

NATURAL HISTORY AND ECOLOGICAL CHEMISTRY OF
THE NEOTROPICAL BUTTERFLY
PAPILIO ANCHISIADES (PAPILIONIDAE)

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ABSTRACT. The life cycle, behavior, and chemical ecology of the neotropical butterfly *Papilio anchisiades idaeus* Fabricius (Papilioninae) were studied, using larvae from a single cluster of eggs obtained in NE Costa Rica. The butterfly places large clusters of eggs on the ventral surface of older (bluish green) leaves of *Citrus*. The larvae are cryptically colored and exhibit communal resting, molting, and nocturnal feeding behavior. Fourth and fifth instars perch on branches and trunk of the host plant. Larvae are parasitized by the braconid wasp *Meteorus* sp., and the ant *Camponotus rectangularis* attacks and kills pupae located on the host plant. Paper wasps do not attack large larvae or pupae, even though their nests are often abundant in *Citrus* trees occupied by *P. anchisiades*. Pupae on substrates away from the host plant may survive ant predation. Larvae readily evert the osmeterium when provoked; a very pungent, disagreeable odor is noticeable (to humans) only in the fifth instar. The principle components of the osmeterial secretions change both qualitatively and quantitatively with the molt to the fifth instar. The major constituents of the secretions of third and fourth instars are sesquiterpenes including "a-bergamotene", a-acoradiene, "a-himachalene", and isomers of farnesene; the main secretion of the fifth instar is dominated by isobutyric acid and 2-methylbutyric acid with sesquiterpenes, aliphatic hydrocarbons, long-chain alcohols, and carboxylic esters constituting minor constituents. The possible adaptive significance of this shift in the chemistry of the osmeterial defensive secretion is discussed.

The neotropical butterfly *Papilio anchisiades idaeus* Fabricius (Papilionidae: Papilioninae) is well known in Mexico, Central and South America (Seitz 1908, Ross 1964a, b). It is a large tailless swallowtail with velvety-black wings bearing white patches dorsally on the forewings, and deep red to lavender blotches dorsally on the hindwings. This butterfly is commonly seen around clumps or groves of *Citrus* trees (Rutaceae), the host plant of the caterpillars (Stoll 1781, Caracciolo 1981, Dewitz 1878, Moss 1919). The life cycle and early stages have been incompletely described (Caracciolo 1891, Dewitz 1878, Ehrlich & Ehrlich 1961, Jones 1881, Moss 1919, Oliveira 1977, Ross 1964a, Stoll 1781). In this paper we describe and illustrate the early stages, and present new information on the behavior of immature stages, on parasitism, predation, and egg placement. In addition, we analyzed osmeterial secretions of third, fourth and fifth instar larvae to compare

the chemistry of these defensive secretions with those of other species of *Papilio*. Since recent investigations demonstrated both qualitative and quantitative changes in the secretions between *Papilio* fourth and fifth instars of other species (Seligman & Doy 1972, Burger et al. 1978, Honda 1980a, b, 1981), we wondered if this was also the case for *P. anchisiades*.

MATERIALS AND METHODS

A cluster of 53 eggs was obtained by observing one female *P. anchisiades* ovipositing on a 4-m high lemon (*Citrus*) tree at the edge of a grassy cattle pasture at "Finca La Tirimbina" at 1300 h on 4 March 1982 in NE Costa Rica. This locality is about 10 km E of La Virgen (10°23'N, 84°07'W, 220 m elev.), Heredia Province, and well within the Premontane Tropical Wet Forest region (Holdridge 1967). The eggs were collected by cutting the branch with the leaf bearing them, and placing the cutting in a clean, air-tight, clear plastic bag. The larvae were reared following previously established methods (Young 1972), which included daily observations and periodic changing of leaves and removal of frass and other debris. The duration of each life stage was measured, and feeding and resting behavior noted. The culture of first instars was transported to Milwaukee, Wisconsin, where the rearing continued until adult emergence. During the Wisconsin rearing period, the larvae were fed leaves from a *Citrus* tree in the greenhouse at the Milwaukee Public Museum.

The rearing period extended from 4 March through 23 April 1982, and during this time osmeterial secretions were collected from all available larvae by instar. These secretions were collected in the standard way: each larva was gently pinched with fine forceps, and the everted osmeterium quickly wiped with a small square of filter paper and dropped immediately into a vial of methylene chloride. Several such "milkings" were done within an instar between 1400–1500 h, and samples were thus obtained for the third through fifth instars. Milkings from different larvae were pooled at each sampling date as follows: 24 third instars milked 1–2 April; 22 fourth instars milked 5–9 April; 22 fifth instars milked 13–19 April. The apparency of odor associated with everted osmeteria was also noted.

Chemical Analyses

Gas chromatography-mass spectrometry subdivides complex compounds into molecular weight fractions. It was done using 15 m × 0.3 mm I.D. OV-17 or SE-30 fused quartz capillary columns (J. and W. Scientific Co., Rancho Cordova, CA) in an LKB 2091 spectrometer, with a splitless injector system (J. and W. Scientific Co.). Confirmation

of the low boiling esters was accomplished on an LKB 900 spectrometer using a 2 m × 2.5 mm I.D. 10% SP-1000 packed column. Synthetic octadecyl and eicosanyl esters were chromatographed on a 2 m × 2.5 mm I.D. 1% OV-17 packed column. Both spectrometers were maintained during scanning at 70 ev, at a source temperature of 270°, and at 270 amp ionizing current.

Synthesis of Eicosanyl Esters of Valeric and Isovaleric Acids

These compounds were prepared for use as standards by combining an excess (1 g) of the corresponding acid chlorides with 1 g of eicosanol in 8 ml of pyridine. After 1 h the mixtures were poured into water and extracted into ether, washed with dilute sodium bicarbonate, and the ether evaporated. The oils were then chromatographed directly providing only one peak on a 1% SE-54 packed column.

Eicosyl valerate. Retention times, the result of component molecular weights and their percent representation in samples at a specified temperature, characterized specific complex compounds with mass spectrometry. Retention temperature 270°, MS: m/z (rel. intensity) 382(0.9 m⁺), 353(0.4), 367(0.060), 340(0.4), 325(2), 280(8), 195(1), 181(1), 167(2), 158(1), 153(2), 139(4), 125(7), 111(14), 103(100, valeric acid + H), 102(17), 97(24), 85(22), 83(23), 82(10), 71(13), 70(9), 69(14), 57(21), 56(7), 43(6).

Eicosyl isovalerate. Retention temperature 265°, MS: m/z (rel. intensity) 382(0.8, m⁺), 367(0.4), 340(0.2), 325(1.5), 280(7), 252(3), 195(1), 181(1), 167(2), 158(1), 153(3), 139(4), 125(6), 111(10), 103(100, valeric acid + H), 97(15), 85(19), 83(14), 71(7), 70(5), 69(9), 57(11), 43(4).

Preparation of methyl esters of osmeterial extract. Diazomethane in ether prepared from N-nitro-N-nitrosomethylguanidine (Aldrich Chemical Co., Milwaukee, WI) was added to 20 μl portions of the extract in methylene chloride until a yellow color persisted. Aliquots of this solution were directly injected.

RESULTS

Description of Early Stages

Eggs (Fig. 1) spherical, sculptured, about 2 mm diam, with lateral pair of ridges fusing into bilobed knob; honey-colored; not changing in color before hatching; duration of stage: seven days.

First instar cylindrical with fine down and slightly bulbous head; initially about 6 mm long; cuticle translucent amber, darkening to "dirty" greenish brown following first feeding on plant tissues; lateral body profile tapered; no tubercles and no discernible markings on cuticle; duration of stadium: seven days.

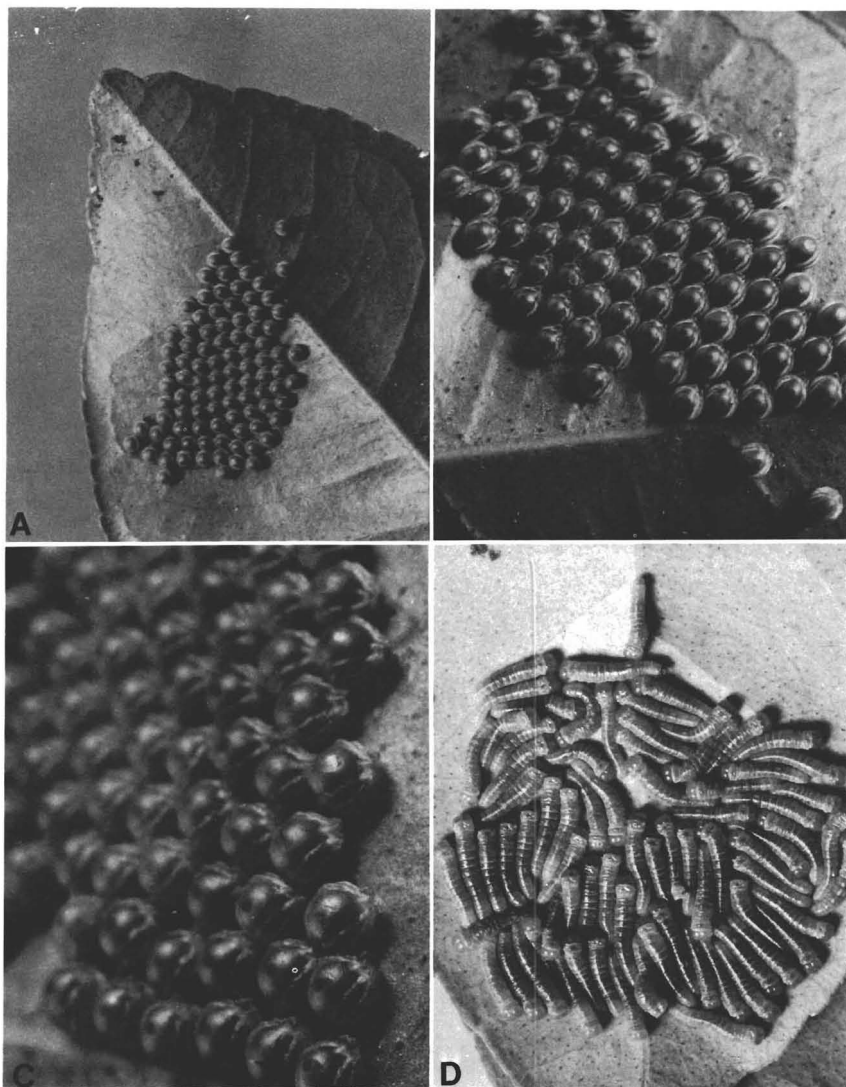


FIG. 1. *Papilio anchisiades*. (A) position of egg cluster on *Citrus* leaf (ventral surface); (B) orientation of individual eggs in cluster; (C) surface sculpturing of individual eggs; (D) second instars.

Second instar (Fig. 1) similar to first but with larger head relative to trunk; more delineation of trunk segments; first three segments and last four dull orange, middle segments greenish; head glossy orange; attained body length of 10–13 mm in seven to nine days.

Third instar (Fig. 2) strikingly different from previous instars; swelled

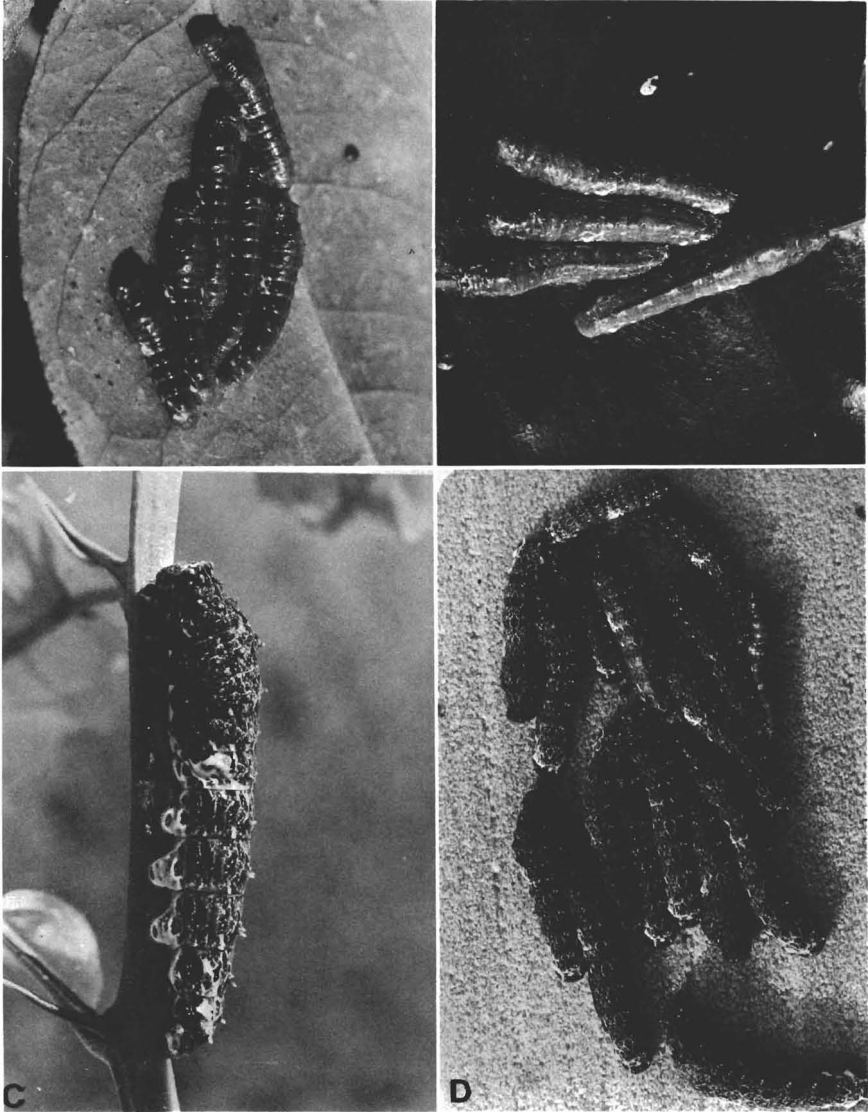


FIG. 2. *Papilio anchisiades*. (A) third instars immediately following molt; (B) fourth instars several days after molting; (C) fifth instar, lateral aspects; (D) aggregative behavior of fifth instars.

thoracic region; coloration a variegated brown and white cuticle with "oily" appearance; head brownish orange and glossy; head hidden and overshadowed by anterior trunk region; attained a length of 20–23 mm in eight days.

Fourth instar (Fig. 2) similar to third but with decrease in thoracic swelling; more pronounced mottling of rich brown and white blotches on trunk; both third and fourth instars with tubercles further described in Oliveira (1977); attained length of 32–36 mm in ten days.

Fifth instar (Fig. 2) with trunk cuticle "lacework" pattern of chocolate-brown background with network of lines and blotches of white; cuticle "warty" due to small tubercles and foldings (further described in Oliveira 1977); prolegs white with brown speckling; head brownish and smaller than anterior trunk; dorsally trunk cuticle bears a series of diamondlike velvety-brown blotches; attained length of 59–62 mm in 20 days. Total larval period: 45 days.

All instars with deep-orange osmeteria, short and stubby in first three instars, long and filamentous in last two. Osmeterium of fifth instar 9–10 mm long. The prepupa (Fig. 3) contracted in body length and darkened in coloration before the final ecdysis.

Pupa (Fig. 3) 37–39 mm long and 21–23 mm at greatest width; resembles broken twig; color pattern a variable mosaic of brown, gray, green, and white, but usually with large, "lichenlike" blotch on posterior two-thirds of wing pads extending posteriorly into dorsal area of abdomen; spiracle openings marked in black; duration of stage: 18–22 days. Overall egg-to-adult time: 70–74 days.

Adults eclosed rapidly, and wings were fully expanded (Fig. 3) within 25 min, and usually between 0800–0900 h. Sex ratio of 25 pupae: 10 females and 15 males.

Behavior of Stages

Eggs placed in tight rows on the ventral surface of mature *Citrus* leaf (Fig. 1); even though "young" or "fresh" (greenish-yellow) leaves available, eggs were placed on older leaf, near the distal end of the branch; butterfly clung to edge of leaf and curled abdomen under while ovipositing for 1 h. When frightened away, it did not return to resume egg laying on the same or several subsequent days. Other observations in Costa Rica indicate that this species oviposits on both mature (greenish-blue) leaves and yellowish-green fresh leaves of *Citrus* in both wet and dry forest regions. All eggs in the cluster touched one another and hatched synchronously taking about 4 h for all larvae to vacate egg shells. Egg shells were immediately devoured by larvae, and larvae remained as one group in the first two instars (Fig. 1). First instars occupied the same leaf as the eggs, and started feeding at the edge of the leaf (Fig. 4). Feeding throughout all instars was synchronous and nocturnal. Breakup into two or more subgroups began in the third instar and continued through the fifth (Fig. 2). Fourth instars

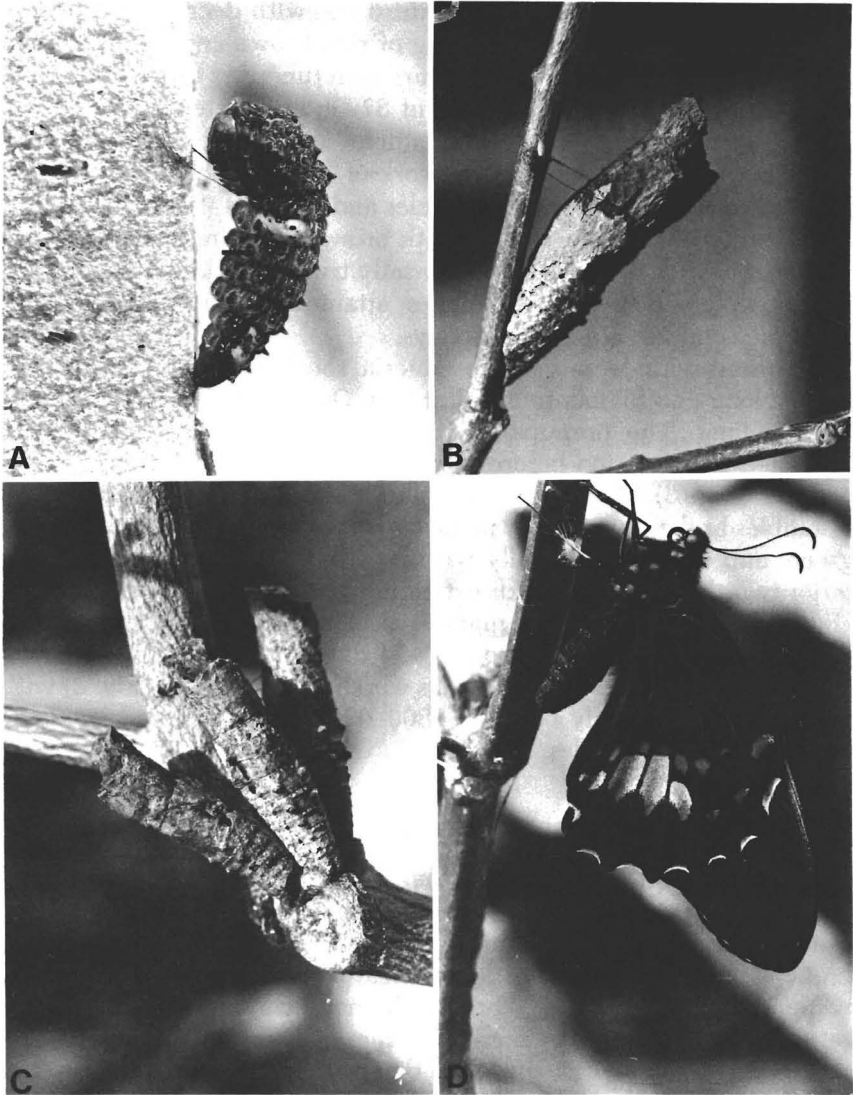


FIG. 3. *Papilio anchisiades*. (A) prepupa; (B) pupa; (C) aggregation of pupae; (D) freshly eclosed adult.

stayed in small groups on the branch rather than on leaves like previous instars.

In field observations fourth and fifth instars aggregated on the trunk of the host, and pupation occurred on the trunk, on nearby buildings or other substrates near the host.

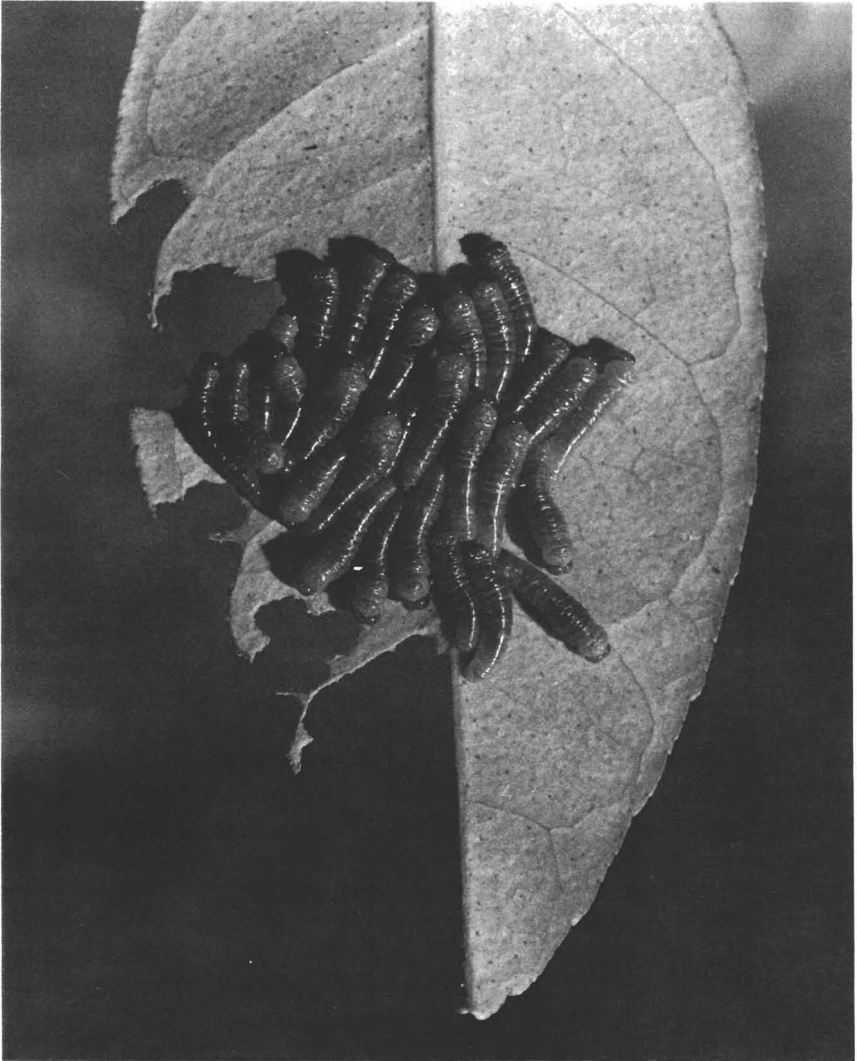


FIG. 4. *Papilio anchisiades*. Feeding pattern of young larvae on a leaf of *Citrus*.

Larvae of all five instars evert the osmeterium when prodded with forceps, but response is much quicker in the first three instars than in the last two. Eversion of the osmeteria in the first three instars was unaccompanied by odor at close range. A strong, disagreeable odor, best described as "sweaty socks," was apparent when the osmeteria of the last two instars were everted. Growth rates of larvae within a group were mostly similar, and molting was synchronous. Molting required

one to two days. However one to three individuals within the group were smaller, and differed by a full instar. Pupation appeared to occur in two "pulses," with larger larvae (presumably females) being the last to pupate.

In field observations, more than one cohort of larval *P. anchisiades* occurred in a single *Citrus* tree, often in trees with nests of paper wasps (*Polistes* and various polybiines). In a *Citrus* tree in a pasture at Palo Verde, Guanacaste Province, Costa Rica, studied between 9–11 November 1969, separate groups of 40–70 larvae were found, and each group contained 3–8 smaller larvae. Most larvae were fifth instar, and the smaller ones second and third instars. A total of nine pupae were scattered on branches, and all were being attacked by the ant *Camponotus rectangularis* Emery. An additional 25 pupae, without ants, were found on a nearby weather-beaten tool shed 5 m from the tree and separated from it by tall grasses. A total of 53 paper wasp nests were in the tree and another 74 nests on the shed. Individual wasps often perched near the pupae on the sides of the shed, but did not attack them. Pupae on the tree were not observed to be attacked by wasps. Successful eclosion of four adults was observed. No pupae on the shed were attacked by *C. rectangularis* during the two days of observation.

Several third instars collected in October 1969 at Naranja, Zaragoza, El Salvador, were parasitized by the braconid *Meteorus* sp. A second unnamed species of the same genus has been recorded from *P. anchisiades* in Venezuela (Paul Marsh, pers. comm.). Together, both records are new, and represent the first reports of parasitism by *Meteorus* on the Papilionidae (Paul Marsh, pers. comm.).

A major feature of larval behavior in *P. anchisiades* in both field and laboratory is the close physical contact among individuals, although laboratory individuals sometimes rested and fed solitarily.

Mass Spectral Analysis of Osmeterial Extracts

Extracts of the fifth instar (Fig. 5) showed a poorly resolved series of short-chain acids and esters, followed by traces of sesquiterpenes eluting from 142–170°, and finally (Fig. 6) a series of hexadecyl, octadecyl, and eicosanyl esters of butyric and valeric acids. The early eluting compounds were ethyl isobutyrate, methyl 2-methylbutyrate, ethyl 2-methylbutyrate, isobutyric acid, isovaleric acid and 2-methylbutyric acid, eluting in that order. Reexamination on a 10% SP-1000 packed column confirmed these assignments and revealed a trace of ethyl 3-hydroxybutyrate eluting just after ethyl 2-methylbutyrate. Also observed were traces of acetic acid and ethyl acetate. All compounds were identified by their mass spectra (Heller & Milne 1976). As found

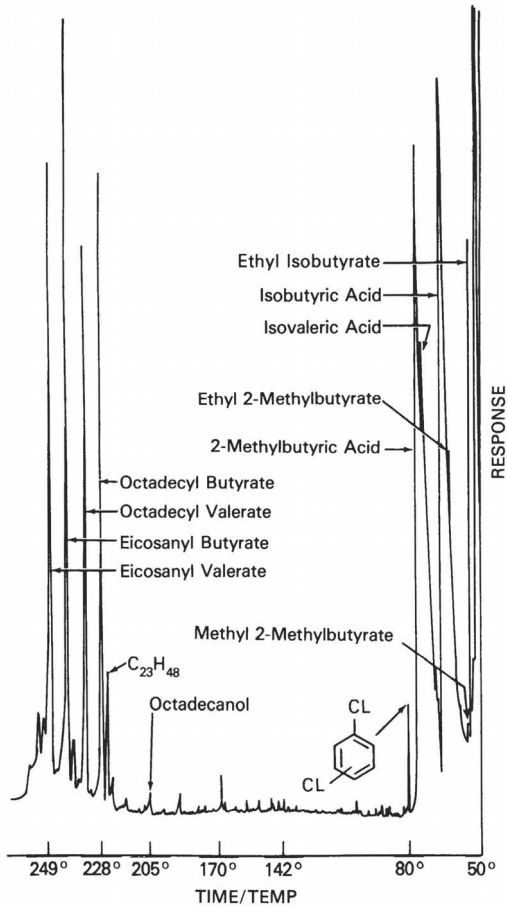


FIG. 5. Chromatographic analysis of fifth-instar osmeterial secretion in *Papilio anchisiades* (SE-30 capillary; 15 m \times 0.30 mm I.D.; 10°/min.).

by Honda (1981) for other *Papilio* species, 2-methylbutyric and isobutyric acids were major components accompanied by smaller quantities of isovaleric acid. Conversion to the methyl esters allowed quantitation of these acids in the ratio 1:0.75:0.021, respectively, as determined on an SE-54 capillary column.

Expansion of the chromatographic region between 80° and 228° (Fig. 6) allowed two terpenes, α -bergamotene and E-b-farnesene (peaks 1 and 2), to be tentatively identified by comparison of their spectra with published compilations (Heller & Milne 1976). Peak 4 was tentatively identified as α -himachalene by similar comparison, while peak 9, assumed to be a sesquiterpene from its mass spectrum (Table 1), was unique to the fifth instar. The mass spectrum of peak 8 was similar

TABLE 1. Mass spectra of sesquiterpenes identified in the osmeterial secretions of *Papilio anchisiades*. Retention temperatures (first numbers) and relative amounts () refer to peaks in Fig. 7.

1.	a-bergamotene: m/z 204(M ⁺ , 2), 161(3), 131(5), 119(53), 93(87), 69(53), 55(40), 43(67), 41(100)
2.	A farnesene isomer: m/z 204(M ⁺ , 5), 161(6), 133(14), 119(7), 93(41), 69(100), 55(20), 43(23), 41(99)
3.	B-accoradiene: m/z 204(M ⁺ , 6), 182(2), 161(6), 133(16), 120(13), 107(8), 93(50), 91(25), 81(25), 79(25), 69(100), 67(25), 57(20), 55(20), 41(90)
4.	"a-himachalene": m/z 204(M ⁺ , 25), 189(7), 161(10), 147(9), 133(17), 121(25), 119(22), 93(100), 79(29), 69(21), 55(31), 43(63), 41(88)
5.	A farnesene isomer (same as 2 above, but mixed with a-himachalene)
6.	A farnesene isomer m/z 204(M ⁺ , 10), 161(18), 133(20), 120(18), 109(16), 93(49), 69(100), 41(79)
7.	"B-selinene": m/z 204(M ⁺ , 15), 189(3), 161(3), 121(22), 119(20), 109(21), 93(100), 80(30), 69(25), 41(42)
8.	Unknown: m/z 220(M ⁺ , 20), 205(5), 177(4), 163(2), 151(6), 149(4), 137(100), 135(53), 110(76), 109(51), 95(46), 82(29), 69(35), 55(40), 43(20), 41(85)
9.	Unknown (only in 5th instar): m/z 204(M ⁺ , 25), 189(13), 169(31), 133(25), 121(50), 119(44), 105(52), 93(69), 91(50), 79(38), 77(31), 69(25), 55(44), 53(38), 43(81), 41(100)

but not identical to the spectrum of caryophyllene oxide reported by Honda (1981). No evidence was found for monoterpenes or the elemene, selinene or germacrene reported by Honda (1981). Also identified in this scan were C₁₄-C₂₂ saturated and unsaturated hydrocarbons as well as naphthalene, dichlorobenzene, and phthalates, all of which are regarded as artifacts.

The acid components of the hexadecyl, octadecyl, and eicosyl esters were expected to be isobutyric and either 2-methylbutyric or isovaleric acids in view of the large quantities of the corresponding free acids that were present (Fig. 5). In fact, comparison of the mass spectra of the last peak with spectra of synthesized samples of eicosyl n-valerate and eicosyl isovalerate reveals that the natural product is the former ester. Thus, the molecular ion of eicosyl isovalerate was slightly less intense relative to high mass peaks, and showed enhanced loss of methyl compared to eicosyl valerate. The other three peaks are also esters of the n-butyric and n-valeric acids by the same reasoning. Fig. 6 also shows the presence of the corresponding alcohols, octadecanol and eicosanol, easily identified by reference to library spectra.

Gas chromatograms of the third and fourth instars were nearly identical, but presented an entirely different picture (Fig. 7). Both short and long chain acids and esters were missing, and only sesquiterpenes were present. As in extracts from the fifth instar, only a-bergamotene, -acoradiene and three farnesene isomers were identified with confidence by comparison with reference spectra. The major component was a compound whose spectrum resembled, but was not identical

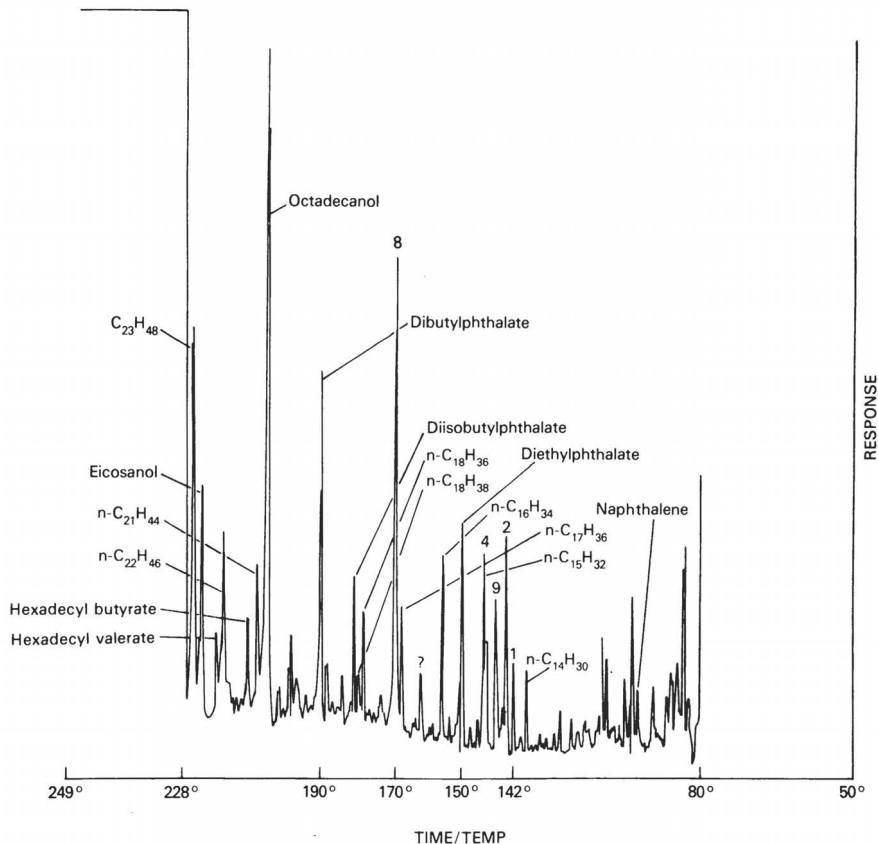


FIG. 6. Mass spectral analysis of fifth-instar osmeterial secretion in *Papilio anchisiades*, highlighting the terpene region of the spectra (SE-30 capillary; 15 m \times 0.3 mm I.D.; 10°/min.).

with, caryophyllene oxide as reported by Honda (1981). Peaks 4 and 7 are very similar, and both resemble library spectra (Heller & Milne 1976) of α -himachalene or β -selinene, but neither corresponds to the β -selinene spectrum reported by Honda (1981). Mass spectra of sesquiterpenes are shown in Table 1.

DISCUSSION

Papilio anchisiades, along with *P. cresphontes* and *P. thoas*, and a few others, exploits various Rutaceae as larval food plants (Brower 1958). It differs from other rutaceous-feeding *Papilio* species by its unique larval aggregative habits, a result of cluster egg placement on the larval host plants. The rutaceous-feeding habit is shared world-

wide by several species of *Papilio* (Munroe 1960), an association perhaps mediated by the rich profiles of specific alkaloids so characteristic of this plant group (Hegnauer 1963). We propose that the widespread abundance of *P. anchisiades* from southern Brazil to Mexico and even southern Texas (Ehrlich & Ehrlich 1961), is in part due to the broad distribution of *Citrus* as an exotic rutaceous host plant coupled with the distribution of other Rutaceae with forest habitats.

Larval host-plant selection in butterflies usually involves a highly structured sequence of visual and olfactory responses (Crane 1955, Swihart & Swihart 1970, Ilse 1937). Vaidya (1969) studied the color preferences of ovipositing *P. demoleus* L. on *Citrus* in laboratory studies, and concluded that both color and scent are required for proper egg placement and noted that butterflies preferred blue-green hues of leaves over yellow-green hues. Within the Rutaceae, differences among genera and species for certain substances in the leaves determine patterns of host specificity in egg-placement behavior among different papilionids (Ichinose et al. 1981). Age-specific differences in odor and color in *Citrus* and other Rutaceae may determine patterns of egg placement in *P. anchisiades* in nature, and the field data for this species in Costa Rica supports a partial preference concept for the older, more bluish-green leaves of host plants. Depending on annual phenological patterns of flushing, such egg-placement substrates may vary in abundance at a locality and influence the abundance of the butterfly population, or result in oviposition on more yellowish green leaves. In a highly seasonal lowland area such as Guanacaste, such effects might be even more pronounced than in less seasonal Atlantic zone habitats in Costa Rica and elsewhere in southern Central America. Ross (1964b) noted that *P. thoas autocles* Rothschild & Jordan frequently oviposits on fresh leaves of the larval host plants, including *Piper* spp. (Piperaceae) and *Citrus* in Mexico. *Papilio aristodemus ponceanus* oviposits on young shoots of *Zanthoxylum fagara* (Rutaceae), "Wild Lime," and first instars readily devour the young leaves without difficulty (Rutkowski 1971). Tough, thick leaves of Rutaceae used by *Papilio* species may retard normal growth and development of caterpillars (Watanabe 1982), thereby selecting for egg placement on young, tender leaves.

Several studies reveal that the attraction of parasitoids to their phytophagous hosts is often mediated by the aromatic substances emitted by the host plant (Herrebut 1969, Read et al. 1970). We suspect that *Papilio* species associated with the highly aromatic Rutaceae are subject to such parasitism, and several larvae within an aggregate of *P. anchisiades* can be killed by the braconid *Meteoris* sp. Such interactions may extend to predatory arthropods such as the ant *C. rectangularis* associated with *Citrus* in lowland Guanacaste, even though

predation by paper wasps under the same conditions may be minimal or nonexistent.

The size of larval groups of *P. anchisiades* in *Citrus* varies, and the group observed in the present study might have been small since the ovipositing butterfly was frightened away. The larvae have been noted to defoliate a tree (Caracciolo 1891), and very large groups of larvae have been found on individual trees (Moss 1919). Pupae are often found on various substrates away from the host tree (Moss 1919), and the present study suggests that mortality from at least one ant species might be less for pupae off the host tree than for those remaining on it.

Although the disagreeable odor from the osmeteria of the older larvae is well known (Stoll 1781, Carracciolo 1891, Moss 1919), the functional role of the secretion remains unknown, although the components are defensive against ants (Honda 1983). The precise egg-placement behavior of *P. anchisiades* suggests that the species is a specialist on Rutaceae, a condition that further suggests coevolved associations with parasitoids and predators that cue into the aromatic properties of *Citrus* and other genera within the family. The cryptic appearance and behavior of larvae of all instars, and the cryptic appearance of the pupa, suggest that this species is palatable to visually foraging predators such as lizards and birds (Brower & Brower 1964). When this first-line defense is penetrated by an attacker, the odor defense associated with the osmeterium might be used to thwart attack (Eisner & Meinwald 1965, Honda 1983). All rutaceous-feeding *Papilio* species appear to have cryptic coloration and habits (Munroe 1960).

We suggest that aggregative behavior in the larval stages of *P. anchisiades* enhances visual crypsis to some predators such as birds and lizards. The combined aggregate of several fifth instars on the bark of the host tree creates the image of a mottled blotch of false lichens and bark on the trunk. Similarly, the tightly packed clusters of younger larvae on the ventral surfaces of *Citrus* leaves resemble dead or dying plant tissue destroyed by a pathogenic microorganism. A large aggregation of fifth instars positioned at the junction between the trunk and main branches of a *Citrus* tree in Trinidad resembled a "clot of wet feces" to both L. P. Brower and P. M. Shepard (L. P. Brower, pers. comm.). Aggregative behavior of the larvae, however, may not deter predation by birds. On 28 July 1986 one of us (A.M.Y.) observed an unidentified jay-size bird pluck off a *Citrus* leaf bearing 50 young third-instar *P. anchisiades* (at 0530 h) at "Finca La Lola" in Costa Rica. The bird then devoured all the larvae in a few seconds.

The osmeterial secretions from third and fourth instars of *P. anchisiades* are similar to those of other *Papilio* species in being domi-

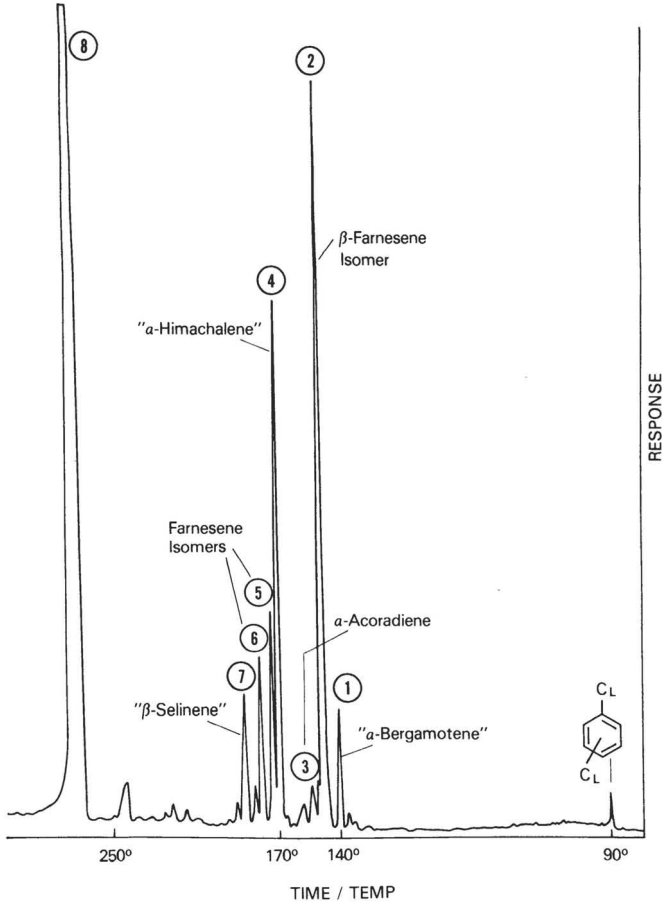


FIG. 7. Mass spectral analysis of fourth-instar osmeterial secretion in *Papilio anchisiades*, highlighting sesquiterpene region of the spectra.

nated by terpenes. Burger et al. (1978) and Honda (1980a, b, 1981) reported that the secretions of earlier instars of several *Papilio* species are made up of mono- and sesquiterpenes. While we did not detect monoterpenes in the secretions of *P. anchisiades*, at least seven sesquiterpenes fortify the osmeterial exudate (Fig. 7). Honda (1981) previously identified sesquiterpenes in the osmeterial secretions of five species of *Papilio*, but several of those produced by *P. anchisiades* appear to be different from those produced by the Japanese species.

Earlier investigations established that the osmeterial secretions of a variety of *Papilio*, *Baronia*, and *Eurytides* species were dominated by isobutyric and 2-methylbutyric acids (Eisner & Meinwald 1965, Crossley & Waterhouse 1969, Eisner et al. 1970, Burger et al. 1978, Lopez & Quesnel 1970). However, it was subsequently demonstrated that this

acidic duet is characteristic of the osmeterial secretions of the fifth instar. In contrast, the secretions of earlier instars of several *Papilio* species lack the short-chain acids produced by fifth instars, and a variety of terpenes are produced by younger larvae (Burger et al. 1978, Honda 1980a, b, 1981).

The osmeterial secretion of the fifth instar of *P. anchisiades* contains isobutyric and 2-methylbutyric acids, but, in addition, isovaleric acid, a compound detected as a minor osmeterial constituent in two other *Papilio* species (Honda 1981). Although isobutyric and 2-methylbutyric acids have been encountered as the acidic moieties of short-chain esters in the osmeterial secretions of *P. anchisiades* and other species (Burger 1978, Honda 1981), long-chain esters containing butyric and valeric acids (Fig. 5) have not been reported previously from papilionid osmeterial secretions. Thus, fifth-instar larvae of *P. anchisiades* are distinctive in producing osmeterial secretions containing esters such as hexadecyl valerate (Fig. 6) and octadecyl butyrate (Fig. 5). It is not clear why the dominant free acids in the secretion—*isobutyric* and *2-methylbutyric*—have not been utilized as the acid moieties of these long-chain esters.

Sesquiterpenes in the osmeterial secretion of the fifth instar is unusual, since this class of compounds has been identified in the secretions of earlier instar *Papilio* (Burger et al. 1978, Honda 1980a, b, 1981). However, one sesquiterpene has been identified in the secretion of *P. protenor* (Honda 1980), and three in that of *P. memnon* (Honda 1981). *Papilio anchisiades* is unusual in having almost as many sesquiterpenes (five) in the secretion of the fifth instar as in that of earlier instars (seven).

The secretions of the last instar of *P. anchisiades* differs from those of any *Papilio* species similarly analyzed in containing aliphatic hydrocarbons and long-chain alcohols (Fig. 7). Nine aliphatic hydrocarbons are present, and these are accompanied by C₁₈ and C₂₁ alcohols. With the presence of esters such as hexadecyl valerate, the distinctiveness of this osmeterial secretion is further evident.

Previous investigators demonstrated that the secretions of younger larvae were qualitatively richer than those of fifth instars (Burger et al. 1978, Honda 1980a, b, 1981); the opposite is true for the secretions of earlier and fifth instars of *P. anchisiades*. Whereas the third and fourth instar secretions contain seven sesquiterpenes (Fig. 7), the fifth instar secretion contains more than 30 compounds (Figs. 5, 6) including acids, hydrocarbons, esters, alcohols, and sesquiterpenes. Qualitatively, the fifth-instar secretion of *P. anchisiades* exceeds that known for any instars of any *Papilio* species.

If it is assumed that the chemical (osmeterial) defenses of earlier-instar *Papilio* evolved as deterrents against predators different than

encountered by the fifth instar, then the differences in the chemistry of these instars is explicable. There is little specific evidence on what organisms constitute enemies for larvae of *P. anchisiades* of any instar. Whatever the selection pressures were for producing the fifth-instar exudate, they have resulted in the most diverse osmeterial secretion encountered in the genus *Papilio*.

The greater heterogeneity and complexity of the osmeterial secretion of fifth instars in *P. anchisiades* suggests that the system becomes most functional in this instar. The pungent odor emitted in the fifth instar results from isobutyric and 2-methylbutyric acids, which are lacking in the earlier instars. The occurrence of some components (sesquiterpenes) of earlier-instar osmeterial secretions in the fifth instar indicates that the biochemical pathways underlying the synthesis of these substances are not completely turned off in the fifth instar. Both qualitative and quantitative changes figure in the regulation of secretion in the fifth instar of *P. anchisiades*.

Our results are largely due to the application of capillary-column gas chromatography, which enabled detecting of minute amounts of specific components in the fifth instar, substances that might have been overlooked otherwise.

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