Ecological Monographs, 76(3), 2006, pp. 399–414

CHEMICAL FACILITATION OF A NATURALLY OCCURRING HOST SHIFT BY *PAPILIO MACHAON* BUTTERFLIES (PAPILIONIDAE)

SHANNON M. MURPHY[1] AND PAUL FEENY

Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, New York 14853-2701 USA

Abstract. Host shifts by herbivorous insects have contributed substantially to current patterns of association between insects and plants. Despite their evolutionary and agricultural interest, however, the plant traits that predispose insects to colonize some plants instead of others are poorly understood. To examine whether ancestral and novel hosts share similar chemical oviposition stimulants that would facilitate a host shift, we investigated a well-substantiated host shift within the *Papilio machaon* group of swallowtail butterflies. *Papilio machaon aliaska* uses three plant species as host plants. *Cnidium cnidiifolium* belongs to the family Apiaceae, the ancestral host-plant family of the *P. machaon* group. *Artemisia arctica* and *Petasites frigidus*, by contrast, belong to the family Asteraceae and were colonized relatively recently by this group of butterflies. *Papilio machaon oregonius*, a close relative of *P. m. aliaska*, feeds and oviposits exclusively on *Artemisia dracunculus*, also in the Asteraceae. We made polar and nonpolar extracts of all four host plants, conducted bioassays with *P. m. aliaska* and *P. m. oregonius* females to test for oviposition stimulants, and found that the polar extracts were the most active. Using high-performance liquid chromatography, we separated the polar extracts into three fractions and again conducted bioassays with *P. m. aliaska* and *P. m. oregonius* females. The fraction containing hydroxycinnamic acid (HCA) derivatives was the only active fraction for all plant species. We further separated the HCA fraction and found two sub-fractions that were active in all of the host-plant species. Co-chromatography indicated that several major constituents of the active fractions are shared between ancestral and novel hosts. In a secondary series of experiments, we investigated chemical fractions of a non-host plant, *Artemisia frigida*, and identified fractions of the *A. frigida* extract that contained oviposition deterrents and a fraction that contained oviposition stimulants for *P. m. aliaska* females. We conclude that the similarity of stimulant profiles in ancestral and novel host plants is consistent with the hypothesis that plant chemistry has played a role in the establishment of this host shift.

Key words: deterrents; herbivory; host-plant chemistry; host-plant selection; host shift; hydroxycinnamic acids; Lepidoptera; oviposition stimulants; Papilio machaon aliaska; Papilio machaon oregonius; *swallowtail butterflies.*

INTRODUCTION

Host shifts by herbivorous insects have contributed substantially to current patterns of association between insects and plants by influencing the diversification and speciation of plant-feeding insects (e.g., Ehrlich and Raven 1964, Bush 1969). Many of our major agricultural pests have colonized crop plants by shifting from a native food-plant host to an abundant crop host. Yet, despite the fundamental implications for evolution and agriculture, the factors that predispose insects to colonize some plants instead of others are poorly understood. Although several remarkable host shifts are known and have been studied in detail (e.g., Thompson and Pellmyr 1991, Carroll and Boyd 1992, Farrell and Mitter 1993, Funk et al. 1995, Schoonhoven et al. 1998, Feder et al. 2003, Forister 2004), there has been recent interest in promoting empirical studies that investigate the ecology of speciation through host shifts, especially the mechanisms that may promote shifts by herbivorous insects (Funk et al. 2002).

Visual cues such as leaf shape (Rausher 1978, Papaj 1986) and olfactory responses to plant volatiles (Feeny et al. 1989, Baur et al. 1993) facilitate the discovery of suitable host plants by phytophagous insects in nature. Contact chemical cues, which are nonvolatile and found on the leaf surface, are perceived once the insect has alighted upon the plant; they strongly influence a female insect's final decision to accept or reject a plant for oviposition (Feeny et al. 1983, 1988, Ohsugi et al. 1985, Honda 1986, 1995, Nishida et al. 1987, Roessingh et al. 1991, Nishida 1995, Haribal and Feeny 1998). Indeed, Heinz and Feeny (2005) recently demonstrated that, for the eastern black swallowtail (*Papilio polyxenes*), a female's response to contact chemical cues is innate, not learned. Chemical similarities between plants often

Manuscript received 20 July 2005; revised 9 December 2005; accepted 20 December 2005. Corresponding Editor: A. R. Zangerl.

[1] Present address: Department of Entomology, 4112 Plant Sciences Building, University of Maryland, College Park, Maryland 20742-4454 USA. E-mail: smurph@umd.edu

have been suggested as a possible explanation for host shifts by insects (Fraenkel 1959, Ehrlich and Raven 1964, Jermy 1984, Renwick 2001), both by members of the Lepidoptera (Miller 1987, Feeny 1992) and Coleoptera (Futuyma and McCafferty 1990, Becerra 1997). The role of plant chemistry during a host shift by an insect, however, has not been tested rigorously by experiment.

Three hypotheses, not mutually incompatible, have been proposed to explain the role of plant chemistry during a host shift by a phytophagous insect species. The behavioral-facilitation hypothesis (Dethier 1941, Jermy 1976, 1984, Feeny 1991) predicts that host shifts are facilitated by the presence of chemical attractants or stimulants in the novel host plant that the insect already uses to recognize hosts. Similarly, host shifts may be abetted by a reduced sensitivity to deterrents that would normally cause a plant to be regarded as an unsuitable host species (Schoonhoven et al. 1998). This hypothesis predicts that host species should have similar chemical profiles that are used by females when deciding to oviposit (Feeny 1995). The metabolic-preadaptation hypothesis (Fraenkel 1959, Ehrlich and Raven 1964, Feeny 1991) states that while plant toxins are metabolic barriers to most insect colonizations, some insect groups have overcome these chemical constraints and are able to incorporate hosts with similar secondary compounds that are otherwise toxic to herbivorous insects. This hypothesis predicts that fewer compounds are responsible for host shifts and that these compounds are generally toxins. The third hypothesis, applicable to insects that derive defense or other benefits from secondary compounds in their host plants, proposes that host shifts may be facilitated by the presence of similar compounds in novel hosts (Ehrlich and Raven 1964, Feeny 1991). Although these hypotheses disagree about the types of chemicals that are most important to host shifts by insects (attractants and deterrents vs. toxins), they all predict that insects are more likely to colonize new hosts containing secondary compounds that are chemically related to the secondary compounds of the ancestral hosts.

To examine whether ancestral and novel hosts share similar chemical stimulants that would facilitate a host shift, we investigated a host shift within the *Papilio machaon* L. group of swallowtail butterflies. *P. machaon* is holarctic in distribution and is made up of a complex assemblage of geographic forms or subspecies (Sperling 1987). Several are recognized by some authors as distinct species (cf. Tyler 1975, Thompson 1995), but molecular evidence has revealed only trivial genetic differences between them (Sperling and Harrison 1994, Caterino and Sperling 1999). Plants of the Apiaceae are the primary larval hosts (Wiklund 1981, Feeny et al. 1983, Sperling 1987, Thompson 1995), although some populations occasionally use plants in the Rutaceae, an ancestral host family for the genus *Papilio* (Scriber 1973, Sperling 1987). In parts of the geographic range of *P. machaon*, however, and most notably in western North America, the larvae also feed on plants in the Asteraceae, a family rarely if ever included among the hosts of other swallowtail species (Scriber 1973). In Alaska and northwestern Canada, *Papilio machaon aliaska* Scud. oviposits and feeds not only on the local apiaceous host, *Cnidium cnidiifolium* (Turcz.) Schischk., but also on *Artemisia arctica* Less. and *Petasites frigidus* (L.) Franch. (Scott 1986), which are both in the Asteraceae. *C. cnidiifolium* is found at low elevations, often along river bluffs, and does not co-occur with *A. arctica*, which is found at higher elevations in tundra meadows. *P. frigidus* grows at both low and high elevations and can be found near both *C. cnidiifolium* and *A. arctica* plants. Enemy-free space evidently plays a significant role in the maintenance of this host-range expansion. In the absence of predators, *P. m. aliaska* larvae survive best on *C. cnidiifolium*, but in the presence of predators, larval survival is greater on the novel host plants (Murphy 2004, 2005).

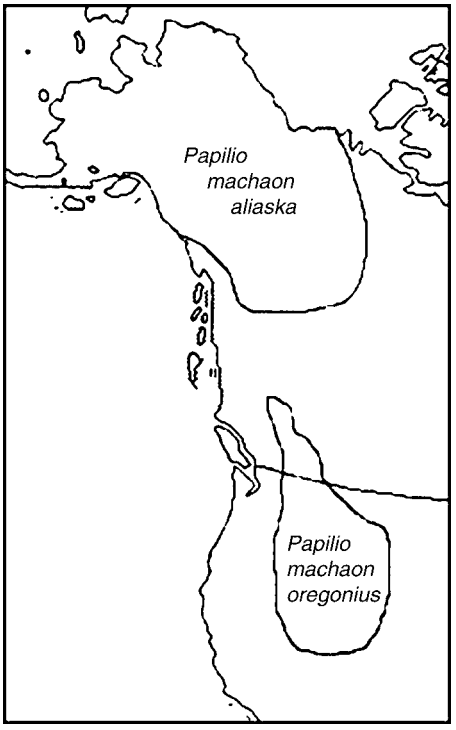

FIG. 1. Approximate distributions of *Papilio machaon aliaska* and *P. m. oregonius* (based on data from Sperling [1987]).

This host range expansion by *P. m. aliaska* appears to represent an intermediate step toward a complete host shift. Elsewhere in western North America, at least two members of the *P. machaon* group are now largely or entirely restricted to the novel host genus *Artemisia*. One of these is *Papilio machaon oregonius* Edwards (also frequently referred to as *P. oregonius*), a close relative of *P. m. aliaska* that occurs in the Great Basin (Fig. 1) and that has shifted completely to feeding on *Artemisia dracunculus* L. (Sperling 1987, Thompson 1988). It is

unclear, however, whether *P. m. aliaska* and *P. m. oregonius* represent a single host shift or two independent host shifts to *Artemisia*. Such shifts may even have happened several times across the range of *P. machaon*.

Many host shifts by phytophagous insects are phylogenetic host shifts between plant species that are closely related, usually within the same family. Recently diverged species are likely to share plant traits, such as chemistry (Wink et al. 1995, Wink 2003), that are attractive to ovipositing females. Thus, the host shift within the *P. machaon* species group from the Apiaceae to the Asteraceae is intriguing. Although both families are included in the Euasterid II clade (Soltis and Soltis 2004), the ancestral and novel host genera do not share recent common ancestry. We hypothesize that the *P. m. aliaska* host plants *C. cnidiifolium*, *A. arctica*, and *P. frigidus*, as well as the *P. m. oregonius* host plant *A. dracunculus*, contain similar chemical stimulants that *P. m. aliaska* and *P. m. oregonius* females use as oviposition cues. Here we describe experiments in which we investigated whether these oviposition cues are volatile or nonvolatile compounds, and we describe a series of bioassays that we performed to isolate the active chemical compounds while controlling for visual cues. In addition to testing chemical extracts from host plants, we also tested chemical extracts from a non-host plant to try to determine why some plants in the Asteraceae are acceptable hosts for ovipositing *P. m. aliaska* females whereas other locally abundant species are not. We selected *Artemisia frigida* Willd. because it is a congener of *A. arctica* and *A. dracunculus* and is frequently found in the field growing next to *C. cnidiifolium* plants. Thus, if *P. m. aliaska* colonized novel hosts that were either closely related to a current host (phylogenetic host shift) or found in abundance near current hosts (ecological-opportunity host shift), then *A. frigida* might be predicted to be a potential host plant. We hypothesized that *A. frigida* is not an acceptable host plant because it either (1) does not contain the active oviposition stimulants found in the other host plants or (2) does contain the active oviposition stimulants, but also contains one or more deterrent compound(s) that negate the activity of the stimulants. We performed a series of bioassays designed to address these hypotheses. In sum, the primary goal of our research was to examine whether ancestral and novel hosts share similar chemical oviposition stimulants that would facilitate a host shift. Our secondary goal was to investigate why non-host plants, such as *A. frigida*, are not acceptable to ovipositing females.

Materials and Methods

Origins of the study populations

Papilio machaon aliaska butterflies are univoltine. Field observations over the past five years indicate that *P. m. aliaska* is a typical hill-topping swallowtail butterfly (cf. Lederhouse 1982). Males emerge a few days earlier in the season than do females. Upon emergence, males fly to hilltops where they defend territories and wait for females to arrive. After mating, females fly downhill toward larval host-plant sites. Populations from the different larval host-plant sites surrounding a hilltop are apparently panmictic and appear to be from a single population (S. M. Murphy, *unpublished manuscript*). Due to the scale of the landscape in the interior of Alaska, it is rarely possible to collect *P. m. aliaska* individuals other than from hilltops.

P. m. aliaska females were collected at five sites in Alaska. Four sites were alpine-tundra hilltops (domes) near Fairbanks, Alaska, USA (see Plate 1): Ester Dome (site 1, 64°52′ N, 148°4′ W, ~720 m a.s.l.), Murphy Dome (site 2, 64°57′ N, 148°21′ W, ~890 m), Wickersham Dome (site 3, 65°13′ N, 148°3′ W, ~977 m), and along the Pinnel Mountain trail southwest of Table Mountain (site 4, 65°25′ N, 145°57′ W, ~1200 m). The fifth site was located along the Yukon River, on the airstrip in Eagle, Alaska (site 5, 64°47′ N, 141°13′ W, ~1000 m). The airstrip site contains high densities of the larval host-plant *Cnidium cnidiifolium* and is used by ovipositing females. The *P. m. aliaska* females in our experiments were the offspring of females that were caught in June 2001 (site 1, $n = 3$ females; site 3, $n = 1$; site 4, $n = 1$), June 2002 (site 2, $n = 2$; site 5, $n = 1$), and June 2003 (site 1, $n = 4$; site 2, $n = 1$; site 3, $n = 5$). The *P. m. aliaska* females from dome sites (1–4) were caught as they arrived at the summit and therefore probably had not already mated. To ensure viable offspring, these females were mated by hand-pairing (Carter and Feeny 1985) in the laboratory with males from the same field site. The female from site 5 had already mated in the field. In the laboratory, females were allowed to oviposit on sprigs of *C. cnidiifolium*, *Artemisia arctica*, and *Petasites frigidus*; their larvae were reared in equal numbers on the three host plants under ambient conditions (24 h light, ~17°C). After the larvae had pupated, they were taken to Cornell University, Ithaca, New York, USA, and were refrigerated (24 h dark, 5°C) for 8–13 months. The following spring and summer (in 2002, 2003, and 2004), the pupae were brought out of the refrigerator in batches and were allowed to emerge in an environmental growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio, USA) set at diapause-breaking conditions (16 h light, 21°C/8 h dark, 15°C) without any plant material. Upon emergence, males and females were numbered according to their site of origin and were fed as described in Carter and Feeny (1985). Females were hand-paired with unrelated males within two days of emerging, and were subsequently tested in bioassays.

P. m. oregonius butterflies are bivoltine. The first brood generally emerges from April to early June and the second brood from late July to early August, but eclosion is not always synchronous and adults can be found any time from April to September (D. McCorkle, *personal communication*). Males and females are com-

PLATE 1. Two domes along the Pinnel Mountain trail near Fairbanks, Alaska, USA. Photo credit: S. Murphy.

mon in dry riverbeds and along the lower slopes of riverbanks. The eight *P. m. oregonius* females used in our experiments were collected either as larvae or as adults from two sites along the Columbia River in Oregon, USA: Philippi Canyon (site 1, 45°40′ N, 120°30′ W) and The Dalles (site 2, 45°36′ N, 121°8′ W). Six of the females were collected as larvae in July 2001 from site 1 ($n = 2$ females) and in August 2003 from site 2 ($n = 4$ females). The larvae were shipped to Ithaca, New York, where they were reared on fennel (*Foeniculum vulgare*) under diapause-inducing conditions (10 h light, 21°C/14 h dark, 15°C); after pupation, they were kept in a cold room (24 h dark, 5°C) for 8–11 months. The following spring and summer (2002 and 2004, respectively), the pupae were allowed to emerge in an environmental growth chamber set at diapause-breaking conditions (16 h light, 27°C/8 h dark, 15°C) without any plant material. Upon emergence, males and females were fed and numbered as described in Carter and Feeny (1985). Females were hand-paired with unrelated males within two days of emerging, and were subsequently tested in bioassays. The other two females were collected from site 1 in August, 2004; one as an adult and the other as a late-instar larva. Both were shipped to Ithaca, New York, where the adult was immediately tested in bioassays. The larva completed its development on fennel under conditions that would not induce diapause (16 h light, 27°C/8 h dark, 15°C); when she emerged from pupation in September, 2004, she was hand-paired with an unrelated male that was collected as a larva from site 2. The female was then tested in bioassays.

Preparation of chemical extracts

Chemical extracts for all host-plant species (*C. cnidiifolium*, *A. arctica, P. frigidus,* and *A. dracunculus*), and for the non-host *A. frigida*, were made using the same technique. Foliage was taken fresh from the field in the vicinity of Fairbanks, Alaska for the four Alaskan plant species, whereas *A. dracunculus* was collected in the vicinity of Pullman, Washington, USA. Foliage from 30–100 plants was collected and then immediately transported, in coolers containing crushed ice, to the laboratory of T. Clausen in Alaska (Chemistry Department, University of Alaska, Fairbanks) or the laboratory of J. Thompson in Washington (Departments of Botany and Zoology, Washington State University). Leaves, separated from stems, were weighed and then evenly divided into two batches. The first batch of leaves, 100 g fresh mass at a time, was soaked in 200 mL methylene chloride (CH_2Cl_2) for 1.5 h and the extract was filtered. The solvent (CH_2Cl_2) extracts nonpolar compounds from the leaves, including the more volatile

compounds that butterflies may detect as they approach the host plant (cf. Feeny et al. 1989, Baur et al. 1993). The extract was concentrated by rotary evaporation in vacuo at <40°C to 1 gle/mL. One gram leaf equivalent (gle) is the amount of material extracted from 1 g fresh mass of leaves. The second batch of leaves, 100 g fresh mass at a time, was soaked in boiling deionized water for 10 min and the extract was filtered. Water, a polar solvent, extracts nonvolatile compounds from the leaves; these include contact stimulants that a female perceives once she has alighted upon the host plant. After filtration, we evaporated the extract to dryness in vacuo at <40°C. At this point, both nonpolar and polar extracts were transported to Ithaca, New York, USA and were stored frozen (−15°C) until they were used in bioassays or fractionated. New extracts were made each summer during the field season, so any effect of year was tested in the statistical analyses for each bioassay.

Polar extracts were separated into fractions by high-performance liquid chromatography (HPLC) using a Hewlett Packard 1100 Series system (Hewlett Packard, Palo Alto, California, USA). Peaks were identified on the basis of UV absorbance spectra, determined by a diode-array detector with monitoring at 254 nm. The polar extracts were first separated into three fractions (F1, F2, F3) on a reverse-phase C-18 column (Phenomenex IB-sil. 10 × 250 mm 5μ) using HPLC-grade water (H_2O), methanol (MeOH), and a solution of 1% acetic acid in water (HOAc) as solvents. The column was eluted at a flow rate of 1.75 mL/min using a stepped gradient: H_2O (initial, 5 min), H_2O-MeOH (60:40, 10 min), H_2O-MeOH (40:60, 20 min), HOAc-MeOH (50:50, 5 min), MeOH (10 min), to H_2O-MeOH (10:90, 15 min). F1 was collected from 0 to 23 min, F2 from 23 to 38 min, and F3 from 38 to 65 min (Appendix F). The F3 fraction was subsequently separated into three fractions (F3a, F3b, F3c) using the same column, but was eluted at a flow rate of 3.0 mL/min using the following stepped gradient: HOAc (initial, 15 min), HOAc-MeOH (80:20, 15 min), HOAc-MeOH (40:60, 10 min), MeOH (10 min), to HOAc (10 min). F3a was collected from 0 to 28 min, F3b from 28 to 35 min, and F3c from 35 to 60 min (Fig. 2). These HPLC gradient programs were developed by M. Haribal (*personal communication*) for efficient separation of flavonoids from hydroxycinnamic acid (HCA) derivatives. Many of the contact stimulants identified as oviposition stimulants for other swallowtail butterfly species belong to one of these two compound classes (Ohsugi et al. 1985, Honda 1986, 1990, Nishida et al. 1987, Feeny et al. 1988, Nishida 1995, Carter et al. 1998, 1999, Haribal and Feeny 1998, Haribal et al. 1998, Ono et al. 2000). For bioassay, aliquots of the parent polar extract, primary fractions (F1, F2, F3), and secondary fractions (F3a, F3b, F3c) were adjusted in volume by rotary evaporation in vacuo at <40°C or by serial dilution to a range of concentrations expressed as gram leaf equivalent per milliliter (gle/mL).

Bioassays

All bioassay trials were conducted in greenhouse facilities at Cornell University, Ithaca, New York, USA. Because females were reluctant to fly in the absence of direct sunlight, all trials were conducted between the hours of 0930 and 1600 on sunny days. The greenhouse was not air-conditioned and ranged in temperature from 18° to 32°C during the trials. Females were fed at least twice each day with a water solution containing 10% honey by volume. Additionally, females were allowed to feed immediately before and after each trial to eliminate any effects of hunger during the experiment. Females were placed in wood-framed cages (0.5 m^3) covered with a fine white mesh (9 holes/cm). Each cage contained a model plant (36 cm in height) made of a wooden "trunk," wire "stems," and sponge "leaves" (5.0 × 3.5 cm triangles, 0.75 cm deep) colored green with a food-coloring solution (McCormick) and glued to the end of each branch (Feeny et al. 1989). The experimental leaves were attached randomly to each position before each trial. In our experiments, we used two bioassay designs. Bioassay design 1 was used in situations when four leaves, either experimental or control, were required. Trials lasted 1 h and the model plants were rotated counterclockwise every 15 min to control for corner biases. Bioassay design 2 was used in situations when three leaves were required. Trials lasted 45 min and models were rotated counterclockwise every 15 min. For all bioassays, females were allowed to fly about and oviposit freely on the sponges during the trial period. The number of times each female landed on each sponge and the number of eggs that she laid on each sponge were recorded. The methods for these bioassays control all visual plant cues; thus differences in female response are determined primarily by differences in preference for the extracts.

Host plants.—To investigate whether the host plants *C. cnidiifolium*, *A. arctica*, and *P. frigidus* contain similar chemical stimulants that *P. m. aliaska* females use as oviposition cues, we conducted a series of bioassays designed to isolate the active chemical compounds. We conducted an identical series of bioassays with extracts of *A. dracunculus*, the *P. m. oregonius* host plant, to determine whether the same chemical fractions as in the *P. m. aliaska* host plants are active.

Our first series of bioassays was designed to compare the stimulant activity of polar and nonpolar extracts of each host-plant species. Using bioassay design 1, we punched a small hole in the center of each sponge leaf and hung a 2-mL cylindrical, open-top vial from the bottom of the leaf so that the rim was even with the upper surface of the leaf. Two of the vials contained 1 gle of the nonpolar host-plant extract while the other two vials contained an equivalent amount of CH_2Cl_2 (1 mL) as a solvent control. Either polar extract (1 gle in 2 mL 50:50 MeOH-HPLC grade H_2O solvent) or an equivalent amount of solvent control was applied to the leaf surfaces. Thus, of the four leaves in the bioassay,

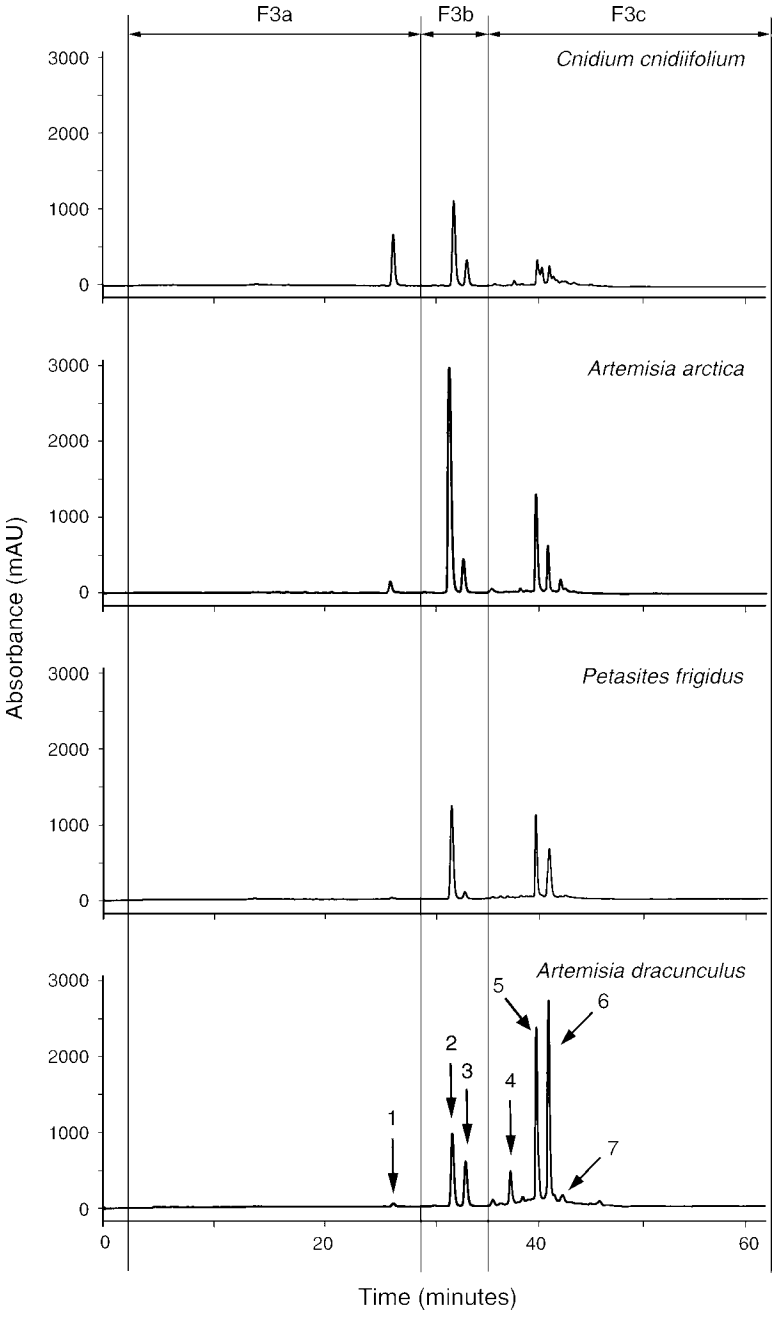

FIG. 2. HPLC chromatograms (monitored at 254 nm) for the F3a, F3b, and F3c fractions of the *Papilio machaon aliaska* ancestral host plant (*Cnidium cnidiifolium*), the *P. m. aliaska* novel host plants (*Artemisia arctica* and *Petasites frigidus*), and the *P. m. oregonius* host (*A. dracunculus*). Equivalent amounts of each host plant were injected for each analysis; peak height represents the amount of compound(s) present in 0.1 gle (gram leaf equivalent) of host-plant extract (mAu is milliabsorbance unit). Numbers 1–7 refer to peaks, not necessarily to individual compounds.

one was treated with polar extract and nonpolar extract (both nonvolatile and volatile plant cues), one with polar extract and nonpolar solvent (nonvolatile plant cues only), one with polar solvent and nonpolar extract (volatile plant cues only), and the last with polar solvent and nonpolar solvent (solvent controls only, no plant cues). This factorial design allowed us to test both polar and nonpolar extracts individually as well as to test for any interactions between them. We tested the responses of *P. m. aliaska* females to polar and nonpolar extracts of *C. cnidiifolium* ($n = 18$ butterflies), *A. arctica* ($n = 20$), and *P. frigidus* ($n = 14$), as well as to extracts of the *P. m.*

oregonius host plant, *A. dracunculus* ($n = 8$). We also tested the responses of *P. m. oregonius* females to polar and nonpolar extracts of *A. dracunculus* ($n = 6$).

All subsequent bioassays were done using only polar fractions and thus did not require the use of the vials. Dose–response curves were constructed to determine what concentration of test extract was required for females to respond. Based on this information, all bioassays of polar fractions were conducted with 0.25 gle extract, which was applied evenly over the sponge by diluting it in 2 mL solvent (50:50 MeOH-HPLC grade H_2O). Control sponges simply had 2 mL solvent (50:50 MeOH-HPLC grade H_2O) applied to them.

After separating the active polar extracts into three fractions (F1, F2, F3; Appendix F), we tested these fractions using bioassay design 1 to determine which fraction contained the stimulant activity of each parent polar extract. The HPLC gradient program that we used to separate the polar extracts produces a F1 fraction containing low-molecular-weight polar compounds such as sugars, salts, and bases (Haribal and Feeny 1998). Based on analyses of UV absorbance spectra, we determined that the F2 fraction consisted largely of flavonoids and the F3 fraction largely of HCA derivatives. We tested the responses of *P. m. aliaska* females to the F1, F2, and F3 fractions of *C. cnidiifolium* ($n = 20$), *A. arctica* ($n = 13$), *P. frigidus* ($n = 18$), and *A. dracunculus* ($n = 4$). Similarly, we tested the responses of *P. m. oregonius* females to the F1, F2, and F3 fractions of *A. dracunculus* ($n = 4$).

We tested the F3 fractions against the corresponding parent polar extracts to determine, in each case, whether the F3 fraction accounted for all of the stimulant activity of the parent extract. If the parent extract proved to be significantly preferred over the F3 fraction, we would know that the F3 fraction required the addition of a compound(s) from either the F1 or F2 fraction to achieve the same level of activity as the parent extract. Synergism between chemical compounds that are either inactive or only slightly active alone, but highly active when combined, has been demonstrated in other swallowtail butterfly species (Ohsugi et al. 1985, Honda 1986, 1990, Nishida et al. 1987, 1990*a*, Feeny et al. 1988, Sachdev-Gupta et al. 1993, Carter et al. 1998, 1999, Nakayama et al. 2003). Using bioassay design 2, we conducted this bioassay with the parent polar extract and F3 fraction for the three *P. m. aliaska* host plants: *C. cnidiifolium* ($n = 16$), *A. arctica* ($n = 15$), and *P. frigidus* ($n = 12$).

Lastly, we separated the active F3 fractions into three sub-fractions: F3a, F3b, and F3c (Fig. 2). Using bioassay design 1, we tested the responses of *P. m. aliaska* females to the F3a, F3b, and F3c fractions of their host plants, *C. cnidiifolium* ($n = 12$), *A. arctica* ($n = 10$), and *P. frigidus* ($n = 6$), to determine which of the fractions contained stimulant activity. We also tested the responses of *P. m. oregonius* females to the F3a, F3b, and F3c fractions of *A. dracunculus* ($n = 2$).

Non-host plant Artemisia frigida.—We conducted bioassays to determine if a non-host plant (*A. frigida*) shares similar contact stimulants as the host plants tested, or whether it contains oviposition deterrents. Using bioassay design 1 (two control leaves and two extract leaves; $n = 7$), we first tested whether *P. m. aliaska* females would lay eggs on a polar extract of *A. frigida*. With this bioassay design, however, we were unable to distinguish whether the extract actually deterred females from ovipositing, or if the females were simply not motivated to oviposit selectively during the trials. For the next bioassay, we tested *P. m. aliaska* females ($n = 15$) using bioassay design 2 with three leaves: (1) a leaf with 0.25 gle of the polar *C. cnidiifolium* extract, which would indicate if females were motivated to oviposit as it is a highly preferred extract; (2) a leaf with 0.25 gle each of *C. cnidiifolium* extract and *A. frigida* extract to test if *A. frigida* extract contains chemical deterrents that would decrease the females' acceptance of *C. cnidiifolium* extract; and (3) a leaf with a solvent control.

We tested fractions of the polar *A. frigida* extract using bioassay design 1 to determine whether any of the fractions (F1, F2, F3) contained oviposition stimulants ($n = 5$). Finally, we conducted a bioassay using design 1 to determine if either the F1 or F2 fractions contained oviposition deterrents ($n = 13$). There were four leaves in this bioassay: (1) a leaf with 0.25 gle of the polar *C. cnidiifolium* extract; (2) a leaf with 0.25 gle each of *C. cnidiifolium* extract and the F1 fraction of the *A. frigida* extract; (3) a leaf with 0.25 gle each of *C. cnidiifolium* extract and the F2 fraction of the *A. frigida* extract; and (4) a leaf with a solvent control.

Statistical analyses

Only females that landed at least 10 times and laid at least five eggs during the course of a trial were considered in the statistical analyses. Additionally, females were tested for corner and period biases using the chi-square goodness-of-fit test (Siegel and Castellan 1988). A female with a corner bias spent most of the trial in one corner of the cage, whereas a female with a period bias only flew during one or two of the 15-minute periods. Females with a corner bias but that flew during all four periods, or females with a period bias but that flew in all four corners, were exposed to all test extracts. However, any females that exhibited both a corner and a period bias were thus not exposed to all test extracts and were discarded from the data set. Counts ranged from 5 to 51 eggs (12.1 ± 0.5 eggs/sponge, mean ± SE).

For all bioassays but one, preferences for test extracts were analyzed using PROC MIXED in SAS Version 8 (SAS Institute 1999). Egg counts were log-transformed to meet with assumptions of normality and equal variance. Treatment (plant extract or fraction), host plant, year, and Julian day (day 1 is 1 March) on which the trial was conducted were fixed effects. Butterfly and site of origin of the butterfly's dam were random factors.

By including butterfly as a random variable, this model accounts for the potential nonindependence of oviposition events when females land on any one of the sponge leaves. Using likelihood ratio tests (Neter et al. 1996), we did not find random factors to account for a significant amount of the variation in any of the models, unless noted. All of these bioassays were also analyzed with the Friedman two-way ANOVA by ranks test (Siegel and Castellan 1988). Both parametric and nonparametric statistical methods agreed in rejection or acceptance of the null hypotheses. The results are given for the analyses using PROC MIXED because they allowed us to test and control for variation in year (new extracts were made each summer), Julian day (bioassays were performed from April through September), and dam (the females were the offspring of several dams); unless otherwise noted, none of these factors had a significant effect in the model. The *P. m. oregonius* bioassay of the primary fractions (F1, F2, F3) of *A. dracunculus* had an extremely small sample size ($n = 4$), so preferences for test extracts were instead analyzed only with the Friedman two-way ANOVA by ranks.

Co-chromatography

Limited time and material did not allow us to isolate and purify the individual components of the F3 extracts for bioassay and chemical identification. Their UV spectra and chromatographic behavior indicate that several, if not all, of the peaks in the F3 fractions are hydroxycinnamic acid derivatives (Feeny et al. 1988, Haribal and Feeny 1998). The baseline separations of these peaks (cf. Fig. 2) and previous experience with equivalent fractions in other plants (Feeny et al. 1988, Haribal and Feeny 1998) suggest that most of the peaks are likely to represent individual compounds. Moreover, the peaks corresponding to caffeoylquinic acid isomers previously identified as oviposition stimulants for *P. polyxenes* (Feeny et al. 1988) and *Eurytides marcellus* (Haribal and Feeny 1998) were among the most prominent in HPLC traces of their respective active fractions.

Hydroxycinnamic acids, such as caffeic, ferulic, and *p*-coumaric acids, are widespread and perhaps universal constituents of higher plants (Sondheimer 1964), but the UV spectra and mass fragmentation patterns of many of their derivatives, especially the various isomers of caffeoylquinic acid, are so similar to one another that their identification would require a series of NMR (nuclear magnetic resonance) experiments (e.g., Haribal et al. 1998) or, at the least, HPLC/MS (mass spectrometry) comparisons with authentic standards. Only one such compound, chlorogenic acid (5-caffeoylquinic acid), was available commercially. Chlorogenic acid, one of the most widespread of the cinnamoylquinic acids (Robinson 1991), is an ester of caffeic acid and quinic acid. Quinic acid has several hydroxyl groups capable of esterification, and each hydroxyl, whether or not esterified, can have an equatorial or axial configuration. Additionally, because the double bond in the caffeoyl group can be either *cis* or *trans*, there are theoretically several dozen possible isomers of this compound, and only a few of their detailed stereochemical structures have been established (Haribal et al. 1998). Plants differ in their profiles of cinnamoylquinic acids (Möller and Herrmann 1983), and butterflies can be quite specific in their responses to particular isomers (Haribal and Feeny 1998).

Sufficient quantities of material remained after the last series of bioassays, however, for co-chromatography to determine which HPLC peaks were common to two or more of the plant extracts (cf. Konczak et al. 2004). For each pairwise combination of host species (*C. cnidiifolium*, *A. arctica*, *P. frigidus*, and *A. dracunculus*), we added equal amounts of their F3 fractions to create six solutions for the co-chromatographic analyses. For each analysis we injected the equivalent of 0.2 gle (in 100 μL) of plant material: 0.1 gle (in 50 μL) of species A and 0.1 gle (in 50 μL) of species B. We then compared the peak heights of these HPLC chromatograms to the "parent" peak heights in the HPLC chromatograms that were created when each species was run alone (0.1 gle in 50 μL; Fig. 2). If the same compound is found in both species, then the height of the peak in the combined extract should equal the sum of the parent peak heights in species A and species B. We devised a Similarity Index (SI) with which to compare the expected and observed peak heights for each combination of extracts. This index expresses the observed increase in height of the larger parent peak as a percentage of the total increase expected if the two parent peaks have identical retention times. It was calculated for each pairwise comparison as 100 × (observed combined peak height − taller parent peak height)/(shorter parent peak height). Even for peaks with identical retention times, we would rarely expect an SI value of 100%, primarily because of experimental errors associated with handling samples of such small volume. We chose a value of 70% as the critical value for the SI: above this value, we do not reject the hypothesis that the retention times of the peak in each of a pair of extracts are identical. We excluded from analysis any pairwise combinations in which the shorter of the two parent peak heights failed to exceed 30% of the taller parent peak. A skewed ratio of peak heights creates the problem that the expected increase after combination may become small enough to approach the range of error in estimating the combined peak height.

The UV absorbance spectrum and retention time of peak 2 indicated that it could be *trans*-chlorogenic acid, known to be one of the oviposition stimulants for *Papilio polyxenes* (Feeny et al. 1988). We made a solution of chlorogenic acid (Sigma) in methanol (0.1 g in 10 mL) and diluted it until a 50-μL injection resulted in a peak height (1343 mAU) comparable to the height of peak 2 in the various host plants (~1000 mAU). We then added the chlorogenic acid solution (50 μL) to each of the host plants' F3 fraction (0.1 gle in 50 μL) and

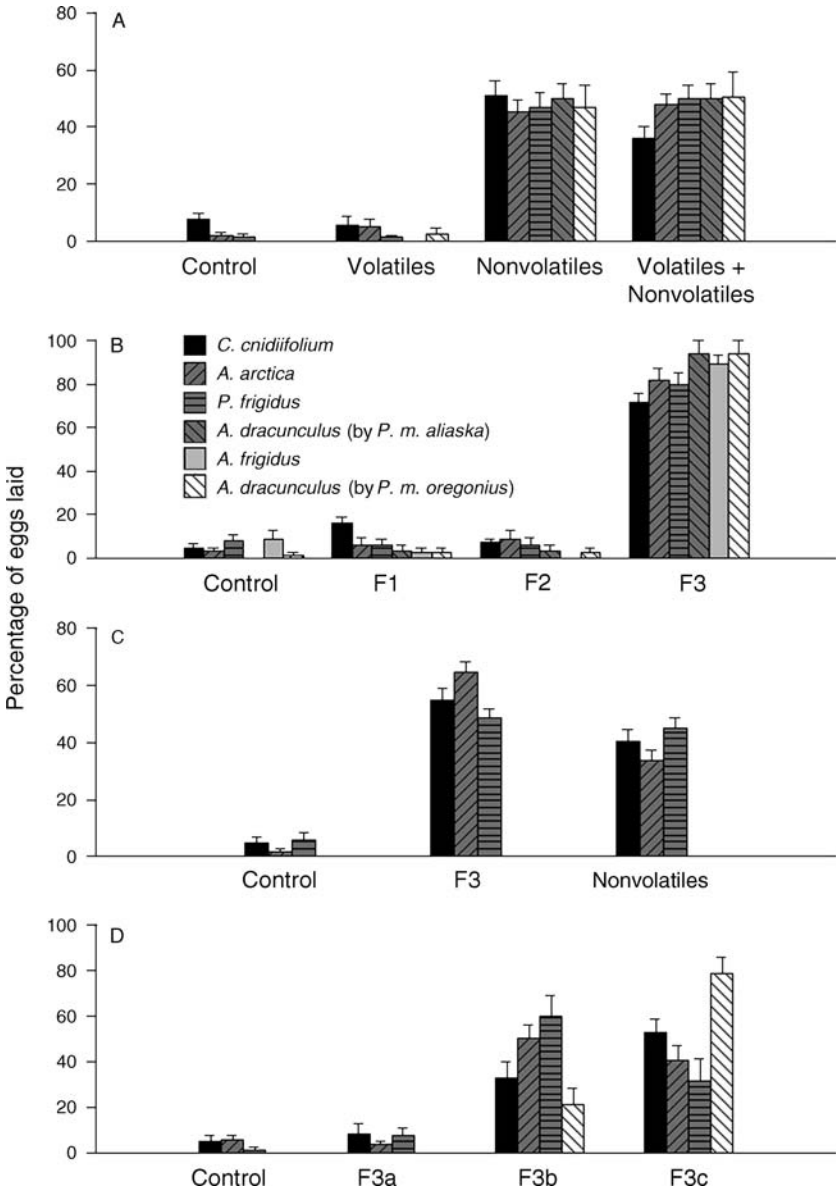

FIG. 3. Percentage of eggs (mean + SE) laid by butterflies (n is the number of female butterflies) on plant extracts of different species tested in bioassays. (A) Percentage of *P. m. aliaska* eggs on nonvolatile (polar) and volatile (nonpolar) extracts of their host plants: *Cnidium cnidiifolium* ($n = 18$), *Artemisia arctica* ($n = 20$), and *Petasites frigidus* ($n = 14$), as well as on the *P. m. oregonius* host *A. dracunculus* ($n = 8$); and the percentage of *P. m. oregonius* eggs on *A. dracunculus* extracts ($n = 6$). (B) Percentage of *P. m. aliaska* eggs on fractions F1, F2, and F3 of *C. cnidiifolium* ($n = 20$), *A. arctica* ($n = 13$), *P. frigidus* ($n = 18$), *A. dracunculus* ($n = 4$), and the non-host plant *A. frigida* ($n = 5$), as well as the percentage of *P. m. oregonius* eggs on *A. dracunculus* fractions ($n = 4$). (C) Percentage of *P. m. aliaska* eggs on the nonvolatile (polar) extract and the F3 fraction of *C. cnidiifolium* ($n = 16$), *A. arctica* ($n = 15$), and *P. frigidus* ($n = 12$). (D) Percentage of *P. m. aliaska* eggs on fractions F3a, F3b, and F3c of *C. cnidiifolium* ($n = 12$), *A. arctica* ($n = 10$), and *P. frigidus* ($n = 6$), as well as the percentage of *P. m. oregonius* eggs on *A. dracunculus* fractions ($n = 2$).

analyzed the HPLC chromatograms. For each combination, we calculated SI values as previously described.

RESULTS

Host plants

Polar vs. nonpolar.—We found that *Papilio machaon aliaska* females preferred polar extracts (nonvolatile plant cues) to nonpolar extracts (volatile plant cues) for all three of their host plants, *Cnidium cnidiifolium*, *Artemesia arctica*, and *Petasites frigidus*, as well as for the *P. m. oregonius* host plant *A. dracunculus* (Appendix A and Fig. 3A). The nonpolar extract of *C. cnidiifolium* was the only nonpolar extract to differ significantly from the solvent control ($F_{1,60} = 4.26$, $P = 0.04$); the solvent control was preferred over the nonpolar extract, but the difference was minimal compared to the difference in

preference between the polar solvent and polar extract (Fig. 3A). *P. m. oregonius* females also preferred polar to nonpolar extracts of their host, *A. dracunculus* (Appendix A and Fig. 3A).

F1 vs. F2 vs. F3 vs. control.—*P. m. aliaska* females laid significantly more eggs on the F3 fraction than on the F1 fraction, F2 fraction, or the control in bioassays of their ancestral host *C. cnidiifolium* ($F_{3,57} = 27.01$, $P < 0.0001$), as well as in bioassays of both of their novel host plants, *A. arctica* ($F_{3,36} = 24.88$, $P < 0.0001$) and *P. frigidus* ($F_{3,48} = 22.37$, $P < 0.0001$) (Appendix B and Fig. 3B). *P. m. aliaska* females also preferred the F3 fraction of *A. dracunculus*, the host plant of *P. m. oregonius* ($F_{3,9} = 20.38$, $P = 0.0002$; Appendix B and Fig. 3B). The F1 and F2 fractions did not differ significantly from the control for any of these host-plant species except for the *C. cnidiifolium* F1 fraction, which was preferred over the control but did not differ from the F2 fraction (Appendix B). The *C. cnidiifolium* extract was the first to be fractioned by HPLC and there were some initial difficulties in purifying the F1 fraction; this fraction is easily contaminated with stimulatory F3 compounds not completely eluted in the previous sample run. The three fractions of *A. dracunculus* also differed significantly in their acceptability to *P. m. oregonius* females (Friedman test, $F_r = 11.00$, df = 3, $P < 0.02$; Fig. 3B). The F3 fraction was the only fraction that was preferred over the control ($P < 0.05$). Thus, for all three of the *P. m. aliaska* host plants, as well as the *P. m. oregonius* host plant, the F3 fraction is the only polar fraction to elicit oviposition by *P. m. aliaska* females. Similarly, *P. m. oregonius* females only respond to the F3 fraction of their host plant, *A. dracunculus*.

F3 vs. polar vs. control.—We found that there was a significant difference between the parent, F3, and control treatments, with more eggs laid on both the parent extract and F3 fraction than on the control ($F_{2,84} = 156.39$, $P < 0.0001$; Appendix C and Fig. 3C). The parent extract and the F3 fraction did not differ in preference for either the *C. cnidiifolium* or *P. frigidus* host plants (Appendix C). Although both the F3 and the parent extract were preferred over the solvent control for the host plant *A. arctica*, the F3 was also preferred over the parent extract (Appendix C). Thus, we conclude that the F3 fraction accounts for the majority, if not all, of the activity of the parent extract. It does not appear that the activity of the F3 fraction would be enhanced significantly by synergism with compounds in the F1 or F2 fractions.

F3a vs. F3b vs. F3c vs. control.—We found a significant difference between the F3a, F3b, F3c, and control treatments; *P. m. aliaska* females preferred fractions F3b and F3c to the control ($F_{3,94} = 47.11$, $P < 0.0001$; Appendix D and Fig. 3D). There was no significant difference in the numbers of eggs laid on either the F3b or F3c fractions for any of the host plants (Appendix D). There was an effect of Julian day ($F_{11,94} = 2.08$, $P = 0.03$). Julian day was not a factor for either the *A. arctica* ($F_{4,32} = 2.18$, $P = 0.09$) or *P. frigidus* ($F_{3,2} = 0.28$, $P = 0.84$) bioassays, but only for the *C. cnidiifolium* bioassay ($F_{5,38} = 2.78$, $P = 0.03$). The effect can be attributed to a single butterfly; she was the only female to lay more than one egg on the control sponge, but she still preferred both the F3b and F3c fractions. If she is discarded from the model, then Julian day no longer has an effect ($F_{11,92} = 1.58$, $P = 0.12$).

Our sample size was not large enough to statistically test *P. m. oregonius* preferences for *A. dracunculus* F3a, F3b, and F3c fractions, as there were only two butterflies in this bioassay. Both females, however, laid eggs only on fractions F3b and F3c, ignoring the F3a fraction and the control (Fig. 3D). Thus, despite our limited sample size, these results agree with findings for the *P. m. aliaska* host plants.

Non-host plant Artemisia frigida

A. frigida (Af) *vs. control.*—We found no difference in the number of eggs laid ($F_{1,6} = 0.75$, $P = 0.42$) or the number of times that females landed ($F_{1,6} = 4.16$, $P = 0.09$) on either the *A. frigida* or the solvent control sponges. There was a significant effect of butterfly ($\chi^2 = 13.1$, $P \leq 0.001$). Five of the seven females refused to lay any eggs on either the *A. frigida* extract or the solvent control, while the other two laid equal numbers of eggs on both the non-host plant extract and the control, indicating that they were not selective about where they laid their eggs.

C. cnidiifolium (Cc) *vs.* Cc+Af *vs. control.*—We found a significant effect of treatment ($F_{2,28} = 45.71$, $P < 0.0001$). The *C. cnidiifolium* extract as well as the *C. cnidiifolium* extract combined with *A. frigida* extract were both preferred over the control (Appendix E). Furthermore, the *C. cnidiifolium* extract was also preferred over the *C. cnidiifolium* extract combined with *A. frigida* extract (Appendix E). Thus, combining *A. frigida* extract with the *C. cnidiifolium* extract significantly decreased the acceptability of the *C. cnidiifolium* extract to ovipositing *P. m. aliaska* females.

F1 vs. F2 vs. F3 vs. control.—After establishing that *A. frigida* extracts contain chemical deterrents, we tested whether any of the polar *A. frigida* fractions contained oviposition stimulants. *P. m. aliaska* females laid significantly more eggs on the F3 fraction of *A. frigida* than on the F1 fraction, F2 fraction, or the control ($F_{3,12} = 20.34$, $P < 0.0001$; Appendix B and Fig. 3B). The F1 and F2 fractions differed neither from the control nor from each other (Appendix B).

Cc *vs.* Cc+Af*F1 vs.* Cc+Af*F2 vs. control.*—Having determined that the F1 and F2 fractions did not contain oviposition stimulants, we next tested whether either of these fractions contained the chemical cues responsible for deterring females from ovipositing on *A. frigida*. There was a significant effect of treatment ($F_{3,36} = 36.29$, $P < 0.0001$) in this bioassay. The *C. cnidiifolium* extract was highly preferred over any other extract or the control (Appendix E). The *C. cnidiifolium* extract with

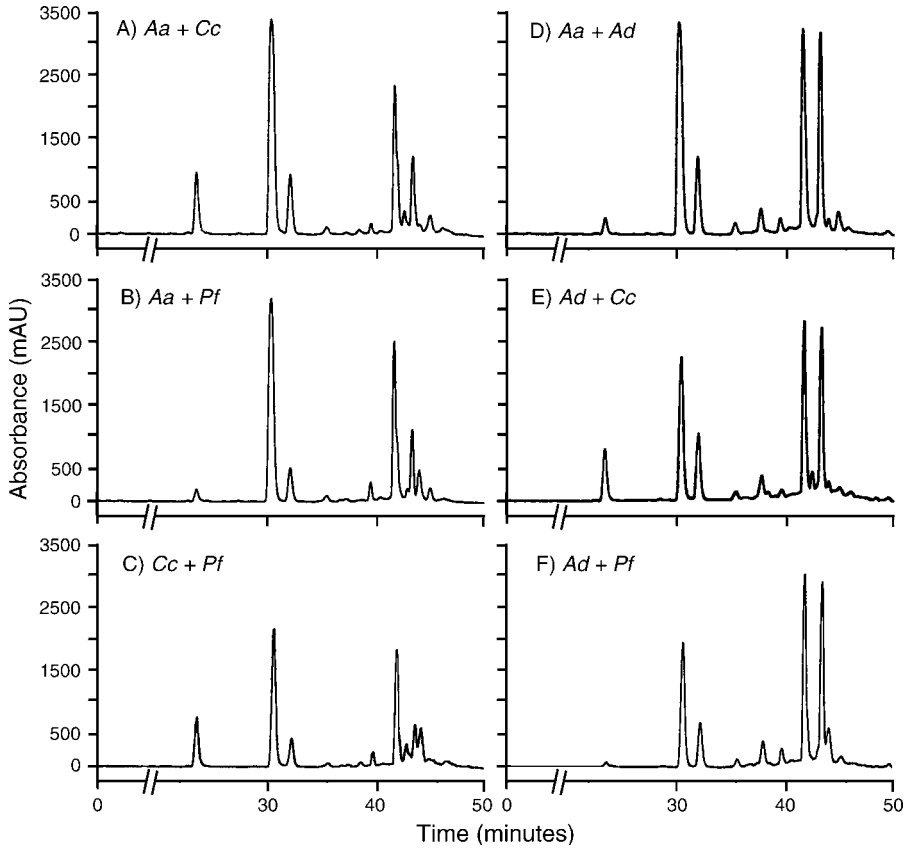

FIG. 4. HPLC chromatograms for the co-chromatography analyses of the *Papilio machaon aliaska* and *P. m. oregonius* host plants. Equivalent amounts (0.1 gle) of each host plant were injected simultaneously. Chromatograms A, B, and C are the *P. m. aliaska* host plants run together, and chromatograms D, E, and F are each of the *P. m. aliaska* host plants run with the *P. m. oregonius* host plant: (A) *Artemisia arctica* and *Cnidium cnidiifolium*; (B) *A. arctica* and *Petasites frigidus*; (C) *C. cnidiifolium* and *P. frigidus*; (D) *A. arctica* and *A. dracunculus*; (E) *A. dracunculus* and *C. cnidiifolium*; (F) *A. dracunculus* and *P. frigidus*. Species abbreviations are as in Table 1.

the F1 fraction of *A. frigida* was less preferred than the *C. cnidiifolium* extract alone, yet was more preferred than the control (Appendix E). The *C. cnidiifolium* extract with the F2 fraction of *A. frigida* did not differ in preference from the control (Appendix E). Hence, the F1 fraction of *A. frigida* appears to be mildly deterrent, whereas the F2 fraction of *A. frigida* is highly deterrent.

Co-chromatography

We found that when the F3 fractions from the different host plants were run together on the HPLC, many of the peaks retained their sharp apex (Fig. 4), indicating that they may contain the same compound(s). By analyzing the heights of the peaks when the plant extracts were run on the HPLC both alone and together, we found several peaks that appear to be found in more than one of the host plants (Table 1). For the host plants of *P. m. aliaska*, five of the seven peaks occur both in the ancestral host, *C. cnidiifolium*, and in *A. arctica*, one of the novel hosts. At least two of these occur also in the other novel host, *P. frigidus* (Table 1). Co-chromatography with a standard solution of *trans*-chlorogenic acid indicated that this compound occurs as peak 2 in *C. cnidiifolium*, *P. frigidus*, and the *P. m. oregonius* host, *A. dracunculus*. However, *trans*-chlorogenic acid is apparently not a major component of peak 2 in *A. arctica* (Table 2).

Although caffeoylquinic acids other than chlorogenic acid are unavailable commercially, we were able to obtain >12 hydroxycinnamic acids and derivatives for comparisons of HPLC retention times and spectra with those of the peaks found in common between two or more of the host plants. Other than chlorogenic acid, none of these commercially available compounds matched any of the host-plant peaks.

DISCUSSION

Our hypothesis, that the *Papilio machaon aliaska* host plants *Cnidium cnidiifolium*, *Artemesia arctica*, and *Petasites frigidus*, as well as the *P. m. oregonius* host plant *A. dracunculus*, share similar chemical stimulants that *P. m. aliaska* and *P. m. oregonius* females use as oviposition cues, was supported. Our first series of bioassays demonstrated that *P. m. aliaska* females prefer

TABLE 1. Co-chromatography results for the F3 fractions of *Cnidium cnidiifolium* (*Cc*), *Artemisia arctica* (*Aa*), *Petasites frigidus* (*Pf*), and *A. dracunculus* (*Ad*) when run alone and together.

Plant species	Peak height (mAU), by peak number						
	1	2	3	4	5	6	7
Cc	813	1267	393	67	574	420	89
Aa	194	3105	517	28	1553	850	207
Pf	18	1008	71	28	1227	526	27
Ad	49	1028	632	384	2490	2871	175
Aa and *Cc*							
Exp.	*1007*	4372	910	95	2127	1270	295
Obs.	*945*	3334	931	90	2323	1227	322
SI (%)	...	18	**105**	**82**	**134**	**90**	**129**
Aa and *Pf*							
Exp.	*212*	4113	*588*	56	2780	1376	*233*
Obs.	*187*	3178	*519*	50	2524	1133	*218*
SI (%)	...	7	...	**79**	**79**	54	...
Cc and *Pf*							
Exp.	*831*	2275	*464*	95	1801	946	*115*
Obs.	*768*	2142	*457*	91	1849	684	*153*
SI (%)	...	**87**	...	**86**	**108**	38	...
Aa and *Ad*							
Exp.	*243*	4133	1149	*412*	4043	*3721*	382
Obs.	*256*	3333	1223	*420*	3248	*3196*	381
SI (%)	...	22	**114**	...	49	...	**100**
Ad and *Cc*							
Exp.	*862*	2295	1025	*451*	*3064*	*3291*	264
Obs.	*824*	2260	1045	*416*	*2838*	*2739*	239
SI (%)	...	**97**	**105**	...	...	...	**72**
Ad and *Pf*							
Exp.	67	2036	*703*	*412*	3717	*3397*	*202*
Obs.	66	1919	*683*	*407*	2993	*2881*	*185*
SI (%)	**94**	**88**	...	...	41	...	...

Notes: If a compound is found in both plant species, then the observed peak height (mAU, milliabsorbance units) when the extracts are run together (Obs.) should equal the expected peak height (Exp.). The expected value is the sum of the peak heights when the plants are run separately. Data in italics were excluded from analysis (ellipses in the SI rows) because the shorter of the two parent peak heights failed to exceed 30% of the taller parent peak (see *Methods*). Expected and observed peak heights for each combination of extracts were compared by calculating a Similarity Index (SI) that expresses the observed increase in height of the larger parent peak as a percentage of the total increase expected if the two parent peaks have identical retention times. For peak combinations with an SI value greater than 70% (indicated in bold), we do not reject the hypothesis that the peaks in the two plant extracts are identical (see *Methods*).

polar over nonpolar extracts for their ancestral host plant (*C. cnidiifolium*), their novel host plants (*A. arctica* and *P. frigidus*), and the *P. m. oregonius* host plant (*A. dracunculus*). *P. m. oregonius* females also preferred polar extracts of *A. dracunculus* over nonpolar extracts (Appendix A and Fig. 3A). Although nonpolar (volatile) compounds may play an important role during earlier stages of host-finding (cf. Feeny et al. 1989), that they do not appear to play a significant role in a female's final decision to oviposit on a host plant. We did not count the number of approaches that females made to the various sponges, which is presumably the stage of host-plant selection that would have been most influenced by the volatile chemical cues (Haribal and Feeny 1998). In a related swallowtail species, *Papilio polyxenes*, Heinz (2002) demonstrated that females learn to associate volatile and visual cues with the presence of contact-stimulant cues. In the future, it would be interesting to investigate the visual and olfactory cues that first attract females to these host species and to test whether any of the species have attractive volatile compounds in common. Our results, however, indicate that the stimulants that cause *P. m. aliaska* and *P. m. oregonius* females to accept or reject hosts as suitable oviposition sites are polar compounds.

Our second series of bioassays demonstrated that the F3 fraction of the polar extract was the only active fraction for all of the host plants (Appendix B and Fig. 3B). Furthermore, the third series of bioassays showed that the F3 fraction was equal in activity to the parent extract for the *P. m. aliaska* host plants *C. cnidiifolium*, *A. arctica*, and *P. frigidus* (Appendix C and Fig. 3C), indicating that compounds in the F3 fraction (HCAs) are active alone and do not rely on synergism with compounds from the F1 fraction (low-molecular-weight polar compounds) or F2 fraction (flavonoids) to function as oviposition stimulants. This finding is rather unusual for butterflies in the *P. machaon* group, or

indeed within the swallowtail family (Papilionidae). Only the zebra swallowtail (*Eurytides marcellus*) previously has been found to respond to a single chemical category of contact stimulant. Isolated from the host plant *Asimina triloba*, the stimulant compound is also a HCA derivative (3-caffeoyl-*muco*-quinic acid) that apparently does not require synergism with other polar plant compounds in order to be active (Haribal and Feeny 1998, Haribal et al. 1998). Other species that have been studied within the genus *Papilio* rely on a complex mixture of primarily HCAs, flavonoids, and N-containing compounds to recognize their host plants; many of these contact oviposition stimulants are inactive or only slightly active when tested alone (Ohsugi et al. 1985, Honda 1986, 1990, Nishida et al. 1987, Feeny et al. 1988, Nishida 1995, Carter et al. 1998, 1999).

Our last series of bioassays with the *P. m. aliaska* host plants showed that two subfractions (F3b and F3c) of the F3 fraction stimulated females to oviposit (Appendix D and Fig. 3D). The two *P. m. oregonius* females that we tested also oviposited only on these two subfractions of their host plant, *A. dracunculus*. The host plants appear to have at least some peaks in common, but none of the compounds appears to be found in all four hosts (Fig. 4, Table 1). Specifically, peak 2 in *C. cnidiifolium*, *P. frigidus*, and *A. dracunculus* appears to be chlorogenic acid, the *trans* isomer of which has been identified as an oviposition stimulant for *Papilio polyxenes* (Feeny et al. 1988), but it is evidently not found in *A. arctica* (Table 2). We found that the compound in F3a is not active on its own (Appendix D), but it could still be a synergist, increasing the stimulant activity of one or more compounds in fractions F3b or F3c. We have not yet investigated whether any of the compounds in F3b or F3c are active alone or if they require the presence of the other compound(s) in their respective fraction; synergistic interactions may also occur between compounds in the F3a, F3b, and F3c fractions, increasing the overall activity of the extracts. Regardless, our results suggest that *P. m. aliaska* and *P. m. oregonius* females use similar compounds, likely to be HCA derivatives, as oviposition cues. Shared chemical cues between both ancestral and novel host plants may have provided the opportunity for *P. m. aliaska* to incorporate host plants in the Asteraceae as well as the Apiaceae, and for *P. m. oregonius* to shift completely to an asteraceous host species.

Why were *A. arctica*, *P. frigidus*, and *A. dracunculus* incorporated into the respective diets of *P. m. aliaska* and *P. m. oregonius* instead of other Asteraceae species? There are many possible explanations, including that the larvae may not be able to survive on other plants, that the novel species may lack the visual or olfactory cues necessary to attract females, or perhaps that the nonhosts inhibit oviposition through the presence of deterrents or lack of oviposition stimulants. We tested the latter possibilities and found that although the polar *A. frigida* extract deters *P. m. aliaska* females from laying eggs on otherwise attractive host extracts (Appendix E), the F3 fraction contains HCA (hydroxycinnamic acid) derivatives that stimulate *P. m. aliaska* females to oviposit (Appendix B). Furthermore, we found that the deterrents are restricted to the F1 and F2 fractions. The F1 fraction, primarily low-molecular-weight polar compounds, is mildly deterrent, whereas the F2 fraction, largely flavonoids, is highly deterrent (Appendix E). Although some flavonoid glycosides, when combined with other compounds, have been characterized as oviposition stimulants for *Papilio polyxenes* (Feeny et al. 1988), *Papilio protenor* (Honda 1986), and *Papilio xuthus* (Nishida et al. 1987), flavonoids are also known to act as deterrents. For instance, *P. xuthus* and *P. protenor* feed on several plants in the Rutaceae, but will not oviposit on other rutaceous plants because of the presence of deterrent flavonoids (Nishida et al. 1990*b*, Honda and Hayashi 1995). The zebra swallowtail (*E. marcellus*) rejects extracts made of late-season leaves from its host plant because of high flavonoid concentrations (Haribal and Feeny 2003). Moreover, even close relatives of *P. m. oregonius* are deterred by the flavonoids found in its host plant. *Papilio polyxenes*, a member of the *P. machaon* species group, will not oviposit on extracts of its host plant, *Daucus carota*, when the flavonoid fraction of *A. dracunculus* is present (K. Woodbury and P. Feeny, *unpublished data*). Perhaps *P. m. aliaska* and *P. m. oregonius* have been able to incorporate novel plants within the Asteraceae into their diets either because the novel hosts do not contain deterrent flavonoids or because *P. m. aliaska* and *P. m. oregonius* have a reduced sensitivity to the presence of deterrent flavonoids. However, many flavonoids, such as those in the nonhost *A. frigida*, remain deterrent and present an effective barrier to colonization despite the presence of oviposition stimulants.

Nearly 20 years ago, as part of a special feature in *Ecology* on insect host-plant associations (Strong 1988), participants discussed the relative merits of ecological factors that drive patterns of insect host use. Many of the articles debated whether predation (Bernays and

TABLE 2. Co-chromatography results for Peak 2 in the F3 fractions of *Cnidium cnidiifolium* (*Cc*), *Artemisia arctica* (*Aa*), *Petasites frigidus* (*Pf*), and *A. dracunculus* (*Ad*) run with chlorogenic acid (chlor.).

Plant species	Peak 2 height (mAU)		SI (%)
	Exp.	Obs.	
Aa and chlor.	4448	3351	18
Cc and chlor.	2610	2259	**72**
Pf and chlor.	2351	2338	**99**
Ad and chlor.	2371	2140	**78**

Notes: For chlorogenic acid alone, peak 2 height is 1343 mAU (milliabsorbance units). Exp. and Obs. are expected and observed peak heights, respectively. The Similarity Index (SI) expresses the observed increase in height of the larger parent peak as a percentage of the total increase expected if the two parent peaks have identical retention times. SI values >70% are indicated in boldface type.

Graham 1988), following in the spirit of arguments for top-down controls of herbivore densities made by Hairston et al. (1960), or host-plant chemistry (e.g., Schultz 1988), play a stronger role in determining host-plant use by herbivorous insects. We believe that the present study, in conjunction with related work by Murphy (2004), demonstrates that both bottom-up (host-plant chemistry) and top-down (predation) controls are necessary for insect host shifts to occur. In this paper, we provide evidence that the initiation of the host-range expansion is likely to have resulted from shared oviposition stimulants among the host plants. The similarity of stimulant profiles in ancestral and novel host plants is consistent with the hypothesis that plant chemistry has played a role in the establishment of the host expansion/shift from Apiaceae to Asteraceae by *P. m. aliaska* and *P. m. oregonius*. Plant chemistry alone, however, cannot explain why an insect would shift completely to the novel host(s), unless the novel host happened to be a nutritionally superior food source compared to the ancestral host. For *P. m. aliaska*, the novel host plants are not nutritionally superior; larval survival in the laboratory is significantly lower on either of the novel hosts than on the ancestral host (Murphy 2004, 2005). Murphy (2004) demonstrated that the inclusion of the novel host plants *A. arctica* and *P. frigidus* in the diet of *P. m. aliaska* is maintained by the relative lack of larval predation on these host plants in comparison with the ancestral host. Together, these studies indicate that similar host-plant chemistry is an essential first step during a host shift by an herbivorous insect, as proposed by Dethier (1941), but that top-down controls, such as enemy-free space, are likely to be the forces that drive host shifts to completion.

Acknowledgments

We thank T. Clausen, T. Hollingsworth, J. Hollingsworth, M. Walker, S. Rothman, P. Bennett, J. Goodman, L. Hough, T. Kast, C. Riggans, A. Moody, A. Borner, S. MacLean, J. Harry, A. Batten, K. Phillips, and the UAF greenhouses for assistance in Alaska. We thank D. McCorkle for help collecting *P. m. oregonius*, along with T. Stoddard, K. Flinn, and T. Hollingsworth. We thank J. Thompson, B. Cunningham, K. Walker, R. Croteau, and A. Black for their assistance at Washington State University. At Cornell University we thank M. Haribal, F. Vermeylen, J. Lavine, C. Tung, C. Swanson, M. K. Wheeler, M. Samuelsen, L. Saunders, J. Fox, D. Ardia, S. Mullen, D. Cousins, P. Cooper, and M. Marsello for their help. M. Geber, A. Renwick, M. Caillaud, A. Kessler, R. Halitschke, the plant–insect discussion group at Cornell University, and two anonymous reviewers gave helpful comments on earlier drafts of this manuscript. This work was supported by the Andrew W. Mellon Foundation, American Museum of Natural History Theodore Roosevelt Memorial Fund, Edna Bailey Sussman Fund, Explorer's Club Exploration Fund, Sigma Xi, Cornell University Department of Ecology and Evolutionary Biology, a NSF Doctoral Dissertation Improvement Grant awarded to S. M. Murphy (DEB-0104560), and an NSF research grant awarded to P. Feeny (IBN-9986250).

Literature Cited

Baur, R., P. Feeny, and E. Städler. 1993. Oviposition stimulants for the black swallowtail butterfly: identification of electrophysiologically active compounds in carrot volatiles. Journal of Chemical Ecology **19**:919–937.

Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. Science **176**:253–256.

Bernays, E., and M. Graham. 1988. On the evolution of host specificity in phytophagous arthropods. Ecology **69**:886–892.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution **23**:237–251.

Carroll, S. P., and C. Boyd. 1992. Host race radiation in the soapberry bug. Natural history with the history. Evolution **46**:1052–1069.

Carter, M., and P. Feeny. 1985. Techniques for maintaining a culture of the black swallowtail butterfly, *Papilio polyxenes asterius* Stoll (Papilionidae). Journal of the Lepidopterists' Society **39**:125–33.

Carter, M., P. Feeny, and M. Haribal. 1999. An oviposition stimulant for spicebush swallowtail butterfly, *Papilio troilus*, from leaves of *Sassafras albidum*. Journal of Chemical Ecology **25**:1233–1245.

Carter, M., K. Sachdev-Gupta, and P. Feeny. 1998. Tyramine from the leaves of wild parsnip: a stimulant and synergist for oviposition by the black swallowtail butterfly. Physiological Entomology **23**:303–312.

Caterino, M. S., and F. A. H. Sperling. 1999. *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Molecular Phylogenetics and Evolution **11**:122–137.

Dethier, V. G. 1941. Chemical factors determining the choice of food plants by *Papilio* larvae. American Naturalist **75**:61–73.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: A study in coevolution. Evolution **18**:586–608.

Farrell, B., and C. Mitter. 1993. Phylogenetic determinants of insect/plant community assembly. Pages 253–266 *in* R. Ricklefs and D. Schluter, editors. Species diversity in ecological communities. University of Chicago Press, Chicago, Illinois, USA.

Feder, J. L., S. H. Berlocher, J. B. Roethele, H. Dambroski, J. J. Smith, W. L. Perry, V. Gavrilovic, K. E. Filchak, J. Rull, and M. Aluja. 2003. Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis*. Proceedings of the National Academy of Sciences (USA) **100**:10314–10319.

Feeny, P. 1991. Chemical constraints on the evolution of swallowtail butterflies. Pages 315–340 *in* P. W. Price, T. M. Lewinsohn, G. W. Fernandes, and W. W. Benson, editors. Plant–animal interactions: evolutionary ecology in tropical and temperate regions. John Wiley, New York, New York, USA.

Feeny, P. 1992. The evolution of chemical ecology: contributions from the study of herbivorous insects. Pages 1–44 *in* G. A. Rosenthal and M. R. Berenbaum, editors. Herbivores: their interactions with secondary plant metabolites. Second edition. Academic Press, New York, New York, USA.

Feeny, P. 1995. Ecological opportunism and chemical constraints on the host associations of swallowtail butterflies. Pages 9–15 *in* J. M. Scriber, Y. Tsubaki, and R. C. Lederhouse, editors. Swallowtail butterflies: their ecology and evolutionary biology. Scientific Publishers, Inc., Gainesville, Florida, USA.

Feeny, P., L. Rosenberry, and M. Carter. 1983. Chemical aspects of oviposition behavior in butterflies. Pages 27–76 *in* S. Ahmad, editor. Herbivorous insects: host-seeking behavior and mechanisms. Academic Press, New York, New York, USA.

Feeny, P., K. Sachdev, L. Rosenberry, and M. Carter. 1988. Luteolin 7-O-(6″-O-malonyl)-β-D-glucoside and transchlorogenic acid: oviposition stimulants for the black swallowtail butterfly. Phytochemistry **27**:3439–3448.

Feeny, P., E. Städler, I. Åhman, and M. Carter. 1989. Effects of plant odor on oviposition by the black swallowtail butterfly,

Papilio polyxenes (Lepidoptera: Papilionidae). Journal of Insect Behavior **2**:803–827.

Forister, M. L. 2004. Oviposition preference and larval performance within a diverging lineage of lycaenid butterflies. Ecological Entomology **29**:264–272.

Fraenkel, G. S. 1959. The raison d'être of secondary plant substances. Science **129**:1466–1470.

Funk, D. J., K. E. Filchak, and J. L. Feder. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica **116**:251–267.

Funk, D. J., D. J. Futuyma, G. Orti, and A. Meyer. 1995. A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): new evidence from mitochondrial DNA. Evolution **49**:1008–1017.

Futuyma, D. J., and S. S. McCafferty. 1990. Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera, chrysomelidae). Evolution **44**:1885–1913.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. American Naturalist **44**:421–425.

Haribal, M., and P. Feeny. 1998. Oviposition stimulant for the zebra swallowtail butterfly, *Eurytides marcellus*, from the foliage of pawpaw, *Asimina triloba*. Chemoecology **8**:99–110.

Haribal, M., and P. Feeny. 2003. Combined roles of contact stimulant and deterrents in assessment of host-plant quality by ovipositing zebra swallowtail butterflies. Journal of Chemical Ecology **29**:653–670.

Haribal, M., P. Feeny, and C. C. Lester. 1998. A caffeoylcyclohexane-1-carboxylic acid derivative from *Asimina triloba*. Phytochemistry **49**:103–108.

Heinz, C. A. 2002. Host finding and recognition by *Papilio polyxenes* (Lepidoptera: Papilionidae): the effects of three host cues and of host-plant experience on oviposition behavior. Dissertation. Cornell University, Ithaca, New York, USA.

Heinz, C. A., and P. Feeny. 2005. Effects of contact chemistry and host plant experience in the oviposition behaviour of the eastern black swallowtail butterfly. Animal Behaviour **69**: 107–115.

Honda, K. 1986. Flavanone glycosides as oviposition stimulants in a papilionid butterfly, *Papilio protenor*. Journal of Chemical Ecology **12**:1999–2010.

Honda, K. 1990. Identification of host-plant chemicals stimulating oviposition by swallowtail butterfly, *Papilio protenor*. Journal of Chemical Ecology **16**:325–337.

Honda, K. 1995. Chemical basis of differential oviposition by lepidopterous insects. Archives of Insect Biochemistry and Physiology **30**:1–23.

Honda, K., and N. Hayashi. 1995. A flavonoid glucoside, phellamurin, regulates differential oviposition on a rutaceous plant, *Pdendron amurense*, by two sympatric swallowtail butterflies, *Papilio protenor* and *P. xuthus*: the front line of a coevolutionary arms race? Journal of Chemical Ecology **21**: 1531–1539.

Jermy, T. 1976. Insect–host-plant relationship: Coevolution or sequential evolution? Pages 109–113 *in* T. Jermy, editor. The host-plant in relation to insect behavior and reproduction. Plenum, New York, New York, USA.

Jermy, T. 1984. Evolution of insect/host plant relationships. American Naturalist **124**:609–630.

Konczak, I., S. Okuno, M. Yashimoto, and O. Yamakawa. 2004. Caffeoylquinic acids generated in vitro in a high-anthocyanin-accumulating sweet potato cell line. Journal of Biomedicine and Biotechnology **5**:287–292.

Lederhouse, R. C. 1982. Territorial defense and lek behavior of the black swallowtail butterfly *Papilio polyxenes*. Behavioral Ecology and Sociobiology **10**:109–118.

Miller, J. S. 1987. Host-plant relationships in the papilionidae (Lepidoptera): parallel cladogenesis or colonization? Cladistics **3**:105–120.

Möller, B., and K. Herrmann. 1983. Quinic acid esters of hydroxycinnamic acids in stone and pome fruit. Phytochemistry **22**:477–481.

Murphy, S. M. 2004. Enemy-free space maintains swallowtail butterfly host shift. Proceedings of the National Academy of Sciences (USA) **101**:18048–18052.

Murphy, S. M. 2005. A host shift by swallowtail butterflies within the *Papilio machaon* species group: evidence for the roles of chemical facilitation and enemy-free space. Dissertation. Cornell University, Ithaca, New York, USA.

Nakayama, T., K. Honda, H. Omura, and N. Hayashi. 2003. Oviposition stimulants for the tropical swallowtail butterfly, *Papilio polytes*, feeding on a rutaceous plant, *Toddalia asiatica*. Journal of Chemical Ecology **29**:1621–1634.

Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. Applied linear statistical models. Fourth edition. Irwin, Chicago, Illinois, USA.

Nishida, R. 1995. Oviposition stimulants of swallowtail butterflies. Pages 17–26 *in* J. M. Scriber, Y. Tsubaki, and R. C. Lederhouse, editors. Swallowtail butterflies: their ecology and evolutionary biology. Scientific Publishers, Gainesville, Florida, USA.

Nishida, R., T. Ohsugi, and H. Fukami. 1990*a*. Oviposition stimulant activity of tryptamine analogs on a Rutaceae-feeding swallowtail butterfly, *Papilio xuthus*. Agricultural and Biological Chemistry **54**:1853–1855.

Nishida, R., T. Ohsugi, H. Fukami, and S. Nakajima. 1990*b*. Oviposition deterrent of a Rutaceae-feeding swallowtail butterfly, *Papilio xuthus*, from a non-host rutaceous plant, *Orixa japonica*. Agricultural and Biological Chemistry **54**: 1265–1270.

Nishida, R., T. Ohsugi, S. Kokubo, and H. Fukami. 1987. Oviposition stimulants of a *Citrus*-feeding swallowtail butterfly, *Papilio xuthus* L. Experientia **43**:342–344.

Ohsugi, T., R. Nishida, and H. Fukami. 1985. Oviposition stimulant of *Papilio xuthus*, a *Citrus*-feeding swallowtail butterfly. Agricultural and Biological Chemistry **49**:1897–1900.

Ono, H., R. Nishida, and Y. Kuwahara. 2000. Oviposition stimulant for a Rutaceae-feeding swallowtail butterfly, *Papilio bianor* (Lepidoptera: Papilionidae): hydroxycinnamic acid derivative from *Orixa japonica*. Applied Entomology and Zoology **35**:119–123.

Papaj, D. R. 1986. Conditioning of leaf-shape discrimination by chemical cues in the butterfly, *Battus philenor*. Animal Behavior **34**:1281–1288.

Rausher, M. D. 1978. Search image for leaf shape in a butterfly. Science **200**:1071–1073.

Renwick, J. A. A. 2001. Variable diets and changing taste in plant–insect relationships. Journal of Chemical Ecology **27**: 1063–1076.

Robinson, T. 1991. The organic constituents of higher plants. Sixth edition. Cordus Press, North Amherst, Massachusetts, USA.

Roessingh, P., E. Stadler, R. Schoni, and P. Feeny. 1991. Tarsal contact chemoreceptors of the black swallowtail butterfly *Papilio polyxenes*: responses to phytochemicals from host- and non-host plants. Physiological Entomology **16**:485–495.

Sachdev-Gupta, K., P. P. Feeny, and M. Carter. 1993. Oviposition stimulants for the pipevine swallowtail butterfly, *Battus philenor* (Papilionidae), from an Aristolochia host plant: synergism between inositols, arsitolochic acids and a monogalactosyl diglyceride. Chemoecology **4**:19–28.

SAS Institute. 1999. SAS PROC MIXED. Version 8. SAS Institute, Cary, North Carolina, USA.

Schoonhoven, L. M., T. Jermy, and J. J. A. Van Loon. 1998. Insect–plant biology: from physiology to evolution. Chapman and Hall, London, UK.

Schultz, J. C. 1988. Many factors influence the evolution of herbivore diets, but plant chemistry is central. Ecology **69**: 896–897.

Scott, J. A. 1986. The butterflies of North America: a natural history and field guide. Stanford University Press, Stanford, California, USA.

Scriber, J. M. 1973. Latitudinal gradients in larval feeding specialization of the world Papilionidae (Lepidoptera). Psyche **80**:355–373.

Siegel, S., and N. J. J. Castellan. 1988. Nonparametric statistics for the behavioral sciences. McGraw Hill, New York, New York, USA.

Soltis, P. S., and D. E. Soltis. 2004. The origin and diversification of angiosperms. American Journal of Botany **9**:1614–1626.

Sondheimer, E. 1964. Chlorogenic acids and related depsides. Botanical Review **30**:667–712.

Sperling, F. A. H. 1987. Evolution of the *Papilio machaon* species group in western Canada (Lepidoptera: Papilionidae). Quaestiones Entomologicae **23**:198–315.

Sperling, F. A. H., and R. G. Harrison. 1994. Mitochondrial DNA variation within and between species of the *Papilio machaon* group of swallowtail butterflies. Evolution **48**:408–422.

Strong, D. R. 1988. Insect host range. Ecology **69**:1.

Thompson, J. N. 1988. Variation in preference and specificity in monophagous and oligophagous swallowtail butterflies. Evolution **42**:118–128.

Thompson, J. N. 1995. The origins of host shifts in swallowtail butterflies versus other insects. Pages 195–203 *in* J. M. Scriber, Y. Tsubaki, and R. C. Lederhouse, editors. Swallowtail butterflies: their ecology and evolutionary biology. Scientific Publishers, Gainesville, Florida, USA.

Thompson, J. N., and O. Pellmyr. 1991. Evolution of oviposition behavior and host preference in Lepidoptera. Annual Review of Entomology **36**:65–89.

Tyler, H. A. 1975. The swallowtail butterflies of North America. Naturegraph Publishers, Healdsburg, California, USA.

Wiklund, C. 1981. Generalist vs. specialist oviposition behaviour in *Papilio machaon* (Lepidoptera) and functional aspects on the hierarchy of oviposition preferences. Oikos **36**:163–170.

Wink, M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry **64**:3–19.

Wink, M., C. Meibner, and L. Witte. 1995. Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. Phytochemistry **38**:139–153.

APPENDIX A

A table showing female *Papilio machaon aliaska* and *Papilio oregonius* oviposition preferences for polar vs. nonpolar host-plant extracts of *Cnidium cnidiifolium*, *Artemisia arctica*, and *Petasites frigidus* (hosts of *P. m. aliaska*) and of *Artemisia dracunculus* (*P. oregonius* host) (*Ecological Archives* M076-015-A1).

APPENDIX B

A table showing female *Papilio machaon aliaska* oviposition preferences for fractions F1, F2, and F3 of the polar extracts of *Cnidium cnidiifolium*, *Artemisia arctica*, and *Petasites frigidus* (hosts of *P. m. aliaska*); *Artemisia dracunculus* (*P. oregonius* host); and non-host *Artemisia frigida* (*Ecological Archives* M076-015-A2).

APPENDIX C

A table showing female *Papilio machaon aliaska* oviposition preferences for the parent polar extract compared to the F3 fraction of *Cnidium cnidiifolium*, *Artemisia arctica*, and *Petasites frigidus* (*Ecological Archives* M076-015-A3).

APPENDIX D

A table showing female *Papilio machaon aliaska* oviposition preferences for fractions F3a, F3b, and F3c of the polar extracts of *Cnidium cnidiifolium*, *Artemisia arctica*, and *Petasites frigidus* (*Ecological Archives* M076-015-A4).

APPENDIX E

A table showing female *Papilio machaon aliaska* oviposition preferences for polar *Cnidium cnidiifolium* extract (Cc) compared to preferences for *Cnidium cnidiifolium* extract combined with either polar *Artemisia frigida* extract (*Cc*+*Af*) or *A. frigida* fractions (*Cc* + *Af* F1 and *Cc* +*Af* F2) (*Ecological Archives* M076-015-A5).

APPENDIX F

A figure showing HPLC chromatograms (monitored at 254 nm) for the polar fractions (F1, F2, F3) of the *Papilio machaon aliaska* ancestral host plant (*Cnidium cnidiifolium*), the *P. m. aliaska* novel host plants (*Artemisia arctica* and *Petasites frigidus*), the *P. oregonius* host (*A. dracunculus*), and a non-host plant (*A. frigida*) (*Ecological Archives* M076-015-A6).