for predator defense and channel them into pheromone synthesis glands (Fig. 3), which sometimes are formed only with PA stimulation (Schneider et al., 1982), and other reproductive tissues, especially spermatophores. The spermatophores are transferred to females during mating and the PAs are similarly spread out over the tegument and channeled into reproductive tissues, eventually being deposited on the eggs for protection. Newly hatched larvae consume the eggshells but quickly lose their PAs unless they find themselves on leaves that contain them.

This chemico-ecological flow scheme (Fig. 12) opens ample perspectives for the localization, selection, and cultivation of PA-producing plants. Indicine N-oxide (Fig. 3K) and at least one isomer (Fig. 3J) are promising anticarcinogenic drugs now in advanced clinical testing; some *Eupatorium* in the right ecologico-evolutionary setting might become important sources for these compounds (Table 6). The free bases, however, and especially the diesters, are very hepatotoxic and carcinogenic, representing a serious problem in human and veterinary medicine. Knowledge of the flow of PAs in nature should be useful for the control of both plants and PA content in natural and agricultural systems. It may also help in the control and evaluation of medicinal plants such as comfrey (*Symphytum*, Boraginaceae), which surely should contain a minimum of PAs when used as herbal teas or fortifying salads, to avoid permanent liver damage to the unsuspecting consumers.

Conclusions, Synthesis, and Perspectives

The integrated ecological, phylogenetic, and biochemical investigation of the Solanaceae/Ithomiinae interface, with the collaboration of *Nephila clavipes*, a large spider that is a major potential predator of the butterflies, has shown that:

(1) Although there exists a reasonable and geographically widespread specificity in the usage of 19 genera of Solanaceae by Ithomiinae larvae, especially at the level of host plant secondary chemistry, there is very little evidence for parallel phylogenetic diversification of these interacting groups over evolutionary time; in general, advancing phyletic lines of butterflies use ever more primitive hosts. The New World Ithomiinae seem to have colonized the already generically diver-

sified Solanaceae through sequential preadaptation to, and encounter and toleration of progressively more toxic secondary chemicals in their food plants. This has undoubtedly affected the distribution, population structure, habit, habitat, phenology, and exceedingly diversified and variable chemistry of these plants, but it should not be called "coevolution."

(2) The variable and diversified Solanaceae toxins are not stored by larval Ithomiinae and do not protect newly emerged adults against the spider predator, which rejects field-caught individuals. Adult Ithomiinae depend heavily on dehydropyrrolizidine alkaloid monoesters for defense and reproduction. These are sought out and sequestered from decomposing Boraginaceae and especially from a constant source, stabilized by mutualistic interaction: flowers of Compositae-Eupatorieae, which place in their nectar a single chemical structure (usually as only one of five different stereoisomers) to attract the pollinators that need these alkaloids. This intimate relationship has undoubtedly determined many aspects of morphology, physiology, population structure, distribution, abundance, and the highly convergent chemistry of these plants and their pollinators.

Interesting additional problems that have arisen during this research, presently under active investigation with similar methodology, include:

(1) The special relationship of the Ithomiinae *Tithorea* and *Aeria* to Apocynaceae-Parsonsieae, which sometimes contain PAs that may be stored by the aposematic larvae of these genera (research under way with J. R. Trigo).

(2) The additional aposematic larvae of Ithomiinae, which feed mostly on tropane-containing plants and *Brunfelsia* (though some *Solanum* are also included), and which may be storing effective predator deterrents from the food plant and, in the case of *Methona* (which contains almost no PAs in the adults), possibly passing them on to the adult butterflies.

(3) Reasons for the apparent nonstorage (and perhaps nonstorability) of most Solanaceae toxins by herbivores.

(4) Possible participation of further compounds, volatile or unstable and derived from PAs or similar precursor, in defense of adult Ithomiinae.

(5) Physiological or behavioral mechanisms for the spreading out of PAs on the tegument of the butterflies and their use in synthesis of various pheromones.

(6) The great diversity and variability of toxins in both the Apocynaceae and the Solanaceae used by larval Ithomiinae and the importance of these in relation to evolution, oviposition, larval feeding, survivorship, and reproduction in their usual herbivores and in other potential enemies.

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