ROLE OF THE OSMETERIAL GLAND IN SWALLOWTAIL LARVAE (PAPILIONIDAE) IN DEFENSE AGAINST AN AVIAN PREDATOR

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ABSTRACT. The importance of the osmeterial gland, a universal characteristic of larvae in the Papilionidae, in defense against vertebrate predators has rarely been examined. In this study, third and fifth instar larvae of *Papilio polyxenes* with and without a functional osmeterium were presented to *Coturnix coturnix* (Japanese quail), a representative avian predator. Virtually all larvae were rejected by *C. coturnix* irrespective of osmeterial function. Larvae of *P. cresphontes* with functional osmeteria were also universally rejected by *C. coturnix*; in contrast, larvae of *P. glaucus* were readily consumed. In view of the fact that osmeterial secretions in these three species are similar in composition, they are unlikely to play a major role in determining palatability of these species to this avian predator.

Additional key words: *Papilio polyxenes, Papilio glaucus, Papilio cresphontes, Coturnix coturnix,* palatability.

The osmeterium, a Y-shaped eversible gland located mid-dorsally behind the head, is a universal characteristic of swallowtail caterpillars (Papilionidae). Its function has long been assumed to be defensive (Merian 1705, as cited in Crossley & Waterhouse 1969). There is indeed evidence of the efficacy of the osmeterial gland and its secretions against invertebrate predators. Eisner and Meinwald (1965) demonstrated that *Papilio machaon* L. larvae use osmeterial secretions to deter ant predation; Damman (1986) determined that the presence of a functional osmeterial gland of *Eurytides marcellus* (Cramer) reduces predation by ants and small spiders, and Chow and Tsai (1989) showed that *Papilio memnon* L. larvae are rejected by praying mantids. Honda (1983) confirmed that many components of osmeterial secretions from a variety of papilionid species are toxic and/or repellent to ants.

The role of the osmeterial gland in defense against vertebrate predators, however, is less clear. Jaervi et al. (1981) demonstrated that the osmeterial gland was ineffective at protecting third instar *Papilio machaon* larvae against predation by the great tit *Parus major* L. (Paridae) (that is, birds refused to eat intact larvae as well as decapitated larvae with osmeteria removed), and Honda (1983) reported that osmeterial

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eversion does not deter Japanese tree sparrow predation on late instar *Papilio protenor* Cramer larvae. However, in many species of swallowtails the chemical composition of osmeterial secretions varies ontogenetically. In six species of *Papilio*, osmeterial secretions of early instar caterpillars consist primarily of mono- and sesqui-terpenes; in later instars (fourth and fifth), osmeterial secretions consist primarily of aliphatic acids and their esters (Honda 1981). Accompanying the change in osmeterial gland chemistry is a major change in morphology. Early instar larvae are black with a white saddle, a pattern generally assumed to be cryptic and reminiscent of bird droppings, and later larvae are solid green with eyespots ("snake mimic"), mottled brown and white, or striped green and black (Tyler 1975). Although most ultimate instar patterns are thought to be cryptic, Jaervi et al. (1981) argue that the striped pattern is aposematic at short distances; indeed, the presence of yellow or orange spots interrupting the black bands on most larvae in the *P. machaon* complex may enhance any aposematic effect.

The possibility exists, therefore, that the defensive importance of the osmeterium changes over the course of development. Accordingly, in this study we tested the palatability of early and late instar larvae, with and without a functional osmeterium, to a representative avian predator. In addition, the differential conspicuousness of different species of swallowtail larvae suggests differences in efficacy of chemical defense against visually orienting predators; cryptic or homotypic species may be relatively vulnerable in comparison with aposematic species. Therefore, in this study we also compared the palatability to an avian predator of larvae of three papilionids differing in ultimate instar coloration.

MATERIALS AND METHODS

All of the swallowtail larvae used in this study were laboratory-reared and originated from adults caught in east central Illinois. *Papilio polyxenes* Fabricius feeds exclusively on herbaceous representatives of Rutaceae and Apiaceae (=Umbelliferae) (Tyler 1975). Early instar larvae are black with a white saddle; fourth and fifth instar larvae are green with black stripes interrupted with yellow spots. Caterpillars in the laboratory were reared on greenhouse-grown foliage of *Petroselinum crispum* (Mill.) Nym. ex A. Hill (parsley) (Apiaceae). *Papilio cresphontes* Cramer feeds exclusively on rutaceous shrubs. Whereas first instar larvae are black with a white saddle, subsequent instars are characterized by a mottled brown and white color pattern (Opler & Krizek 1984). In the laboratory, *P. cresphontes* larvae were reared on foliage of greenhouse-grown *Citrus limon* (L.) Burm. (Rutaceae). *Papilio glaucus* L. has the broadest food plant range of any swallowtail species and is reported to feed on foliage of trees in over a dozen plant

families (Tyler 1975). Early instar *P. glaucus* larvae are brownish-black with a white saddle, but late instar larvae are green and snake-like in appearance with conspicuous eyespots on the thorax. *P. glaucus* larvae were reared in the laboratory on foliage of *Liriodendron tulipifera* L. (Magnoliaceae) or *Prunus serotina* Ehrh. (Rosaceae) collected from wild trees.

The avian predator used in this study was *Coturnix coturnix* L., the Japanese quail (Phasianidae). *C. coturnix* feeds freely on insects and has been used previously in investigations of insect palatability as a representative ground-feeding insectivorous bird (Wiklund & Jaervi 1982, Wiklund & Sillen-Tullberg 1985). The individuals used in this study were reared from egg hatch on commercial chicken feed and had no prior exposure to insect prey.

Palatability trials were similar in design to those described by Wiklund and Sillen-Tullberg (1985). Trials were run in one of two large (2.13 m × 2.13 m) cages containing 14 quail. In the center of the cage, two watchglasses were placed on the floor 76.2 cm apart. Five mealworms were placed in each dish and were consumed by the quail. This process was repeated with five additional mealworms in each dish after the first five were consumed. After the second ten mealworms were eaten, five swallowtail caterpillars were placed in one watchglass and five mealworms in the other watchglass. Observations were made for a ten-minute interval. This process was repeated four times so that a total of 20 swallowtail larvae were exposed to the quail (with the exception of *P. cresphontes*, for which only a limited number of larvae of the appropriate age were available).

Two separate experiments were conducted. In the first series of trials, the palatability of larvae of three different species was compared. In all species, the osmeterium was fully functional. For *P. polyxenes* and *P. glaucus*, trials were run with both third instar larvae (bird-dropping morphs in both species) and late instar larvae (striped or "snake" morph, respectively). In the second series of trials, two developmental stages—third instar and fifth instar larvae—of only one species, *Papilio polyxenes*, were examined. Within each developmental stage, the palatability of larvae with and without a functional osmeterium was tested. Osmeterial glands were rendered nonfunctional by the method of Damman (1986). A dab of Liquid Paper correction fluid (Liquid Paper Corp., Boston, Maryland) was placed directly on the fold from which the osmeterium everts. Larvae treated in this fashion were unable to evert their osmeterium even when prodded by the investigators. A dab of Liquid Paper was also placed on mealworms in the control watchglasses at a comparable location behind the head.

Differences in the numbers of individuals eaten or not eaten over

TABLE 1. Survival of *Papilio* caterpillars with functional osmeteria in the presence of *Coturnix coturnix*.

Species[a]	Instar	No. eaten	No. not eaten
P. cresphontes	4/5	0	8
P. glaucus	3	20	0
P. glaucus[b]	4/5	8	0
P. polyxenes	3	0	20
P. polyxenes[c]	5	0	20

[a] Survival is not independent of species ($\chi^2 = 36$, $P < 0.05$)
[b] Survival of *P. glaucus* is independent of instar (Fisher exact $P = 0.09$)
[c] Survival of *P. polyxenes* is independent of instar (Fisher exact $P = 1.00$)

the trial period were evaluated with a test of independence; in comparing late instar individuals of the three different species, a chi-square test was used and for all 2 × 2 tables a Fisher's exact test was used.

RESULTS AND DISCUSSION

The three species of *Papilio* examined in this study differ in their susceptibility of predation by Japanese quail (Table 1). Whereas 100% of *P. polyxenes* larvae (40/40) and *P. cresphontes* larvae (8/8) survived encounters with quail, 100% (28/28) of the *P. glaucus* larvae were consumed. The unpalatability of *P. polyxenes* was unaffected by developmental stage; both third and fifth instar larvae were consistently rejected. By the same token, the palatability of *P. glaucus* was unaffected by developmental stage; all caterpillars were consumed irrespective of instar. The unpalatability of *P. polyxenes* was also independent of the presence of a functional osmeterium; 39/40 caterpillars with occluded osmeteria survived encounters with quail (Table 2).

In general, while *C. coturnix* showed no reluctance to seize and consume ultimate instar *P. glaucus* caterpillars, they were hesitant to touch the green and black striped ultimate instar *P. polyxenes* larvae. They showed less reluctance to sample the third instar *P. polyxenes* "bird dropping" morphs and in fact picked up several individuals out of the watchglass and entirely consumed one. This inclination to sample third instar larvae was reported also by Wiklund and Jaervi (1982), who noted that *C. coturnix* seized 13 of 18 *P. machaon* larvae in a similar feeding trial; of these 13, 12 were subsequently dropped and one eaten.

The basis for rejecting third instar *P. polyxenes* larvae does not appear to be visual; indeed, from the human perspective, third instar *P. glaucus* larvae, which are seized and consumed by Japanese quail, are virtually indistinguishable from third instar *P. polyxenes* larvae. Moreover, the unpalatability of third instar *P. polyxenes* larvae cannot be attributed solely to the osmeterial gland, since larvae that could not evert their

TABLE 2. Survival of *Papilio polyxenes* larvae with and without functional osmeteria in the presence of *Coturnix coturnix* (osmeteria rendered nonfunctional by occlusion with Liquid Paper).

Instar	Osmeterium	No. eaten	No. not eaten
3	functional	0	20
3[a]	nonfunctional	1	19
5	functional	0	20
5[b]	nonfunctional	0	20

[a] Survival of third instar larvae is independent of osmeterial function (Fisher exact $P = 1.0$)
[b] Survival of fifth instar larvae is independent of osmeterial function (Fisher exact $P = 1.0$)

glands when seized were equally as unacceptable to *C. coturnix* as were larvae with functional osmeteria. It is also unlikely that osmeterial secretions are primarily responsible for differences in acceptability of late instar *Papilio* species as well, since the known chemical composition of osmeterial secretions is extremely similar in the three species (Eisner et al. 1970).

The fact that, of the three species of *Papilio* examined in this study, two oligophagous species were unpalatable and one polyphagous species was palatable suggests that larval diet may be involved in relative acceptability of swallowtail larvae to *C. coturnix*. Oligophagous Lepidoptera, including papilionids, have long been known to sequester toxins from their food plants; sequestration of aristolochic acids from *Aristolochia* food plants, for example, is documented throughout the Troidini (Papilionidae) (Rothschild 1972). Although sequestration of food plant allelochemicals has not been demonstrated for *Papilio* species, the possibility exists that oligophagous species in the genus sequester toxins from food plants for defensive purposes. Consistent with this suggestion is the report by Jaervi et al. (1981) that the "obnoxious properties" of *P. machaon* larvae (which are oligophagous, as are *P. polyxenes,* on Apiaceae and Rutaceae) are detectable in the cuticle by birds; the presence of diet-derived defensive compounds in cuticle has been reported in other species (Bernays & Woodhead 1982). The rutaceous food plants of *P. crespohontes* are known to contain a variety of toxic allelochemicals, including furanocoumarins and furanoquinoline, beta-carboline, and benzylisoquinoline alkaloids; the apiaceous food plants of *P. polyxenes* contain sesquiterpene lactones, furanocoumarins, and polyacetylenes (Hegnauer 1973), which may be sequestered (Berenbaum 1990). On the other hand, despite the presence of toxic allelochemicals such as cyanogenic glycosides, benzylisoquinoline alkaloids, sesquiterpene lactones, and saponins in food plants of *P. glaucus* (Scriber et al. 1987), including those species used as food plants in this study, these polyphagous caterpillars are not demonstrably distasteful

to predators and give no other indications of any ability to sequester food plant toxins.

Osmeterial eversion in the presence of vertebrate prey may not be without survival value in all cases. The possibility exists that other vertebrate predators (e.g., those with a long ecological association with these North American species) may be adversely affected by osmeterial secretions. Another possibility is that osmeterial secretions, while not themselves toxic, may serve a function analogous to that of pyrazines in many species of unpalatable butterflies (Rothschild et al. 1984)—that is, they may serve as olfactory aposematic signals. The high volatility and pungent aroma of osmeterial secretions, particularly the aliphatic acid esters typical of late instar *Papilio* larvae (Eisner et al. 1970, Honda 1981), may provide a warning signal of distastefulness to potential predators. The value of such an olfactory signal is that it may reduce the risk of handling and subsequent rejection based on contact chemoreception of cuticular defenses and increase the probability that an individual caterpillar would survive an encounter with a would-be predator (Wiklund & Jaervi 1982, Wiklund & Sillen-Tullberg 1985). Naive quail, unfamiliar with insect prey in general and swallowtail caterpillars in particular, may require prior experience with distasteful individuals to learn to associate osmeterial secretions with unpalatability. If osmeterial secretions are indeed primarily warning signals, rather than effective allomones in their own right, the possibility exists that palatable caterpillars such as *P. glaucus* may actually be olfactory Batesian mimics of sympatric unpalatable papilionids. This suggestion must remain speculative until additional information is obtained on the composition and mode of action of osmeterial secretions of both palatable and unpalatable swallowtails.

ACKNOWLEDGMENTS

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