

PART I

EVOLUTION OF POPULATIONS
AND SPECIES

Chemical Mediation of Host-Plant Specialization: The Papilionid Paradigm

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Understanding the physiological and behavioral mechanisms underlying host-plant specialization in holometabolous species, which undergo complete development with a pupal stage, presents a particular challenge in that the process of host-plant selection is generally carried out by the adult stage, whereas host-plant utilization is more the province of the larval stage (Thompson 1988a, 1988b). Thus, within a species, critical chemical, physical, or visual cues for host-plant identification may differ over the course of the life cycle. An organizing principle for the study of host-range evolution is the preference-performance hypothesis (Jaenike 1978). According to this hypothesis, ovipositing females should maximize their fitness by selecting plants on which offspring survival will be high; in other words, over a range of potential host plants, adult female preference should be correlated with larval performance. Evidence in support of this hypothesis has been mixed (Thompson 1988a, 1988b; Scheirs and deBruyn 2002). Poor correlations have been attributable in some cases to opposing evolutionary forces that include conflicting selection pressures exerted by trophic factors such as predation and parasitism (Camara 1997; Heisswolf et al. 2005); ecological factors such as relative rarity of most-suitable hosts (Rauscher 1980), recent invasion of less-suitable hosts into an otherwise coevolved community (Wiklund 1975), or thermal constraints on voltinism patterns (Scriber 1996, 2002); phenotypic plasticity of host-plant selection (Mercader and Scriber 2005); or parent-offspring conflict in the case of species in which adults are also phytophagous (Roitberg and Mangel 1993). Although positive correlations are frequently found in other taxa (viz., Craig and Itami, this volume), such correlations are exceptional in lepidopterans.

[AQ1]

The dichotomy between adult behavioral preference and larval physiological performance in Lepidoptera is key to longstanding debates over the evolution of specialization in general. The timing and nature of adaptation to plant

chemistry throughout the life cycle are central to these debates. Almost 60 years ago, Dethier (1948) suggested that “the first barrier to be overcome in the insect-plant relationship is a behavioral one. The insect must sense and discriminate before nutritional and toxic factors become operative.” Thus, Dethier argued for the primacy of adult preference, or detection and response to kairomonal cues, in host-plant shifts. In contrast, Ehrlich and Raven (1964) reasoned that “after the restriction of certain groups of insects to a narrow range of food plants, the formerly repellent substances of these plants might . . . become chemical attractants,” arguing for the primacy of shared allomonal phytochemistry and larval detoxification in initiating host shifts (with the evolution of kairomonal responses following). Oviposition “mistakes,” in which females lay eggs on host plants that do not support larval growth (e.g., Berenbaum 1981b), are cited as evidence for the importance of behavioral cues in initiating host shifts; colonization of novel host plants that share the same range of defense compounds is claimed as evidence of the primacy of larval performance in the process (see Berenbaum 1990 for a review).

[AQ2]

[AQ3]

In view of the fact that the physiological and behavioral changes necessary to effect a host shift arise by random mutation, primacy is in a sense irrelevant; host shifts are completed when the necessary behavioral and physiological traits are in place, irrespective of the order in which they occur (Berenbaum 1990). Complicating the resolution of this issue are recent experimental studies indicating that preference and performance traits involved in host shifts may not be entirely under heritable genetic control and are instead influenced by epigenetic and even nongenetic factors. Evidence suggests that larval feeding experience in at least some taxa may influence adult oviposition preference either via “chemical legacy” (retained chemical signals) or via some form of retained memory (Barron 2001; Rietdorf and Steidle 2002; Akhtar and Isman 2003; but see van

Emden et al. 1996). No real resolution of such debates can take place until the biochemical and genetic bases of preference and performance are elucidated for a broad array of species.

How Lepidopterans Prefer

A priori prediction of which chemicals influence oviposition preference and larval performance and whether linkages in chemical perception or processing between developmental stages exist is hampered by the fact that, within the Lepidoptera, caterpillars and adults experience very different chemical environments, with very different attendant behavioral and physiological consequences. Adult females, with relatively few exceptions, search for host plants while flying; complementing visual cues for identifying host plants are volatile odorant cues. Long-distance orientation to host plants by female butterflies is generally mediated by volatile signals detected by antennae (Feeny et al. 1989); in that this process involves upwind anemotaxis, it might be expected to resemble pheromone plume-following behavior and rapid antennal processing in males. Male moths orienting in an odor plume benefit from the ability to assess changes in ambient pheromone concentrations and accordingly rely on rapid processing of signals to facilitate rapid assessment (Wang et al. 1999). How female butterflies process volatile kairomonal cues is virtually unexplored.

Once host plants are encountered, suitability assessment generally involves contact chemoreception, mediated by tarsal and in some cases ovipositor receptors. Oviposition kairomonal cues tend to be contact chemical cues restricted to plant surfaces or tissues close to the surface (Heinz and Feeny 2005). In contrast with volatile cues, rapid clearance may not be of such paramount importance in the absence of upwind anemotaxis, nor is directionality of the signal at issue. As well, plant chemicals present on the surface constitute a small subset of the total allelochemical inventory of the plant, and these chemicals tend to be present on the plant surface in concentrations orders of magnitude smaller than those in internal tissue (Brooks et al. 1996; Brooks and Feeny 2004).

Signal sorting differs qualitatively and quantitatively between immature and adult stages of Lepidoptera. The ability to fly exposes ovipositing females to a far more chemically complex environment than that experienced by the vast majority of lepidopteran larvae. Adult females must locate and identify particular host plants within a community of dozens or even hundreds of potential nonhosts; the ability to differentiate among complex chemical signals is at a premium. In contrast, caterpillars, with few exceptions, are restricted to walking and thus “sample” a vastly smaller subsection of the plant community. In most species, caterpillars remain on the plant on which they hatch and are rarely if ever called upon to differentiate among potential host-plant species. Host-plant acceptance is mediated primarily by contact chemoreception. Yet there is a fundamental difference even in contact chemoreception between adult and larval

lepidopterans; whereas “tasting” by ovipositing females is intermittent and brief, foraging larval lepidopterans may be in constant contact with surface chemicals. Moreover, for species hatching from eggs laid within plant tissues, neonates are essentially immersed in a chemical cauldron and are potentially exposed to locally high concentrations of phytochemical cues (Kreher et al. 2005).

The neurological burden imposed by the necessity of differentiating among host and nonhost signals has been suggested to represent a constraint on the evolution of host preferences in insects (Bernays 2001). In what was purportedly a test of the so-called information processing hypothesis, Janz and Nylin (1997) compared the discriminatory capacity of two monophagous nymphalids, *Polygonia satyrus* and *Vanessa indica*, with that of the polyphagous nymphalids *P. c-album* and *Cynthia cardui*. As adults, the specialists were better able to distinguish and discriminate against “bad-quality” nettle than were the generalists, consistent with the idea that generalists are well equipped to sort through a large number of signals but less capable of evaluating subtle differences in particular signals. In this study, the fact that larvae of the generalist species displayed no growth impairment on the “poor-quality leaves” makes interpreting these results more ambiguous in that so-called poor quality did not manifest itself in performance differences.

How Lepidopteran Larvae Perform

Allomones, or deterrent compounds, as well as kairomones, or attractants, play a role in determining preference and performance in both larval and adult Lepidoptera. Toxicological consequences of exposure to plant chemicals, however, differ dramatically between larval and adult stages, due to qualitative differences in composition of toxins and quantitative differences in toxicological loads. Whereas the exposure of an ovipositing female to particular host-plant chemicals may be on the order of seconds or minutes, caterpillars are exposed to host-plant chemicals more or less continuously for the duration of larval life, which can extend for days or weeks. Caterpillars that are capable of ingesting their body weight in plant material are potentially exposed to enormous quantities of compounds that often possess detrimental biological activities at high concentrations. Moreover, by virtue of consuming leaf or other plant tissue in its entirety, in all but a few cases caterpillars are exposed to a much broader inventory of host-plant chemicals. Skeletonizers and leaf miners may avoid indigestible plant material, but even so, their intake of plant allomones is extremely likely to exceed that of ovipositing adults.

Although chemical complexity has been thought to act as a constraint on ovipositing adults via limits on neural information processing, the idea that chemical complexity acts as a constraint on larvae via limits on detoxification processing has received considerably less attention of late. There has been a longstanding assumption that the principal detoxification enzymes in Lepidoptera (e.g., cytochrome

TABLE 1.1

Phenotypic Correlations Between Leaf and Fruit Furanocoumarin Content of *Pastinaca Sativa* (From Berenbaum et al. [1986], Zangerl et al. [1997], and Unpublished Data). $N = 124$, Values are Correlation Coefficients, and Significant Correlations ($p < 0.05$) are in Bold

Leaf Content	Seed Content				
	Imperatorin	Bergapten	Isopimpinellin	Xanthotoxin	Sphondon
Imperatorin	-0.022	0.016	0.033	0.032	0.258
Bergapten	0.016	0.045	-0.068	0	-0.003
Isopimpinellin	0.046	-0.051	-0.157	0.01	0.067
Xanthotoxin	-0.031	-0.106	-0.142	-0.077	0.035
sphondin	-0.061	0.006	-0.032	-0.156	0.134

P450 monooxygenases) are broadly substrate specific, as they generally are in vertebrates (Berenbaum 1999), and thus well designed to accommodate novel substrates; indeed, this idea is the basis for the hypothesis that enzymes that process plant allelochemicals are preadaptations for the evolution of insecticide resistance (Gordon 1961). In fact, as more detoxification enzymes are characterized at the biochemical and molecular level, it has become abundantly clear that broad substrate specificity is the exception, particularly in oligophagous species (Berenbaum 2002; Mao et al. 2005).

Thus, chemically mediated interactions between host plant and lepidopteran differ profoundly over the course of development. In addition, by virtue of the fact that in essentially all plants, chemistry changes with time and development, ovipositing females must make choices based on chemicals that may not be predictive of the chemistry that will be experienced by their progeny. The time between egg deposition and egg hatch can be on a scale of hours, in which case chemical changes in host plants may be trivial. However, for eggs that undergo seasonal diapause, the time displacement between oviposition and hatch can be on a scale of months, in which case chemical changes in host plants may be dramatic. The parsnip webworm *Depressaria pastinacella*, for example, feeds as a caterpillar exclusively on the developing buds, flowers, and fruits of the biennial forb *Pastinaca sativa*. Larval performance in this species is profoundly affected by furanocoumarin content of the reproductive tissues of its host plants (Berenbaum and Zangerl 1993), yet the number of eggs laid by ovipositing females is independent of the furanocoumarin chemistry of foliage (Zangerl and Berenbaum 1992). Adult females oviposit in late spring before this biennial plant has produced a flowering stalk; the only tissue available at the time of oviposition for assessment is foliage. Furanocoumarin content of foliage present before production of the flowering stalk is in virtually no case significantly correlated with furanocoumarin chemistry of the reproductive structures in this plant (Table 1.1). Larvae of this species

do respond behaviorally to octyl esters, which are present in reproductive tissues that serve as food and absent in foliage, even though, upon ingestion, these compounds are toxic (Carroll et al. 2000). The within-plant distribution of these compounds may allow larvae to move from oviposition sites on the leaves to feeding sites within the reproductive structures. This example also illustrates the difficulty of classifying plant infochemicals as either kairomones (host-recognition cues) or allomones (plant-defense compounds).

Preference-Performance Relationships in Lepidoptera

An enormous amount of information exists about preference and performance in lepidopterans (Table 1.2); over a dozen studies have been conducted to determine the relationship between oviposition preference and larval performance. In over half of these studies, no correlation could be found. The lack of correlation may result from any number of possible explanations, not the least of which is the difficulty in measuring these attributes. Thompson's (1988a, 1988b) definition of preference as the "hierarchical ordering of plant species by ovipositing females when the plants are presented in equal abundance and availability" is widely but not universally accepted (Singer et al. 1992). As such, "preference" is in reality a series of behaviors, including orientation in flight, decision to land, and decision to oviposit. "Performance" is even more nebulous experimentally, generally measured by survival but also by such fitness proxies as development time, growth rate, or pupal weight (Moreau et al. 2006).

Operational problems notwithstanding, the underlying genetics of preference and performance characterized in numerous systems offer little support for a mechanistic link between these attributes. In general, interspecific differences in female preference are associated with the X chromosome (Thompson 1988a, 1988b; Thompson and Pellmyr 1991); in Lepidoptera the female is the heterogametic sex, and in reciprocal crosses oviposition preferences map onto the male

TABLE 1.2

Tests for Significant Positive Correlations Between Preference (pref) and Performance (perf) Involving Lepidopterans

Family	Taxon	Pref/perf Correlation	Author
Papilionidae	<i>Papilio machaon</i>	No	Wiklund 1974
	<i>Papilio glaucus</i>	Yes (Ohio population)	Bossart 2003 Scriber 1996, 2002
		No (Florida population)	
	<i>Battus philenor</i>	No	Rausher 1980
	<i>Eurytides marcellus</i>	Yes	Damman and Feeny 1988
Pieridae	<i>Ascia monuste</i>	No	Carta-Preta and Zucoloto 2003
	<i>Eucheira socialis</i>	No	Underwood 1994
	<i>Pieris rapae crucivora</i>	Yes	Chen et al. 2004
	<i>Pieris rapae melete, napi</i>	Yes	Ohsaki and Sato 1999
Nymphalidae	<i>Polygonia c-album</i>	Yes	Nylin and Janz 1996
	<i>Polygonia c-album</i>	No	Nylin and Janz 1996
	<i>Junonia coenia</i>	No	Prudic et al. 2005
	<i>Junonia coenia</i>	No	Camara 1997
	<i>Melitoea cinxia</i>	No	Van Nouhuys et al. 2003
	<i>Danaus plexippus</i>	Yes	Mattila and Otis 2003
	<i>Danaus plexippus</i>	Yes	DiTommaso and Losey 2003
	<i>Danaus plexippus</i>	No	Ladner and Altizer 2005
Lycaenidae	<i>Polyommatus icarus</i>	Yes	Bergstrom et al. 2004
	<i>Mitoura</i> spp.	Yes	Forister 2004
	<i>Glaucopsyche lygdamus</i>		Carey 1994
Noctuidae	<i>Spodoptera exigua</i>	No	Berdegue et al. 1998

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source population (Scriber et al. 1991; Thompson and Pellmyr 1991). X-linked traits are thought to evolve rapidly if mutations are recessive because such traits are exposed to selection in the heterogametic sex (Charlesworth et al. 1987); this idea has lent support to the idea that adult preference is key to initiating host shifts. In addition, the disproportionate influence of X-linked traits on oviposition preferences may facilitate the evolution of adaptive gene complexes due to reduced recombination (Jaenike 1989). Within-species variation in preference, however, does not appear to be predominantly sex-linked (Janz 1998). Low levels of additive genetic variance for within-species variation in oviposition preference argue for involvement of major genes. In the rare cases in which larval preference has been genetically mapped, it is not necessarily genetically correlated with adult preference, although it can be X-linked (Nylin and Janz 1996).

Chemical Mediation of Preference and Performance: Papilionids as Paradigm

Despite the fact that butterflies have been the subject of chemical ecology studies for over a century, the chemistry of host-plant preference and performance has been eluci-

dated for only a handful of groups. One group in which host- utilization patterns are closely associated with particular plant allelochemicals is the family Papilionidae, the swallowtail butterflies (Fig. 1.1). The swallowtails have been a paradigm group for the study of chemical coevolution for over 50 years (Feeny 1991, 1992; Scriber et al. 1995). In this cosmopolitan family with over 500 species, host-plant use patterns are remarkably conservative. Only 21% of the 281 species with known host associations use more than a single plant family (Scriber et al. 1991). Of the plant families utilized by papilionids, five dominate; these are the Annonaceae, Apiaceae, Aristolochiaceae, Lauraceae, and Rutaceae. Two tribes, Troidini and Zerynthiini, are restricted to Aristolochiaceae; caterpillars in these groups sequester toxic aristolochic acids from their host plants and acquire chemical defense against predators that is carried over to the adult stage. In contrast, over 75% of species in the tribe Papilionini, comprising the genus *Papilio*, utilize host plants in the families Rutaceae and Apiaceae, with the North American *P. machaon* complex of approximately a dozen species accounting for most of the specialists on Apiaceae (Sperling 1987). The genus *Papilio* also contains the most polyphagous of papilionids: species in the *P. glaucus* com-

[AQ6]

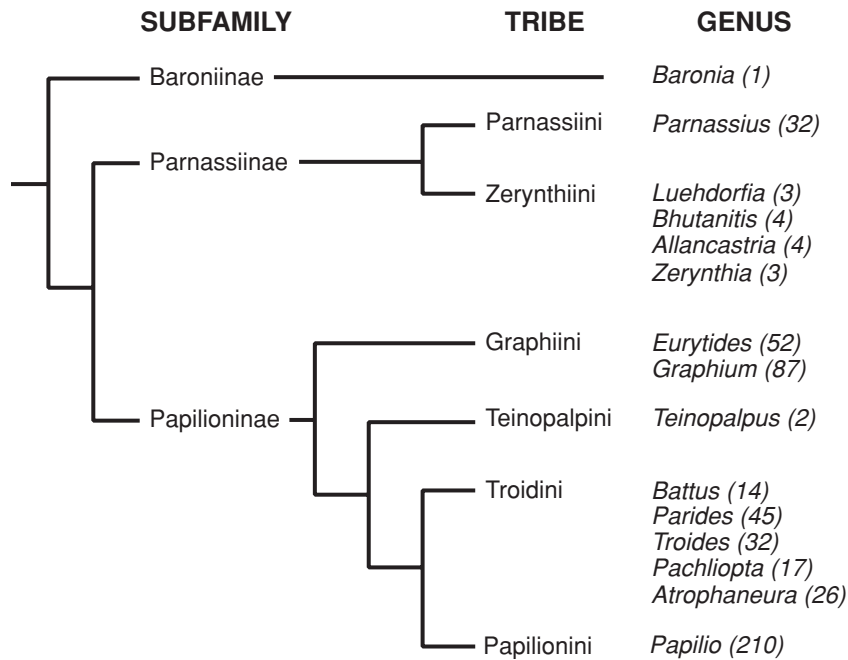


FIGURE 1.1. Phylogeny of the family Papilionidae, based on Hancock (1983), Miller (1987), and Caterino et al. (2001). Figures in parentheses indicate approximate numbers of species in each genus. Not listed are several small genera, each containing one or two species. Recent molecular evidence (Caterino et al. 2001) suggests that the subfamily Parnassiinae is not monophyletic and that the tribe Zerynthiini, with or without *Luehdorfia* (cf. Zakharov et al. 2004), and the subfamily Papilioninae may be sister taxa.

[AQ32]

plex. *Papilio glaucus* and *P. canadensis* have distinct but overlapping host ranges encompassing several families of early successional trees, including species in Rosaceae, Oleaceae, and Tiliaceae.

Host Search and Oviposition

Long-distance orientation to host plants by papilionids involves the processing of visual cues. Females of the zebra swallowtail, *Eurytides marcellus*, approach understory shrubs of various nonhost species as well as shrubs of their larval host plants (*Asimina* spp.). After close-range inspection by a slowly fluttering female, a nonhost shrub is about twice as likely as a host shrub to be rejected by a female without landing. Nevertheless, about one-third of nonhosts approached are not rejected until after a female landed and had access to contact chemical cues (Damman and Feeny 1988). Although *E. marcellus* females are strongly attracted to host-plant volatiles, at least at short range (Haribal and Feeny 1998), the initial process of filtering potential oviposition sites from surrounding vegetation is strongly dependent on responses to visual cues such as the ratio of young to mature leaves and the presence of leaf buds (Damman and Feeny 1988).

Females of the pipevine swallowtail, *Battus philenor*, in southeast Texas lay small batches of eggs on two herbaceous *Aristolochia* species in the forest understory. Individual females can be found landing preferentially either on narrow-leaved or on broad-leaved plants, the large majority of which are nonhosts (Rausher 1978). A female's leaf shape preference is determined by her recent contact experience with the narrow-leaved host, *A. serpentaria*, or the broad-leaved host, *A. reticulata*. Contact alone, even without oviposition, is sufficient to bring about this associative

learning (Papaj 1986). Since the relative availability of the two host species as suitable oviposition substrates changes seasonally, short-term learning of leaf shapes can result in greater searching efficiency (Rausher 1995).

Papilio species possess six classes of spectral receptors, including UV, violet, blue, green, red, and broadband (Arikawa 2003). Physiological receptivity to colors is reinforced by chemical cues; *B. philenor* displays the capacity to learn to associate different color cues with sucrose (representative of nectar plant chemistry) and leaf extracts (representative of oviposition substrate chemistry). Landings and probes on nontrained colors were biased toward the training alternatives, suggesting neural limitations on processing visual information (Weiss and Papaj 2003), akin to that proposed for processing chemical information (Kelber and Pfaff 1999).

The role of olfaction in the oviposition behavior of swallowtails has remained less clear despite the early demonstration by Saxena and Goyal (1978) that volatile extracts of the host plant *Citrus limettioides* (lime) elicited significant oviposition by *P. demoleus* females. In laboratory bioassays with model plants, females of the black swallowtail, *P. polyxenes*, were significantly more active when host volatiles were added to artificial leaves treated with contact stimulant extracts. They also landed more frequently and laid more eggs (Feeny et al. 1989). Activity levels and landing rates were significantly reduced, however, in the presence of volatiles from cabbage, a nonhost (Feeny et al. 1989). In comparable experiments with *E. marcellus*, females were highly responsive to model leaves treated with both contact stimulants and volatiles but volatiles alone were sufficient (in combination with visual cues) to stimulate some oviposition (Haribal and Feeny 1998). Recently, Heinz (2002) has shown that responses to volatiles by *P. polyxenes* females are

largely innate but that the females are capable of learning combinations of volatile and leaf-shape cues.

Once a female has landed on a leaf, contact stimulants are of crucial importance in determining whether a potential oviposition site is accepted or rejected (Städler 1992; Renwick and Chew 1994). The stimuli are perceived by tarsal contact chemoreceptors during drumming behavior, in which the females “taste” the leaf surface with their forelegs (Ilse 1956; Feeny et al. 1983). In the pierid butterfly *Pieris brassicae*, Ma and Schoonhoven (1973) showed that each taste sensillum on the foretarsi of females contains five receptor cells, including at least one cell that responds to the glucosinolates that characterize the crucifer host plants. Among swallowtails, cells responding to contact stimulants from carrot, *Daucus carota*, have been detected in the foretarsi of *Papilio polyxenes* females (Roessingh et al. 1991). The stimulant activity of host-plant extracts typically results from synergistic interactions between several ingredients that are seldom active alone (Honda 1986; Feeny et al. 1988; Ohsugi et al. 1991; Sachdev-Gupta et al. 1993; Nishida 1995). Contact receptors in butterflies, including swallowtails, can also respond to compounds that inhibit rather than stimulate oviposition (Nishida et al. 1990; Sachdev-Gupta et al. 1990). Although a particular compound or class of compounds may contribute the major sensory cue, it is the total complex that forms the basis for perception and behavioral response (cf. Dethier 1976; Renwick and Chew 1994).

Responses of *P. polyxenes* females to contact chemical cues are innate and not altered by specific host-plant experience. Females responded to extracts of two host plants, carrot (*D. carota*) and poison hemlock (*Conium maculatum*) differentially but without regard to the plant previously experienced (Heinz and Feeny 2005). The postalighting response is unaffected by host-plant experience; however, Heinz (2002) has shown that the females are capable of learning all three major cue types (contact stimulants, volatile stimulants, and visual cues) in free-flight bioassays, especially when the cues were presented in pairs. Females also demonstrated learning in whole-plant bioassays, being most likely to approach and land on the same species with which they had prior oviposition experience (Heinz 2002). Such chemically mediated learning is likely to enhance the rate of egg laying in an oligophagous species such as *P. polyxenes*, successive generations of which may encounter shifting arrays of potential host-plant species that differ significantly in chemistry and leaf shape (Heinz 2002).

Identifying the Chemical Cues: Kairomones and Allomones

Oviposition Kairomones: Volatile Attractants

Little is known about the identities of volatile compounds used as oviposition kairomones by swallowtails, probably because bioassays of volatiles are much more time-consuming than are those for contact stimulants and concentra-

tions of active material are typically much smaller. Electroantennogram recordings revealed that the antennae of females of *P. polyxenes*, *P. machaon*, and *P. troilus* respond selectively to several compounds in the volatiles of their host plants (Baur and Feeny 1995). *Cis*- and *trans*-sabinene hydrate, 4-terpineol, and *cis*-3-hexenyl acetate are among the dozen or so components of carrot volatiles that evoke responses in *P. polyxenes* antennae, but their role in oviposition behavior is not yet known; several of the compounds evoke antennal responses in males as well as females (Baur et al. 1993). More likely candidates for volatile oviposition kairomones should perhaps be sought among the volatile phenylpropanoids and other compound classes that are more characteristic of the Apiaceae, Rutaceae, and other typical swallowtail host families.

Although they have to date eluded chemical characterization, it is becoming clear that volatile stimulants may be no less significant than contact kairomones in the evolution of host preference in swallowtails. First, there is increasing experimental evidence that host volatiles represent a major component of the oviposition-stimulant profiles to which many swallowtails respond. Second, the work of Heinz (2002) indicates that responses to volatile stimulants are heritable and hence potentially more conserved during swallowtail evolution than previously suspected. And third, the circumstantial evidence is compelling; the host plants of swallowtails are commonly aromatic, and both terpenoid and phenylpropanoid volatiles have been reported from virtually all the major food-plant families (Feeny et al. 1983 and references therein). Moreover, several reports of oviposition “mistakes” by swallowtails are suggestive of a role for a common theme in the volatile profiles of swallowtail food plants. Within the genus *Papilio*, in particular, there have been numerous observations of females of one species laying eggs on host plants used more typically by larvae of other species (references in Feeny et al. 1983). Stride and Straatman (1962), for example, found that females of *P. aegaeus*, a Rutaceae feeder, would oviposit on *Cinnamomum camphora* (Lauraceae), a larval food plant of *Graphium* species. Berenbaum (1981) found two eggs of *P. glaucus* on leaves of *Angelica atropurpurea*, a most unusual food plant for this tree-feeding generalist, but a potential food plant of *P. polyxenes* and other umbellifer-feeding *Papilio* species. D. A. West (personal communication) found an egg of *P. troilus*, a Lauraceae-feeding species, on *Aristolochia macrophylla*, a host plant of the pipevine swallowtail, *Battus philenor*. In such examples, it seems more plausible to invoke a role for shared volatiles, rather than similar contact stimulants or visual cues, in triggering the mistakes.

Oviposition Kairomones: Volatile Attractants

Contact oviposition stimulants have been at least partially characterized for eight species (genus *Papilio*) in the tribe Papilionini, two species (*B. philenor* and *Atrophaneura alcinous*) in the tribe Troidini, and one species each in the

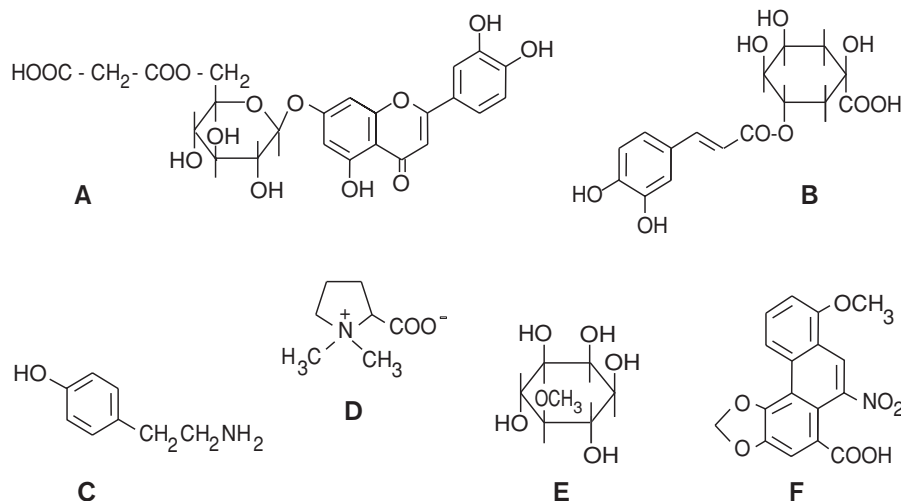


FIGURE 1.2. Examples of classes of compounds identified as contact oviposition stimulants for swallowtail butterflies. A. Luteolin 7-O-(6''-O-malonyl)- β -D-glucopyranoside, a flavonoid glycoside (*Papilio polyxenes*); B. *trans*-chlorogenic acid (5-caffeoylquinic acid), an hydroxycinnamic acid derivative (*Papilio protenor*, *P. polyxenes*); C. tyramine, a simple base (*P. polyxenes*); D. stachydrine, a zwitterion compound related to proline (*P. xuthus*, *P. protenor*); E. sequoyitol, an inositol (*Atrophaneura alcinous*, *Battus philenor*); F. aristolochic acid I, an aristolochic acid (*A. alcinous*, *B. philenor*).

Graphiini (*Eurytides marcellus*) and Zerynthiini (*Luehdorfia japonica*) (Nishida et al. 1994; Honda 1995; Nishida 1995; Honda et al. 1997; Carter et al. 1998, 1999; Haribal and Feeny 1998; Ono et al. 2000a, 2000b; Nakayama et al. 2003; Nakayama and Honda 2004). In the genus *Papilio*, host recognition is mediated by a mixture of compounds belonging to at least two of several chemical categories (Fig. 1.2). These include flavonoid glycosides (e.g., Fig. 1.2A), hydroxycinnamic acid derivatives (e.g., Fig. 1.2B), simple bases (e.g., adenosine, bufotenine and tyramine, Fig. 1.2C), amphoteric substances (e.g., proline and stachydrine, Fig. 1.2D), and simple aliphatic hydroxy compounds such as inositols (e.g., (+)-*chiro*-inositol and sequoyitol, Fig. 1.2E) and organic acids of low molecular weight (e.g., quinic acid and 2-C-methyl-D-erythronic acid) (Nishida 1995; Nakayama et al. 2003). In *P. polyxenes*, for example, specific components of the mixture eliciting oviposition include the flavonoid luteolin 7-O-(6''-O-malonyl)- β -D-glucopyranoside (L7MG) (Fig. 1.2A), *trans*-chlorogenic acid (5-caffeoylquinic acid) (Fig. 1.2B), and tyramine (Feeny et al. 1988; Carter et al. 1998). Roessingh et al. (1991) identified contact chemoreceptors responsive to L7MG on the female foretarsi of *P. polyxenes*; the specificity of this receptor is suggested by the fact that luteolin glucoside, lacking the malonyl moiety of L7MG, is ineffective in stimulating a response. Ono and Yoshikawa (2004) characterized a tyramine receptor (PxTYR1, AB182633) from cDNA from female foretarsi of the related species *P. xuthus*, which feeds on rutaceous host plants. This receptor may play a role in recognizing bases in the multicomponent oviposition stimulants.

In the Troidini, sister tribe to the Papilionini (Miller 1987), the picture is quite different. Females of both species so far examined are stimulated by a mixture of aristolochic acids (alkaloid derivatives, e.g., aristolochic acid I, Fig. 1.2F) and a methylinositol (pinitol and/or sequoyitol) (Nishida and Fukami 1989a; Papaj et al. 1992; Sachdev-Gupta et al. 1993). The lack of responses by troidines to flavonoids is curious in view of the general occurrence of such compounds in that

family (Nishida 1995). Of particular interest, therefore, is the finding that a primary contact oviposition stimulant for females of *L. japonica* (tribe Zerynthiini), which oviposit on *Heterotropa aspera* (Aristolochiaceae), is a flavonoid (Nishida 1994). *Luehdorfia japonica* does not respond to aristolochic acids, nor are these present in the *Heterotropa* host plants (Nishida 1994, 1995). This suggests that responses to flavonoids may be quite ancient in the swallowtail lineage and that their absence from the stimulant profiles of troidine swallowtails represents a secondary loss.

The primary contact oviposition stimulant for *Eurytides marcellus* (tribe Graphiini) in foliage of its major host plant, pawpaw (*Asimina triloba*, Annonaceae), is 3-caffeoyl-muco-quinic acid (3-CmQA) (Haribal and Feeny 1998; Haribal et al. 1998). Surprisingly, this compound alone accounted for the activity of host-plant extracts and required no synergists (Haribal and Feeny 1998). The same compound is also an oviposition stimulant for the spicebush swallowtail, *Papilio troilus*, from the foliage of sassafras (Lauraceae), although in this case as part of a synergistic mixture (Carter et al. 1999). The strong responses of *E. marcellus* females to host volatiles may compensate for the absence of other contact stimulants for this species (Haribal and Feeny 1998; Heinz 2002). There is evidence for stereospecificity in the responses of swallowtails to caffeoyl-quinic acid (CQA) isomers. Of several isomers occurring in pawpaw extracts, 3-CmQA significantly stimulated oviposition while other diastereomers did not (Haribal and Feeny 1998). Neither *E. marcellus* females (Haribal and Feeny 1998) nor those of *P. troilus* (Carter et al. 1999) responded to 5-CQA (chlorogenic acid), a stimulant for *P. polyxenes* (Feeny et al. 1988) and *P. protenor* (Honda 1990). The use of CQA isomers as contact stimulants by species in both the Papilionini and Graphiini suggests that the use of hydroxycinnamic acid derivatives as oviposition stimulants may have been an ancestral feature of the subfamily Papilioninae. Consistent with this suggestion is the finding by Nishida (1995) that 3-hydroxy-4-methoxycinnamoylmalic acid is an

oviposition stimulant, along with sequoyitol and aristolochic acids, for *Atrophaneura alcinous* (tribe Troidini).

Whereas the oviposition kairomones of butterflies in some other families, such as the Pieridae, include compounds used by the host plants as allomones, those used by most swallowtails studied thus far belong to classes of compounds that are typically innocuous to insects. A conspicuous exception is the use of aristolochic acids as kairomones by troidine swallowtails. These compounds can serve as powerful feeding deterrents and toxins for insects (Miller and Feeny 1983). Sequestration of aristolochic acids and their role in insect defense may have provided strong selection for the use of these plant allomones as oviposition kairomones (Nishida and Fukami 1989a, 1989b). It is probably not a coincidence, however, that many of the compounds characteristic of swallowtail food-plant families are derived biosynthetically from the phenylpropanoid pathway (Feeny et al. 1983). The central compounds in phenylpropanoid biosynthesis are the hydroxycinnamic acids, derived from phenylalanine. Several such compounds are known to be oviposition kairomones for swallowtails. Umbelliferone, the basic hydroxycoumarin, is simply derived from a hydroxycinnamic acid and in turn serves as precursor for the furanocoumarins, among the notable allomones of many swallowtail food plants. Although the same compounds may not typically serve as both kairomones and allomones, it is nevertheless curious that these frequently share a common biosynthetic ancestry, perhaps increasing the probability of biochemical linkages between performance and preference in swallowtails.

Deterrent compounds also play a role in mediating oviposition (Haribal and Feeny 2003). *Papilio polytes* is a specialist on a narrow range of rutaceous hosts; whereas methanolic extracts of acceptable hosts stimulated oviposition (*Toddalia asiatica*, a major host, as well as the marginal host *Melicope triphylla*), methanolic extracts of host plants not normally utilized in the field did not stimulate oviposition (e.g., *Murraya paniculata*). Larval mortality was also higher on the rutaceous species not normally utilized in the field, suggesting the possibility that the same or similar compounds deterring oviposition also act as larval antifeedants (Nakayama et al. 2002). Ono et al. (2004) confirmed that a hydroxybenzoic acid derivative in the foliage of the non-host rutaceous species *Orixa japonica* acts as both oviposition deterrent and antifeedant in *P. xuthus*.

Larval Allomones

In terms of allomones, of the more than 200 species in the genus *Papilio*, over 75% use plants containing allomones called furanocoumarins (Berenbaum 1983). These plants present a toxicological challenge to herbivores because UV-activated furanocoumarins covalently bind to DNA and protein and interfere in cellular processes in a broad range of organisms (Berenbaum 1991, 1995). Furanocoumarins occur in two structural configurations: linear fura-

nocoumarins (with the furan ring attached at the 6,7 positions of the benz-2-pyrone nucleus), which cross-link opposing strands in DNA helices, and angular furanocoumarins (with the furan ring attached at the 7,8 positions), which form only monoadducts with DNA. Linear furanocoumarins occur in over 10 plant families and are most abundant in the Rutaceae and Apiaceae, the preferred hosts for most *Papilio* species. Angular furanocoumarins are restricted to a few genera in three families and are most abundant in two tribes in the Apiaceae (Murray et al. 1982).

Among the swallowtails, only species in the tribe Papilionini, which normally encounter furanocoumarins, can tolerate ecologically relevant concentrations of these compounds in their diet; representative troidine and graphiine larvae die when confronted with host-plant foliage supplemented with xanthotoxin (Heininger 1989). Within the genus *Papilio*, the ability to tolerate furanocoumarins is commensurate with the frequency with which they are encountered within host plants. Whereas *P. troilus*, a specialist on lauraceous host plants lacking furanocoumarins, cannot consume these compounds, the polyphagous *P. glaucus* and to a lesser extent *P. canadensis*, which occasionally encounter at least one rutaceous host, containing furanocoumarins, have an intermediate ability to tolerate these compounds. By comparison, Rutaceae and Apiaceae specialists within the genus (including *P. multicaudatus* and *P. polyxenes*) can tolerate concentrations exceeding 1% of the dry weight of food ingested (Berenbaum 1981; Mao et al. [AQ10] 2006). In at least one species, *P. polyxenes*, the presence of furanocoumarins in the diet is associated with enhanced survivorship, although these compounds do not appear to act directly as feeding stimulants (Berenbaum 1981). [AQ11]

Adaptation also occurs in swallowtails to combinations of chemicals in their host plants. Myristicin, an essential oil constituent of many apiaceous hosts of papilionids, is a methylenedioxyphenyl derivative; many such compounds act as toxin synergists by virtue of their ability to inhibit cytochrome P450-mediated detoxification enzymes. *Papilio polyxenes*, which routinely encounters both furanocoumarins and myristicin in its many apiaceous hosts (Harborne et al. 1969), displays relative immunity to the synergistic potential of myristicin (Neal and Wu 1994).

Retention of the ability to detoxify or otherwise tolerate chemical defenses associated with ancestral host plants is a recurrent pattern within the Lepidoptera. Janz et al. (2001) determined that within the Nymphalini most species, irrespective of contemporary host-plant use, can survive on species in the ancestral host family Urticaceae. Similarly, *P. polyxenes* larvae, restricted to feeding on Rutaceae and Apiaceae, nonetheless can tolerate the benzylisoquinoline alkaloids characteristic of lauraceous host plants (Miller and Feeny 1989), and *P. canadensis*, a polyphagous species with a northern distribution mostly beyond the range of furanocoumarin-containing rutaceous host plants, nonetheless can metabolize these compounds (Li et al. 2001). Mechanistically, retention of the capacity to detoxify allomones charac-

teristic of ancestral plants could arise in at least two ways: ancestral detoxification enzymes may be retained unmodified as new enzymes evolve by gene duplication events; alternatively, gene duplication events may lead to the acquisition of novel substrates without a loss of the ability to metabolize ancestral allomones because the genetic changes facilitating novel substrate acquisition occur in places in the protein that are not involved in ancestral allomone metabolism.

Preference and Performance Genes

Even less well understood than the chemical cues mediating preference and performance are the actual genes influencing the processing of chemical signals. Although the process by which host-plant shifts occur is widely recognized to involve genetic changes, documenting such genetic change has proved difficult. Examples are vanishingly rare, even among the well-studied butterflies. The idea that independent gene complexes regulate preference systems in larval and adult swallowtails has been embraced for over a quarter century (Wiklund 1975), at least in part because of the abundance of evidence that host-plant ranges of larvae and adults are generally not congruent.

In swallowtails, what little genetic work has been done with respect to mapping preference and performance genes provides support for the idea of independent gene complexes. The adult oviposition preference hierarchy in *P. zeliccaon* is controlled by at least one locus on the X chromosome and at least one locus that is on an autosome [AQ12] (Thompson 1988, 1993; Thompson et al. 1994). Indications are that preference strength is not regulated by a locus on the X chromosome. In contrast, hybridization studies demonstrate no X-chromosome contribution to any component of larval performance; thus, X-linkage of preference and performance is eliminated as a possibility. Pupal mass and development time and survival on host and nonhosts may be Y-linked or the result of nongenetic maternal effects (Thompson et al. 1990).

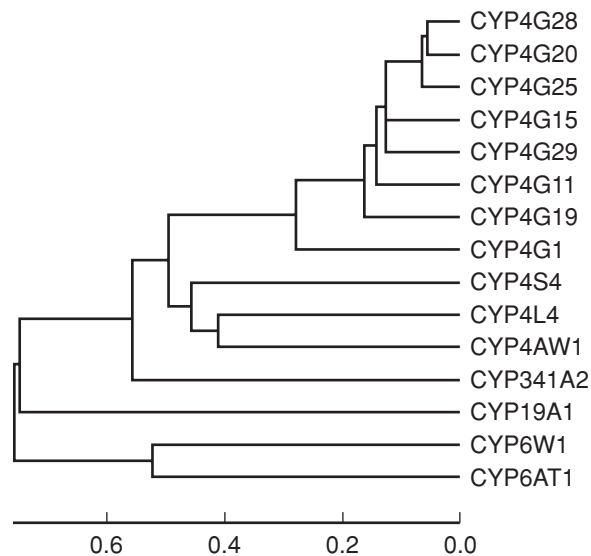
In the polyphagous tiger swallowtail *P. glaucus*, Scriber [AQ13] et al. (1991) demonstrated X-chromosome influence over oviposition preference. Bossart and Scriber (1995) evaluated variation in oviposition preference and found differences not only in relative fidelity to specific host plants, but also differences in the preference rank of particular host plants. Heritable variation was detectable in relative fidelity toward less-preferred hosts but not in preference rank. That host-plant choice is influenced by past experience and physiological state in this species argues against strict genetic correlation between adult preference and larval performance. Bossart (2003) examined covariance between preference and performance (as evidenced in the proxy measures of larval development time, relative growth rate, pupal mass, and mortality) in strains of *P. glaucus* that differ in fidelity—a locally monophagous population from Florida and a locally polyphagous population in Ohio. Covariance was detected independently within each population, although relative

performance was consistent across host plants, independent of adult preferences. Although preference-performance correlations were in the expected direction in the Ohio population (whereby performance measures were correlated with preference for the typical host), in Florida, the correlation was in a direction opposite to that expected. Ovipositing females with the strongest preference for the unusual host plant (*Liriodendron tulipifera*) produced progeny attaining the greatest pupal weight. Based on their findings, “multiple genetic control mechanisms (e.g., pleiotropy and coadaptation)” were invoked to account for preference-performance relationships (Bossart 2003). [AQ14]

Candidates

In terms of characterizing specific genes mediating host-plant specialization, one gene superfamily—the cytochrome P450 monooxygenases (P450s)—appears to play a role in both preference and performance in lepidopterans in general and swallowtails in particular. P450s catalyze the NADPH-associated reductive cleavage of oxygen to produce a functionalized product and water. The genes encoding these enzymes constitute one of the largest superfamilies known (Nelson et al. 1996; <http://drnelson.utmem.edu/CytochromeP450.html>). The enormous proliferation of these proteins is in part a reflection of their functional versatility. Reactions catalyzed by P450s include such oxidative transformations as monooxygenations, dehydrogenations, and peroxidase-type oxidations (Mansuy 1998), so P450s participate in a wide variety of biosynthetic and detoxification reactions. In insects, P450s are involved in the biosynthesis of pheromones, cuticular hydrocarbons, and hormones and are also responsible for metabolism and detoxification of exogenous substrates such as insecticides and plant allomones (Feyereisen 1999).

Gonzalez and Nebert (1990) suggested that the P450 superfamily began to diversify over 400 million years ago, concomitant with the colonization of terrestrial habitats by plants and herbivorous animals. Rapid diversification via a series of gene duplication events may have been a consequence of reciprocal selection pressures whereby plants evolved biosynthetically novel defense compounds, and insects (and other herbivores) overcame the toxins with novel detoxification pathways. Multiple gene duplication events allowed P450s to acquire new functions in the presence of new environmental stress factors while retaining ancestral metabolic capabilities. Protein diversification is, however, only one of several possible evolutionary mechanisms; evolution may also proceed by alterations in gene expression. In insects, P450 expression is regulated in response to a wide range of factors, including developmental stage and exposure to both endogenous and exogenous substances. Examining P450-mediated metabolism of host-plant chemicals within a group of closely related species with well-characterized host-utilization patterns can provide insights into how P450s evolve and diversify in response to environmental selective agents.



[AQ33] FIGURE 1.3. Simple amino acid distance tree of putatively
 [AQ34] chemosensory P450s (CYP4G28 from *Papilio polyxenes* [Mao et al. in preparation]; sequences other than *P. polyxenes* from Genbank).

P450s contribute to preference and performance in herbivorous insects in two ways. Along with glutathione-S-transferases and carboxylesterases, they are the principal detoxification system for metabolizing plant allelochemicals in caterpillar midguts (Berenbaum 2002). They are also key enzymes in chemosensory systems of adults, functioning as degradative enzymes that break down plant and other odorants bound to receptors, thereby regenerating these receptors (Vogt 2003). How P450s that function as degradative enzymes in insect chemoreception relate to those involved in larval midgut detoxification is subject to speculation; in no one species has the full inventory of P450s been characterized functionally. Perireceptor phenomena are not involved in the signal transduction cascade per se but rather are involved with the disposition of the chemical signal itself—uptake, delivery to neuronal receptors, and eventual release of chemicals to regenerate the system. In both vertebrate and invertebrate systems, proteins mediate these processes. Whereas odorant-binding proteins play a role in transporting relatively lipophilic signal substances through the aqueous medium bathing the sensory neuron to receptor proteins in the membrane, degradative enzymes may act extracellularly to facilitate termination of signals by breaking down the odorant. Among the odorant-degrading enzymes, including esterases, glutathione-S-transferases, epoxide hydrolases, and aldehyde dehydrogenases, P450s contribute to chemical signal clearance in both vertebrates and invertebrates (Gu et al. 1999) (Fig. 1.3). While the specificity of P450s involved in degrading pheromones has not been widely assessed, it is assumed that activity and specificity levels are high. With respect to insects, cytochrome P450 and NADPH-P450 reductase genes have been identified that are preferentially expressed in antennae of *Drosophila melanogaster* (Hovemann et al. 1997), suggesting a function in odorant clearance.

Maibeche-Coisne et al. (2002) report that CYP4L4 is expressed preferentially in the antennae of the moth *Mamestra brassicae*, and CYP4S4 expression is limited to the sensilla trichodea in the antennae responsible for odor detection.

In the most compelling study to date implicating P450s in odorant degradation, Leal (2001) showed that enzymatic degradation of the sex pheromone of the scarabaeid beetle *Phyllopertha diversa* is mediated by P450s (Wojtasek and Leal 1999). Subsequent work (Maibeche-Coisne et al. 2004) led to the cloning of three P450s from male antenna cDNA: CYP4AW1, which is antenna specific, CYP4AW2, and CYP6AT1, which is antenna rich. To date, there have been no reports on female-specific expression of P450s associated with sensory organs involved in perceiving chemical cues from plants. For Lepidoptera, then, chemical mediation of preference and performance should converge when adults and larvae assess the same host-plant tissues—when oviposition is directly on tissues eventually consumed by the larvae and when the time between egg hatch and larval development is short (so that chemical changes associated with plant maturation are minimal). Kairomone-degrading P450s, by virtue of exposure to lower and less toxic concentrations of plant chemicals than larval midgut allomone-degrading P450s, should have broader substrate specificity. As well, kairomone-degrading P450s, by virtue of encountering only a subset of the chemical content of host-plant foliage, should be fewer in number than allomone-degrading P450s.

The nature of P450s contributing to allelochemical processing in both larval and adult preference remains a subject for speculation. To date, one P450, CYP4G28, has been characterized from female *Papilio polyxenes* adults that has highest identity (ca. 85%) with two other lepidopteran P450s in the CYP4 subfamily that have been implicated in chemosensory processing (Fig. 4). Expression patterns of CYP4G28 are consistent with a chemosensory function. Real-time polymerase chain reaction analysis with head, thorax, abdomen, wing, antennae, and thorax transcripts showing highest levels of expression in tarsi and antennae, structures that in adult females are associated with detection of plant kairomones. The precise substrate specificity of CYP4G28 and, indeed, other CYP4 P450s expressed in tarsi awaits characterization. [AQ15]

P450s IN LARVAL SWALLOWTAIL PERFORMANCE

P450s in *Papilio* display differences both in constitutive activity against furanocoumarins and in inducibility in response to furanocoumarin ingestion (see Berenbaum 1995 for a review). In general, these differences correspond to the frequency of ecological exposure to furanocoumarins. The genus *Papilio* has historically been divided into five sections (Munroe 1961). Of these, sections II, III, and IV have some association with furanocoumarin-containing plants. Constitutive activity against xanthotoxin, a linear furanocoumarin, is high in *P. cresphontes*, a section IV specialist on linear furanocoumarin-containing Rutaceae, the putative ancestral host-plant family of the group. Activity is

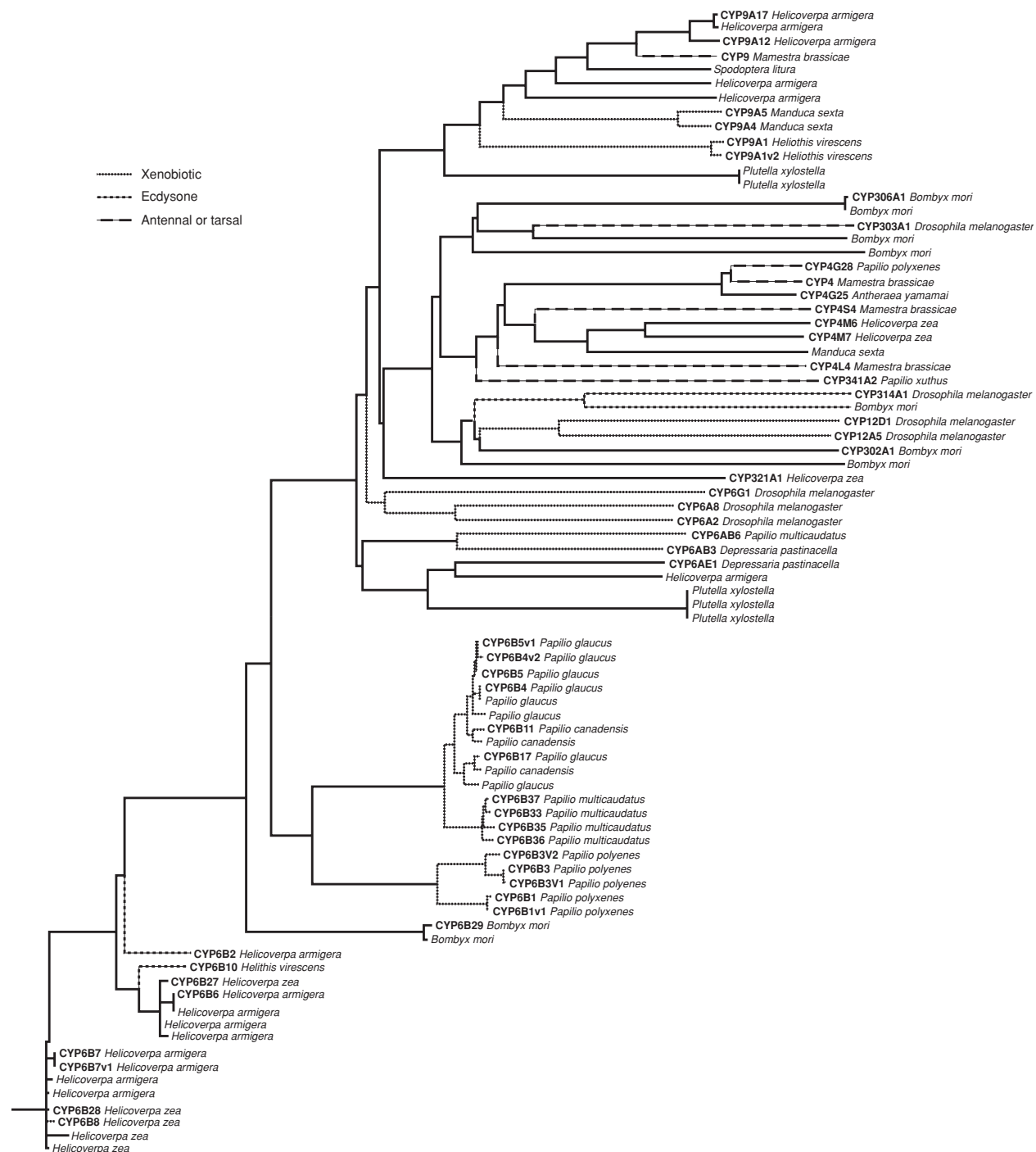


FIGURE 1.4. Rooted tree of lepidopteran P450 proteins generated with the CLUSTALW program in Biology Workbench (ver. 3.2), employing defaults for all settings except that the “Blosum series” was employed for the weight matrix. Known or putatively xenobiotic-metabolizing P450s (involved in larval performance) are represented by dots, known or putatively kairomone-processing P450s (involved in adult preference) by long dashes, and P450s involved in processing endogenous substrates (e.g., hormone or pheromone synthesis) by short dashes. [AQ35]

high as well in *P. polyxenes* and *P. brevicauda*, section II specialists on Apiaceae containing furanocoumarins. In contrast, P450-mediated metabolism of xanthotoxin is undetectable in *P. troilus*, a section III specialist on Lauraceae, which lack furanocoumarins (Cohen et al. 1992).

The molecular basis of furanocoumarin resistance has been investigated extensively in *P. polyxenes*. CYP6B1 transcripts are inducible by xanthotoxin (Cohen et al. 1992), and the protein it encodes metabolizes linear and to a lesser degree angular furanocoumarins (Ma et al. 1994).

[AQ16] Transcripts of another P450, CYP6B3 (Hung et al. 1995), are induced by a wider range of linear and angular furanocoumarins but encode a P450 capable of only low levels of furanocoumarin metabolism (Wen et al. 2003). Consistent with a function of allelochemical detoxification, both of these genes are expressed only in feeding stages of the life cycle; no expression is detectable in eggs, pupae, or adults, which do not consume furanocoumarin-containing host-plant tissue (Harrison et al. 2001).

Coexpression of CYP6B1 and P450 reductase (Wen et al. 2003) resulted in high turnover rates for the linear furanocoumarins xanthotoxin and psoralen; in contrast, substrate binding and turnover rates for angular furanocoumarins are low to undetectable in heterologous expression systems (Ma et al. 1994; Wen et al. 2003). This relative ranking is consistent with the relative frequency of occurrence of these furanocoumarins in host plants; linear furanocoumarins such as xanthotoxin and bergapten are widespread among apiaceous as well as rutaceous host plants, whereas angular furanocoumarins are restricted to a handful of genera in the Apiaceae (Berenbaum 1983).

Comparing two closely related CYP6B4 and CYP6B17 groups in the polyphagous congeners *P. glaucus* and *P. canadensis*, Li et al. (2003) found that, generally, P450s from *P. glaucus*, which feeds occasionally on furanocoumarin-containing host plants, displays higher activities against furanocoumarins than those from *P. canadensis*, which normally does not encounter furanocoumarins. These P450s in turn catalyze a larger range of furanocoumarins at lower efficiency than CYP6B1 from *P. polyxenes*, a specialist on furanocoumarin-containing host plants. Reconstruction of the ancestral CYP6B sequences using maximum likelihood predictions and comparisons of the sequence and geometry of their active sites to those of contemporary CYP6B proteins indicate that substrate specificity is related to host-plant diversity; P450s of oligophagous swallowtails have a narrower range of substrates, but higher activity toward those substrates, than do P450s of polyphagous swallowtails.

The broadening of P450 substrate specificity that appears to have accompanied the evolution of polyphagous feeding within the genus *Papilio* is associated with remarkably few genetic changes. Substitution of aliphatic amino acids for the aromatic Phe484 and Phe371 of CYP6B1 results in an enzyme with a more open and accessible catalytic pocket and, as a consequence, a broader range of substrates in these polyphagous species. Along the lineage leading to *Papilio* P450s, the ancestral, highly versatile CYP6B protein presumed to exist in a polyphagous ancestor evolved through time into a more efficient and specialized CYP6B1-like protein in *Papilio* species with continual exposure to furanocoumarins. Further diversification of *Papilio* CYP6Bs has likely involved interspersed events of positive selection in oligophagous species and relaxation of functional constraints in polyphagous species (Li et al. 2003).

Determining whether changes in the specificity of regulatory pathways accompanies change in substrate specificity

within a lineage requires comparative analysis of promoter regions of these genes (Hung et al. 1996). The CYP6B1 promoter possesses a xanthotoxin-responsive regulatory element required for basal transcription and xanthotoxin inducibility (Prapaipong et al. 1994; Petersen et al. 2003). XRE-xan appears to be conserved in all swallowtail CYP6B genes and may account for the furanocoumarin inducibility of these genes even in species that rarely encounter furanocoumarins in their host plants (McDonnell et al. 2004).

In addition to these elements, the CYP6B1 promoters also contain putative XRE-AhR elements identical to the aryl hydrocarbon-response elements present in mammalian phase I detoxification genes. Transfections of CYP6B4 and CYP6B1 promoters containing EcRE/ARE/XRE-xan and XRE-AhR elements indicate that both are induced significantly by benzo(α)pyrene, an aryl hydrocarbon widespread in the environment, as well as by xanthotoxin, an allelochemical encountered in their host plants (McDonnell et al. 2004). In the mammalian CYP1A1 gene, the benzylisoquinoline alkaloid berberine interacts with XRE-AhR (Vrzal et al. 2005); if the promoter elements function similarly in the CYP6B genes, the presence of XRE-AhR may reflect conservation from ancestral species within the genus associated with magnoliaceous host plants, possibly contributing to the ability of other section II Rutaceae-feeding swallowtails (e.g., *P. aegaeus*) to feed and develop on such ancestral host plants as *Magnolia virginica*, *Michellia champaca*, and *Cinnamomum camphora* (J. M. Scriber, personal communication) and may share regulatory elements responsive to ancestral toxins. [AQ17]

P450s and Host-Use Evolution

The potential involvement of *P. polyxenes* cytochrome P450s in both host-plant selection by adults and host-plant utilization by larvae provides an extraordinary opportunity to examine within a single gene superfamily some of the mechanistic bases for host-use evolution. Although progress is being made in understanding the genetic architecture of host-plant formation within the *Rhagoletis pomonella* complex with respect to odor perception (Linn et al. 2003), comparable studies have not yet been done within the Lepidoptera, and to date there have been no studies examining the involvement of allelochemical metabolism in host-plant perception and host-plant consumption within the same species. Whether P450s recruited as degradative enzymes in chemoreception are similar in function to those recruited as degradative enzymes in allelochemical detoxification remains an open question. Rapid evolution of host shifts may be facilitated if in fact these two groups of P450s share similar substrate specificities or regulatory pathways. Chlorogenic acid, a component of the oviposition stimulant for *P. polyxenes*, is in fact a substrate for CYP6B8, a P450 in the midgut of the generalist *Helicoverpa zea*. Extensive background information on the chemical mediation of oviposition behavior and larval performance in *P. polyxenes* coupled with the unique inventory of cytochrome P450s

characterized from the genome of this species provide a unique and timely opportunity to investigate the mechanistic basis of host-use patterns in Lepidoptera.

Among the other groups of environmental response genes (Berenbaum 2002) that are likely to influence both preference and performance are olfactory and gustatory receptor genes. The absence of a *Papilio* genome project presents a tremendous obstacle to characterizing these genes. In *Drosophila melanogaster*, for example, odor perception depends on activation of a family of receptors sharing seven transmembrane domains; 60 Or genes have been identified in the *D. melanogaster* genome (Clyne et al. 1999; <http://flybase.org/genes/>). Only a subset of these genes expressed in larvae are also expressed in adults: 13 in adult antennae and 1 in the maxillary palp (Kreher et al. 2005). The intriguing possibility exists that genes such as these may provide a mechanistic link between larval and adult preference. Gustatory receptors are less well characterized, but these genes may also have conserved function throughout development. There are 68 gustatory receptor genes (*Gr*) (Amrein and Thorne 2005). Little is known of their function, and expression levels are low, even in known taste neurons. A subset of these receptors is expressed in both larvae and adults and may mediate perception of compounds that are deterrent in both life stages.

The fact that certain chemicals are deterrent to larval and adult stages of certain swallowtail species (e.g., hydroxybenzoic acid and *P. xuthus* [Ono et al. 2004]) is suggestive of a “congruent sensory mechanism between the tarsal chemoreceptors of adults and the gustatory receptors of larvae” (Nishida 2005). Specific gustatory receptors expressed throughout the life cycle may thus represent a genetic link between nonpreference in butterflies and nonperformance in caterpillars, as it were. Similarly, certain compounds function as host-recognition cues in both life stages (e.g., aristolochic acids in troidines such as *Atrophaneura alcinous* [Nishida and Fukami 1989]), and specific receptors may also link preference and performance genetically.

Conclusions

With respect to the relationship between preference and performance in the evolution of host-plant specialization, whether *Papilio* is in fact representative of the 120,000 species of Lepidoptera (or, indeed, of just the 20,000 species of butterflies) is an open question. If in fact the genus is typical, then the expectation of a tight correlation between these two important aspects of host-plant utilization is perhaps unrealistic. Among the purported selective advantages of a holometabolous lifestyle is the opportunity to allow for more efficient exploitation of different environments, and with those environments come vastly different selective pressures. Nonetheless, the host plant is more than just a source of caterpillar food in the life of most butterflies. Over and above serving as a substrate for depositing eggs, it can be a rendezvous site for mating, and a source of chemical

protection for eggs and adults, among other things (Nishida 2002). Because of these commonalities, it is a reasonable expectation that certain sensory and physiological traits will be shared across life stages. Just how many are held in common is likely a function of the specific ecological association. As both preference and performance are more precisely defined at the genetic level, and as more Lepidoptera genomes become available, the specific linkages are likely to be uncovered, to shed new light on questions that have bedeviled investigators for the better part of a century.

Acknowledgments

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