Inconsistent use of host plants by the Alaskan swallowtail butterfly: adult preference experiments suggest labile oviposition strategy

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Abstract. 1. The Alaskan swallowtail butterfly (*Papilio machaon aliaska*) uses three unrelated plant species as hosts: *Cnidium cnidiifolium* (Apiaceae), *Artemisia arctica* (Asteraceae), and *Petasites frigidus* (Asteraceae). The research presented here investigated whether there are any consistent patterns in host choice by *P. m. aliaska* females.

2. The first two experiments were designed to test if *P. m. aliaska* host preference is constant or if it changes from day to day. If host preference is labile, the experiments were designed to also test whether a female's diet breadth narrows or expands over time.

3. The third experiment tested the host preferences of female offspring from several wild-caught *P. m. aliaska* females. If *P. m. aliaska* individuals are specialised in their host use, then all of the offspring from a single female would likely prefer the same host-plant species. This experiment was also designed to test the Hopkins' host selection principle; does the food plant on which a female is reared as a larva influence her future choices when she is searching for host plants for her own offspring?

4. The results from all of these experiments indicate that *P. m. aliaska* females vary greatly in their oviposition behaviour and in their preferences for the three host plants. Most populations appear to consist of generalists with labile oviposition behaviour. There is no evidence to support the Hopkins' host selection principle.

5. It is suggested that the generalised selection of host plants by *P. m. aliaska* females may be a 'bet-hedging' strategy and that this strategy may maximise reproductive fitness in an unpredictable environment.

Key words. Herbivory, host-plant preference, host-plant selection, host shift, Lepidoptera, oviposition behaviour, *Papilio machaon aliaska*.

Introduction

Why and how insects choose to feed on some plant species and not others has been a subject of considerable research for several decades. Host selection can be based on the insect's physiological state (Singer, 1971, 1982) or the ecological abundance of the host plants (Fox & Morrow, 1981). Host use is often determined by adult behaviour (Jermy, 1984; Futuyma, 1986) and a number of studies have shown that many Lepidoptera have distinct preference hierarchies when selecting from a range of potential host plants (Singer, 1971; Wiklund, 1981; Singer, 1982; Nylin & Janz, 1993; Janz & Nylin, 1997; Thompson, 1998; Forister, 2004). A female's choice of oviposition site has significant impacts on her offspring, especially for relatively immobile, newly hatched larvae.

Swallowtail butterflies from the *Papilio machaon* group use plants of the Apiaceae as their primary hosts (Wiklund, 1981; Feeny *et al.*, 1983; Sperling, 1987; Thompson, 1995). Apart from occasional use of plants in the family Rutaceae, an ancestral host family for the genus *Papilio* (Sperling, 1987), *P. machaon* swallowtails have rarely incorporated non-apiaceous plants into their diet. In Alaska and north-western Canada, the univoltine swallowtail *P. m. aliaska* Scud. oviposits and feeds not only on the local apiaceous host, *Cnidium cnidiifolium* (Turcz.)

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Schischk., but also on *Artemisia arctica* Less. and *Petasites frigidus* (L.) Franch. (Scott, 1986) in the distantly related family Asteraceae. This host-range expansion by *P. m. aliaska* appears to represent an intermediate step towards a complete host shift. There is at least one example of a species in the *P. machaon* group that is now restricted to the novel host genus *Artemisia* (Sperling, 1987); *Papilio oregonius* Edwards, a close relative of *P. m. aliaska* (Sperling & Harrison, 1994; Caterino & Sperling, 1999), has shifted completely to the novel host-plant family Asteraceae and is monophagous on *Artemisia dracunculus* L. (Thompson, 1988c). It is unclear, however, whether *P. m. aliaska* and *P. oregonius* represent a single host shift or two independent host shifts to *Artemisia*.

When a specialised insect species, such as *P. m. aliaska*, uses three unrelated plant species as hosts, the question arises as to whether females follow a generalised or specialised oviposition strategy. Do individual *P. m. aliaska* females use both the ancestral and novel hosts or do some females specialise on only one of the three host plants? Localised specialisation has been documented before (e.g. Fox & Morrow, 1981) and it may be possible for *P. m. aliaska* to be locally monophagous given that its host plants are found in different environments. *Cnidium 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. *Petasites frigidus* grows at both low and high elevations in moist habitats; at high elevations it may co-occur with *A. arctica* plants while at low elevations it is often found in bogs, although it can sometimes be found in the vicinity of *C. cnidiifolium* plants (pers. obs.). Previous work has demonstrated that these host plants are not equal in terms of larval survival in the field (Murphy, 2004) or the laboratory (Murphy, 2005). In the absence of predators, *P. m. aliaska* larvae survive best on the ancestral host plant, *C. cnidiifolium*, but in the presence of predators, larval survival is greater on the novel host plants. In the field, *A. arctica* and *P. frigidus* plants seem to offer larvae enemy-free space that is not found on the ancestral host plant *C. cnidiifolium*. This enemy-free space appears to result from the diverse environments in which the novel host plants occur, and is not due to any particular trait of the plants themselves (Murphy, 2004).

Despite the environmental differences and physical distance between the locations where the host plants can be found,

P. m. aliaska is thought to be a typical hill-topping swallowtail butterfly (cf. Lederhouse, 1982) and field observations over the past 5 years appear to support this assumption (Murphy, 2005). 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 towards larval host-plant sites. Populations from the different larval host-plant sites surrounding a hilltop are thus assumed to be panmictic; females and males from surrounding larval sites arrive at the top of the tundra domes within a few days of each other and mate. Host races therefore seem unlikely because this behavioural adaptation presumably maintains gene flow between populations from different larval host-plant sites by reducing associative mating near the host plants.

The research presented here was designed to investigate whether there are any consistent patterns in host choice by *P. m. aliaska* females. Are there host-associated populations that exhibit specialised oviposition preferences for one of the three host plants? An ideal way to answer this question would be to follow females from larval host-plant sites to mating sites and subsequently observe their oviposition behaviours. Unfortunately it is physically impossible to follow females in the field for more than a few metres because of rugged terrain and because host-plant sites are often miles apart and separated by several hundred metres in elevation. Instead, experiments were designed to test a female's host-plant preferences in oviposition bioassays on two consecutive days. With this information, it is possible to determine whether host specificity is constant or changes over time. Furthermore, if host preference is labile, do females become more generalised and accept a greater proportion of the host plants over time or do females become more specialised and eventually focus on a single host species?
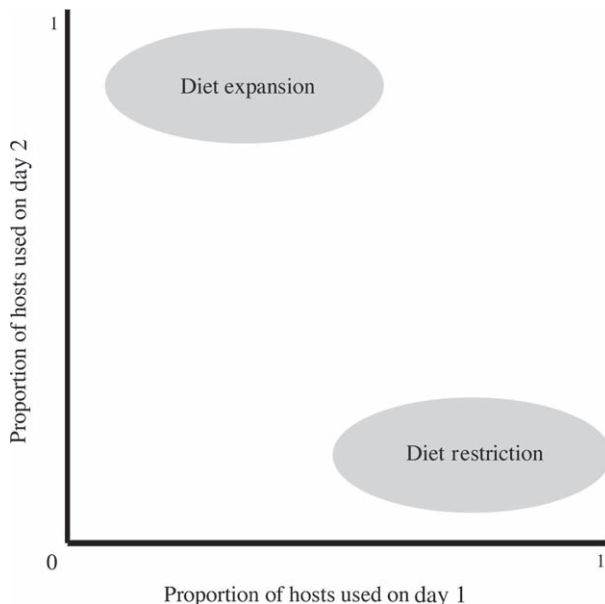
There are three predicted outcomes of experiments in which females are asked to rank their preferences for the host plants over two consecutive days (Fig. 1). First, females prefer a certain plant species in favour of other species and do not vary their preferences through time. These females demonstrate high host fidelity. Second, there may be no pattern in host choice from one day to another, indicating that the females belong to a population of generalists. Some females may prefer one host, other females another host while yet other females switch between hosts or use multiple hosts. Third, females may prefer one plant species the
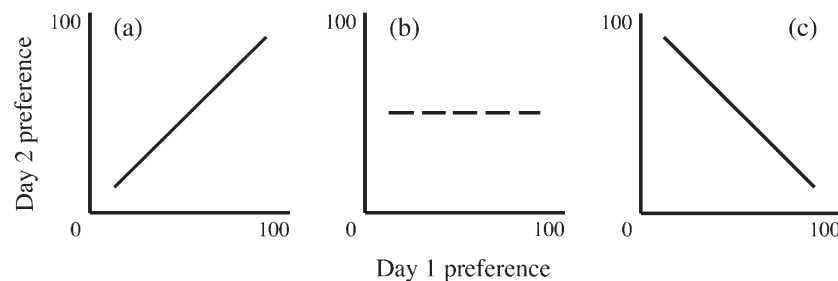
Fig. 1. Potential outcomes of preference experiments testing adult *Papilio machaon aliaska* females. 'Day 1 preference' is the percentage of eggs that a female lays on any one of the host plants (e.g. the ancestral host) on the first day of the experiment while 'Day 2 preference' is the percentage of eggs that the female lays on the same host plant on the second day of the experiment. (a) Females tend to prefer certain plants and do not vary their preferences over time. (b) No real trend in the data, which indicates that this is a population of generalists. (c) Females change their preferences over time, which indicates that this is a population of generalists.

first day yet prefer another species the next day. It is possible to expand on this third outcome and analyse whether a female's diet breadth narrows or expands during the experiment (Fig. 2). If a female uses only one of the available host plants the first day but uses two or three of them the second day, then she falls into the diet expansion category. Conversely, if a female reduces the number of hosts that she will use from day to day, then she is part of the diet restriction category. Females that are consistent in their use of host plants through time should lie between these two areas in Fig. 2. The goal of these experiments is to determine if *P. m. aliaska* females are consistent in following a specialised oviposition strategy or whether females are labile in their host use and follow a more generalised oviposition strategy.

In a final experiment, the preferences of *P. m. aliaska* females for chemical extracts of the three host plants were tested. These extracts have been previously demonstrated to contain the active chemical compounds that females use as oviposition cues upon contact with the host plants (Murphy & Feeny, in press). The bioassays tested several offspring from each female under the assumption that if *P. m. aliaska* individuals are specialised in their host use, then all of the offspring from a single female would likely prefer the same host-plant species. If the siblings preferred different host plants, then this would indicate that host-plant specialisation is not a highly conserved trait from generation to generation within *P. m. aliaska*. This bioassay was designed to also investigate if the food plant on which a female is reared as a larva influences her future choices when she is

searching for host plants for her own offspring. The Hopkins' host selection principle, sometimes suggested in an effort to explain oviposition behaviour of adult female insects, proposes that females are predisposed to lay eggs on the host plant on which they themselves fed as larvae (Dethier, 1954). Two previous studies have demonstrated that swallowtails in the *P. machaon* group do not possess this type of larval memory for their host plants as adults (Wiklund, 1974; Heinz & Feeny, 2005). Thus, it does not seem likely that larval host plant would influence choices by *P. m. aliaska* adults.

Materials and methods

Experiment 1 – whole plants in large cages

Five *P. m. aliaska* females were collected on 10–19 June 2000, from three alpine-tundra hilltops (domes) near Fairbanks, Alaska. Due to the scale of the landscape in the interior of Alaska, it is difficult to collect *P. m. aliaska* individuals other than from hilltops. One female was collected from Ester Dome (64°52′N, 148°4′W, ≈ 720 m), one female from along the Pinnel Mountain trail south-west of Table Mountain (65°25′N, 145°57′W, ≈ 1200 m) and three females from Wickersham Dome (65°13′N, 148°3′W, ≈ 977 m). The females were caught as they arrived at the summit of the domes and were therefore assumed not to have already mated. During a previous field season, females were similarly caught as they arrived at the summit of a dome, but were left unmated; these females never laid fertilised eggs (S. M. Murphy, unpublished data). To ensure viable offspring, females were mated by hand pairing (Carter & Feeny, 1985) in the laboratory with males from the same field site.

This bioassay was conducted in a greenhouse near Fairbanks, Alaska, that did not contain any plants other than those used in the experiment. The greenhouse was not air-conditioned and ranged in temperature from 18 to 30 °C during the trials. Females were placed individually in wood-framed cages (0.66 m^2) covered with a fine white mesh (five holes per centimetre, Jo-Ann Fabric, Ithaca, New York). Each cage contained three potted plants of approximately equal size: a *C. cnidiifolium* plant, an *A. arctica* plant, and a non-deterrent, non-host bean plant (*Vicia faba*) to serve as a control. The bean plants were grown from seed (Agway, Ithaca, New York). The *C. cnidiifolium* plants were transplanted into pots from a steep river bluff near the Bonanza Creek Long-term Ecological Research (LTER) Experimental Forest (64°42′N, 148°18′W, 185 m) and the *A. arctica* plants were transplanted from Ester Dome. Females were fed at least twice per day with a water solution containing 10% honey by volume (Carter & Feeny, 1985); in addition each cage was provisioned with a Petri dish full of honey water so females could feed freely during the bioassays.

The bioassays were 12 h long and began between 6.00 and 7.00 hours. Females were placed in the cages the morning after they were caught and were allowed to oviposit freely for 12 h. The plants were then searched and the number of eggs that each female laid on each plant was recorded. The experiment began on the day that the female laid at least five eggs on any of the

Fig. 2. Potential outcomes of preference experiments testing adult *Papilio machaon aliaska* females. The proportion of hosts that a female uses on the first day of the experiment is plotted against the proportion of hosts that she uses on the second day of the experiment. If females become less selective over time, then they should be found in the area of the graph labelled 'Diet expansion'. If females become more selective over time, then they should be found in the area of the graph labelled 'Diet restriction'.

plants. For three of the females this occurred on the first day, for one female it was the second day and for the last female it was her fourth day of captivity. The positions of the plants in the cages were randomised and the bioassay was repeated the next day. During the experiment, females laid between 6 and 50 eggs per day (mean $= 26.3 \pm 5.0$).

Experiment 2 – plant sprigs in clear boxes

Twelve *P. m. aliaska* females were collected on 10–19 June 2003, from three domes near Fairbanks, Alaska. Six females were collected from Ester Dome (E1–E6), one female from Murphy Dome (M1; 64°57′N, 148°21′W, $\approx$ 890 m), and five females from Wickersham Dome (W1–W5). The females were caught as they arrived at the summit of the domes and were mated in the laboratory with males from the same field site.

This bioassay was conducted in a laboratory with natural light near Fairbanks, Alaska. Each morning at 7.00 hours, the females were fed and then individually placed in clear, plastic boxes ($31 \times 16.5 \times 8$ cm) that were provisioned with sprigs of all three host plants: *C. cnidiifolium*, *A. arctica*, and *P. frigidus*. Females were allowed to oviposit freely for 12 h after which the number of eggs laid on each plant as well as on the box was recorded. The females were fed again and placed in a large net cage for the night. The experiment began on the day that the female laid at least five eggs on any of the plants provided in her box. For all but two of the females this occurred on the first day that they were placed in the bioassay box, for the other two it was their second day of captivity. During the experiment, females laid between 6 and 108 eggs per day (mean $= 37.0 \pm 6.6$).

Host-plant sprigs measured approximately 10 cm in length and the boxes were large enough so that the three plant species did not touch each other. The sprigs were placed in florist aquapiks to prevent desiccation and were replaced with fresh foliage twice per day. The position of the sprigs in the boxes was randomised each morning. *Cnidium cnidiifolium* was collected from two sites, both of which were broadleaf woodlands on steep river bluffs (site 1 = 64°42′N, 148°18′W, 185 m; site 2 = 64°57′N, 147°38′W, 220 m). *Artemisia arctica* was collected from two high-elevation tundra meadows (site 1 = 64°52′N, 148°4′W, 710 m; site 2 = 64°57′N, 148°21′W, 880 m). *Petasites frigidus* was collected at one of the same sites as *A. arctica* (64°57′N, 148°21′W, 880 m) as well as from a lower-elevation, poorly drained bog (64°52′N, 147°50′W, 175 m).

Experiment 3 – plant extracts in large cages

The females used in this experiment were the offspring of the females in experiment 2. Each female's offspring are most likely full siblings, but if the females had already mated in the field, the offspring could be half-siblings given that the females were mated in the laboratory after they were caught. The offspring of each female were reared in equal numbers on the three host plants under ambient conditions (24 h light, $\approx$ 17 °C). After the larvae had pupated, they were taken to Ithaca, New York, and refrigerated (24 h dark, 5 °C) for 8–13 months. The

following summer (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) 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 fed as described in Carter and Feeny (1985). Females were hand-paired with unrelated males within 2 days of emerging and were subsequently tested in bioassays.

All bioassay trials were conducted in greenhouse facilities on the Cornell University campus. Females were reluctant to fly in the absence of direct sunlight, hence all trials were conducted between the hours of 9.30 and 16.00 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 per day and 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^2) covered with a fine white mesh (nine holes per centimetre). Each cage contained a model plant (36 cm in height) made of a wooden 'trunk', four wire 'stems', and sponge 'leaves' (5.0×3.5 cm triangles, 0.75 cm deep) coloured green with a food-colouring solution (McCormick & Company, Inc., Hunt Valley, Maryland) and glued to the end of each branch (Feeny *et al.*, 1989). Chemical extracts from the three host plants were applied individually to experimental leaves and were attached randomly to each position before each trial. The fourth leaf was a control and had only the chemical solvent applied to it, without any plant material.

Chemical extracts for all host-plant species (*C. cnidiifolium*, *A. arctica*, and *P. frigidus*) were made using the same technique. Foliage was taken fresh from the field from the same sites as described in experiment 2 during June 2003. Foliage from 30 to 100 plants was collected and then immediately transported, in crushed ice, to the laboratory of Dr T. Clausen in Alaska (Chemistry Department, University of Alaska, Fairbanks). Leaves, separated from stems, were weighed and were soaked (100 g fresh weight at a time) in boiling de-ionised water for 10 min and the extract filtered. Water, a polar solvent, extracts non-volatile compounds from the leaves; these include contact stimulants that a female perceives once she has alighted upon the host plant and have been demonstrated to be highly attractive to *P. m. aliaska* females (Murphy & Feeny, in press). After filtration, the extract was dried *in vacuo* at < 40 °C. The extracts were transported to Ithaca, New York and stored frozen (–15 °C) until the following summer (2004) when they were rehydrated with a 50:50 solution of methanol and HPLC-grade water (MeOH-H$_2$O). In preparation for the bioassays, the extract was diluted with MeOH-H$_2$O until it reached a concentration of 1 gle/2 ml solution. One gram-leaf equivalent (gle) is the amount of material extracted from 1 g fresh weight of leaves. Each experimental leaf in the bioassay had 1 gle plant extract (2 ml of solution) applied to it; the control leaf had 2 ml MeOH-H$_2$O applied to it, which controlled for any potential effects of the solvent.

Trials lasted 1 h and the model plants were rotated counter-clockwise every 15 min to control for corner biases. 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 as well as the number of eggs that she laid on each sponge was recorded. During the experiment, females laid between 5 and 41 eggs per day (mean $= 12.0 \pm 1.0$). The methods for these bioassays eliminate all visual plant cues, and thus female response is determined entirely by preference for the extracts.

Statistical analyses

Regression analyses of the experimental results from experiments 1 and 2 were performed using SigmaPlot (SPSS Inc., 2000). For experiment 3, differences in female preferences when grouped within dam were analysed with the Friedman two-way ANOVA by ranks test (Siegel & Castellan, 1988). Females W1 and E4 were not included in these analyses as they each only had one surviving female offspring. Differences in female preferences when grouped within larval host plant were also analysed with the Friedman two-way ANOVA by ranks test (Siegel & Castellan, 1988). The host plant *P. frigidus* was not

included in this analysis because only one female that was reared on *P. frigidus* was tested.

Results

Experiment 1 – whole plants in large cages

Females did not present a clear pattern in their host-plant preferences from the first to the second day of this experiment (Fig. 3a; $R^2 = 0.02$, $F_{1,3} = 0.06$, $P = 0.82$). One female was consistent in only ovipositing on *C. cnidiifolium*. Two females preferred *C. cnidiifolium* the first day but then switched to other hosts the second day while the other two females shunned *C. cnidiifolium* the first day but accepted it to varying degrees the second day. The proportion of hosts used during the experiment also varied widely among females (Fig. 3b; $R^2 = 0.02$, $F_{1,8} = 0.17$, $P = 0.69$); because there are three potential host plants, females used either 1/3, 2/3, or 3/3 of the hosts on any given day.
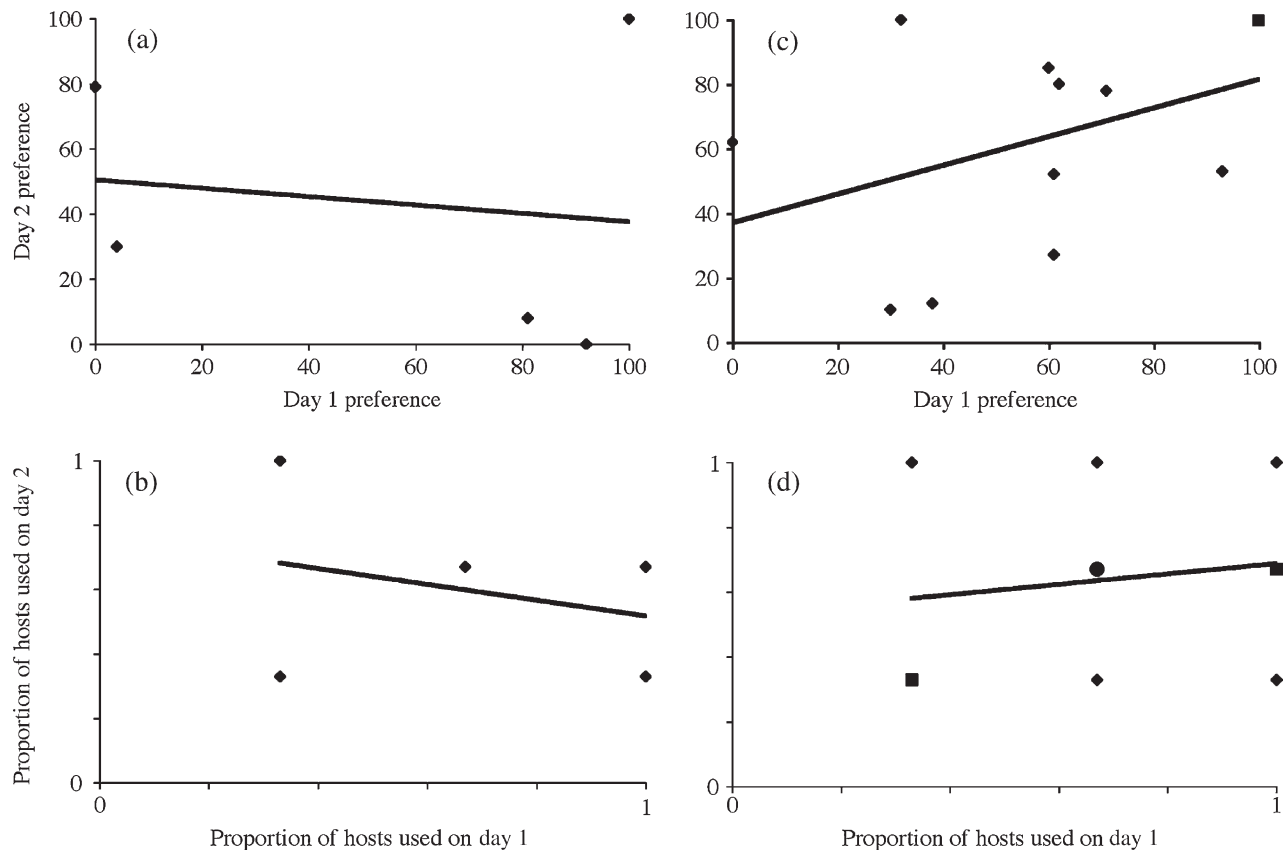
Fig. 3. Regression analyses for bioassays of female *Papilio machaon aliaska* host-plant preferences. In all graphs, diamonds represent the response of a single individual, squares represent two individuals and the circle represents three individuals with the same response. (a) Whole plant preferences of *P. m. aliaska* females on the first and second days of experiment 1 ($R^2 = 0.02$, $F_{1,3} = 0.06$, $P = 0.82$). The ancestral host plant *Cnidium cnidiifolium* is used as the reference species. (b) Proportion of the host plants that *P. m. aliaska* females used on the first and second days of experiment 1 ($R^2 = 0.02$, $F_{1,8} = 0.17$, $P = 0.69$). (c) Host-plant preferences of *P. m. aliaska* females on the first and second days of experiment 2 ($R^2 = 0.17$, $F_{1,10} = 2.0$, $P = 0.19$). The ancestral host plant *C. cnidiifolium* is used as the reference species. (d) Proportion of the host plants that *P. m. aliaska* females used on the first and second days of experiment 2 ($R^2 = 0.03$, $F_{1,10} = 0.27$, $P = 0.62$).

Experiment 2 – plant sprigs in clear boxes

Female preferences were erratic from the first day of the experiment to the second day (Fig. 3c; $R^2 = 0.17$, $F_{1,10} = 2.0$, $P = 0.19$). Two females only oviposited on *C. cnidiifolium* while the other females varied in their use of *C. cnidiifolium* between the two days. Females varied in the proportion of host plants that they would accept on the two days of the experiment (Fig. 3d; $R^2 = 0.03$, $F_{1,10} = 0.27$, $P = 0.62$). Some females expanded the number of hosts they used from the first day to the second while others restricted the number of hosts that they used.

Experiment 3 – plant extracts in large cages

The female offspring of many dams, regardless of their original field site, tended to prefer the ancestral host plant (Table 1). There do not appear to be any clear distinctions in female preference determined by either dam or the dam's original field site. These results do not include female E1 because only two of her female offspring emerged and neither was attracted to any plant extracts. Several females preferred two host plants equally; when this happened, it was most often a tie between *C. cnidiifolium* and *P. frigidus* (Table 1). Female offspring of all dams preferred one or more of the host plants to the control (Table 2, Fig. 4). There were no clear patterns in preference for single host plants either within single dams or among dams from a single field site. All dams had offspring that were attracted to several of the host plants, some significantly so (Table 2, Fig. 4). Indeed, there were only four females that laid all of their eggs on a single host-plant species. Two females specialised on *C. cnidiifolium*; they were offspring of dams M1 and E6 and were both reared on *A. arctica*. The other two females specialised on *P. frigidus*; they were offspring of dams E5 and W3 and were both reared on *C. cnidiifolium*.

The plant species that the females were reared on as larvae does not appear to affect their preference for host plants as adults (Tables 1 and 2, Fig. 5). A majority of the females reared on *A. arctica* preferred *C. cnidiifolium* during the oviposition trials (Table 1). Females that were reared on both *A. arctica* and *C. cnidiifolium* preferred host-plant extracts to the solvent control, but females reared on *A. arctica* were particularly attracted to *C. cnidiifolium* extracts (Table 2, Fig. 5). Finally, of the four females that specialised on a single host plant, none were reared on that particular host plant.

Discussion

Papilio machaon aliaska females vary greatly in their oviposition behaviour and their preferences for the three host plants. In the first two experiments, neither of the regression models explained a significant portion of the variance in female preference (Fig. 3a,c). Hence, based on the original predictions, neither the model in which females prefer certain plants (Fig. 1a), nor the model in which females change their preferences over time (Fig. 1c) consistently predicted female behaviour. That the population is composed mainly of generalists is the remaining explanation (Fig. 1b). Although most females were generalists, a few pursued a more specialised strategy. Three females accepted only a single host plant; all of these specialists chose *C. cnidiifolium*, the ancestral host plant (Fig. 3). The other females laid eggs on at least two of the host plants and often accepted all three host plants on at least 1 day of the experiment (Fig. 3).

Neither regression model explained the variance associated with the proportion of the hosts that were accepted (Fig. 3b,d). Given the predictions (Fig. 2), some females exemplified 'diet expansion', accepting more plant species as time progressed, while other females were part of the 'diet restriction' group and narrowed their range of acceptable host plants. Still other

Table 1. Number of female offspring that preferred the polar extracts of *Cnidium cnidiifolium* (*Cc*), *Artemisia arctica* (*Aa*), or *Petasites frigidus* (*Pf*) either singly or equally between species. Females are first grouped within dam; dam nomenclature refers to whether she was caught on Wickersham dome (W), Ester dome (E), or Murphy dome (M). Females are next grouped within the host-plant species on which they were reared as larvae.

	Preferred one species most			Preferred two species equally			
	Cc	*Aa*	*Pf*	*Cc & Aa*	*Cc & Pf*	*Aa & Pf*	Total no.of female offspring
Dam							
W1	1						1
W2	3	1					4
W3	5	1	1		1		8
W4	2	2	2		1		7
W5	1		3	1	1		6
E2		1			1		2
E3	3	1			1		5
E4		1					1
E5	2	1	2		2		7
E6	3	1					4
M1	3		2				5
Larval food plant							
Cc	7	5	5		3		20
Aa	16	3	6	1	3		29
Pf		1					1

Table 2. Female preferences for the polar extracts of *Cnidium cnidiifolium* (Cc), *Artemisia arctica* (Aa), *Petasites frigidus* (Pf), or the solvent control. F_r and *P*-values represent the results of non-parametric Friedman tests of extract ranks. For each comparison d.f. = 3. Females are first grouped within dam; dam nomenclature refers to whether she was caught on Wickersham dome (W), Ester dome (E), or Murphy dome (M). Females are next grouped within the host-plant species on which they were reared as larvae.

Female grouping	*n*	F_r	*P*-value	Specific comparisons	
				P < 0.05	*P* < 0.1
By Dam†					
W2	4	8.7	<0.05	Cc > control	
W3	8	12.7	<0.01*	Cc > control	Aa, Pf > control
W4	7	8.5	<0.05		Cc, Pf > control
W5	6	10.9	<0.02*	Pf > control	Cc > control
E2	2	12.2	<0.01*	n.a.§	
E3	5	14.2	<0.01*	Cc > control	Pf > control
E5	7	13.4	<0.01*	Cc, Pf > control	
E6	4	8.0	<0.05		Cc > control
M1	5	13.4	<0.01*	Cc > control	
By larval food‡					
Cc	20	25.0	<0.001**	Cc, Aa, Pf > control	
Aa	29	56.4	<0.001**	Cc, Aa, Pf > control; Cc > Aa	

*Remains significant (a < 0.05) after Bonferroni adjustment to correct for Type I error.
**Remains significant (a < 0.01) after Bonferroni adjustment to correct for Type I error.
†Does not include the two females that only had one surviving female offspring.
‡Does not include the single female that was reared on *P. frigidus*.
§Sample size too small to make comparisons.

females were consistent in the number of hosts that they used each day. Although their absolute number of acceptable hosts did not change over the experiment, some of these females substituted one species for another from one day to the next and thus may not be as consistent as they appear in Fig. 3. The results from this part of the experiment correspond with the previous results and indicate that the populations appear to be composed mostly of generalists.

Singer *et al.* (2002) cautioned that because females in the wild do not select host plants randomly, oviposition studies that randomly select host plants from the field may not discover a female's real preference hierarchy. The plants randomly selected by the researcher may not be the same plants that the ovipositing insect would choose. Perhaps *P. m. aliaska* females tended to prefer *C. cnidiifolium* because acceptable *A. arctica* and *P. frigidus* plants were not included in the bioassay cages. This seems unlikely, however, because the plants that were included in the bioassays were not shunned and some females did prefer them on at least 1 day during the experiments. It seems unlikely that variation in preference for either *A. arctica* or *P. frigidus* would outweigh the interspecific variation in preference between *A. arctica*, *P. frigidus*, and *C. cnidiifolium*.

Any potential variation in the acceptability of particular plants was controlled for in the third experiment. In this experiment, preference hierarchies were established by testing chemical extracts of the host plants that were created from at least several dozen plants of each species. Females tended to rank *C. cnidiifolium* extracts highest in preference hierarchies (Tables 1 and 2), but also laid more eggs on both of the other host plants than the control (Fig. 4). Just four of the 50 females tested accepted only a single host plant: two accepted only *C. cnidiifolium* and the other two only *P. frigidus*. In comparisons of the

preferences among siblings, there was no clear hierarchy of host-plant preference (Table 1). General trends in host-plant hierarchy also did not exist between the offspring of dams from the same field site (Tables 1 and 2). If certain populations had begun to diverge into host races, then females from one site (e.g. Ester dome) may have been expected to rank host plants differently than females from another site (e.g. Wickersham dome). Finally, this experiment lent no support for the Hopkins' host selection principle. Females reared on *C. cnidiifolium* ranked any of the three host plants as most preferred and females reared on *A. arctica* were actually most likely to prefer *C. cnidiifolium* to the other host plants (Table 1). Both the females reared on *C. cnidiifolium* and *A. arctica* preferred all three host plants to the control (Table 2, Fig. 5).

Female preference for host plants, despite being a trait expressed only in females, has been shown to be paternally inherited in two other *P. machaon* swallowtails (Thompson, 1988b). Thus, if the assumption that females were unmated when collected was incorrect, then mating field-collected females with new males in the laboratory may have inadvertently created half-sibs with different preference alleles. This scenario seems unlikely, however, because the females were caught as they approached the top of the dome, often several hours after all of the territorial males had been caught, and would therefore not have had previous opportunities to mate. Also, all of the females appeared fresh and young, without any visible damage to indicate that they were older and had already mated. Finally, during a previous field season, females that were caught in the same manner were left unmated and they laid unfertilised eggs (S. M. Murphy, unpublished data). The possibility that the females had mated previously in the field cannot be discounted, however, as other closely related swallowtail species are known to mate
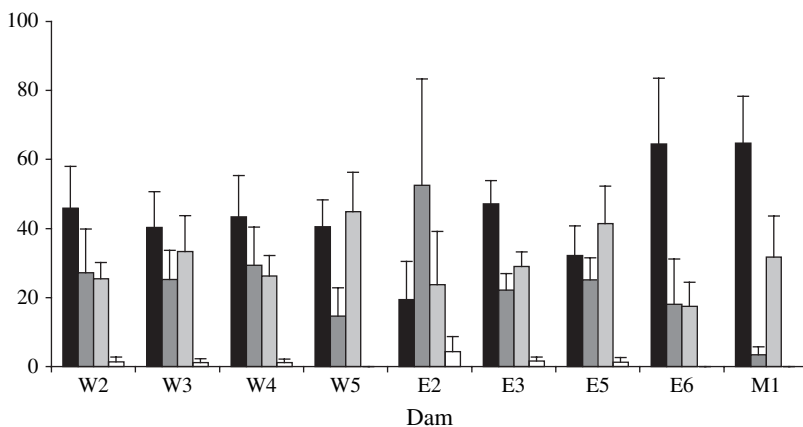
Fig. 4. Mean percentage of eggs laid by female *Papilio machaon aliaska* siblings on whole plant extracts (+ SE). The females were the offspring of nine wild-caught *P. m. aliaska* females from Wickersham dome (W2, *n* = 4; W3, *n* = 8; W4, *n* = 7; W5, *n* = 6), Ester dome (E2, *n* = 2; E3, *n* = 5; E5, *n* = 7; E6, *n* = 4) and Murphy dome (M1, *n* = 5). The females in this bioassay were tested on host-plant extracts of *Cnidium cnidiifolium* (black bars), *Artemisia arctica* (dark grey bars), and *Petasites frigidus* (light grey bars) as well as a solvent control (open bars). See Table 2 for comparisons between host plants.

multiple times (e.g. *Papilio polyxenes*, Lederhouse, 1981). If the females in the bioassays were indeed half-sibs, the result of multiple mating, then this may partially explain why some female offspring preferred one species while others preferred another plant species. The several females that found two species equally attractive, however, would not be explained by this premise and their behaviours are least supportive of any potential host specialisation or host race formation.

Host races are usually invoked in studies of sympatric speciation (Bush, 1969; Feder *et al.*, 1988; Caillaud & Via, 2000; Filchak *et al.*, 2000; Berlocher & Feder, 2002; Dres & Mallet, 2002), which seems unlikely in the case of *P. m. aliaska* because many of the factors believed to facilitate host race formation (e.g. mating on the host plant) are not fulfilled. Conclusive identification of host races is difficult to achieve because ecological, behavioural, and genetic studies are all needed to establish that a population is at least partially reproductively isolated from conspecific populations and that this isolation is a direct result of adaptation to a specific host plant (Diehl & Bush, 1984). Until genetic studies of *P. m. aliaska* population struc-

ture and gene flow resolve the question, these results suggest that populations of *P. m. aliaska* have not yet differentiated into host races, at least in central Alaska.

Female preference for the three host plants does not appear to be inherently fixed. Host selection also does not depend on where the female is from or what host plant she fed upon as a larva. Females often preferred *C. cnidiifolium* and a few selected it exclusively. *Cnidium cnidiifolium* is generally the most suitable food plant for developing larvae in the laboratory (Murphy, 2004, 2005), yet the majority of females oviposited on all three hosts and some even preferred one of the other hosts to *C. cnidiifolium*. Female preference and larval performance need not necessarily correspond, especially if the relative suitability of host plants or their environments change through time or space (Rausher, 1979; Thompson, 1988a).

It is proposed that the data presented here suggest that the generalised selection of host plants by *P. m. aliaska* females is a 'bet-hedging' strategy. Shared chemical cues in ancestral and novel host plants may have provided the opportunity for the establishment of the host expansion onto the two novel host species (Murphy & Feeny, in press). Predators are common in the ancestral host plant's environment and larval mortality on *C. cnidiifolium* can be very high in the field; enemy-free space on the novel host plants may be the selective pressure maintaining the host expansion, possibly driving the incipient host shift to completion (Murphy, 2004). In years when predation risk is great, eggs laid on the novel hosts (*A. arctica* and *P. frigidus*) would be more likely to survive than eggs laid on the ancestral host (*C. cnidiifolium*) where predators are common. By contrast, in years with lesser predation risk, larval fitness would be greater on the ancestral host than on either of the novel hosts. In environments and periods of time when predation risk is inconsistent, laying a few eggs on each of the hosts may be the most prudent strategy to ensure the success of at least some progeny. If predator numbers decline, then selection may favour females that specialise on the ancestral host because it seems to be a better host for most larvae (Murphy, 2005). Conversely, if predation risk remains high for several years, then selection may favour a complete host shift to the novel host plants and a final abandonment of the ancestral plant (Murphy, 2004). Thus, consistent host-associated predation may lead to selection for more
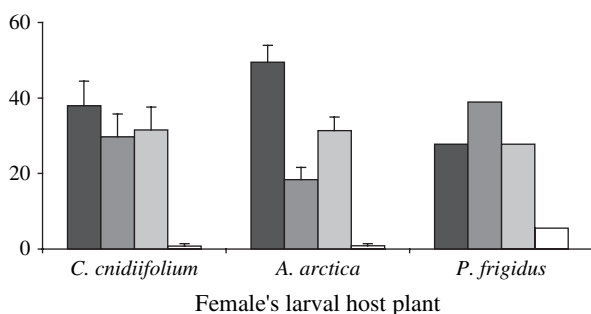
Fig. 5. Mean percentage of eggs laid by female *Papilio machaon aliaska* siblings on whole plant extracts (+ SE). The females were the offspring of nine wild-caught *P. m. aliaska* females and reared on *Cnidium cnidiifolium* (*n* = 20), *Artemisia arctica* (*n* = 29), or *Petasites frigidus* (*n* = 1). The females in this bioassay were tested on host-plant extracts of *C. cnidiifolium* (black bars), *A. arctica* (dark grey bars), and *P. frigidus* (light grey bars) as well as a solvent control (open bars). See Table 2 for comparisons between host plants.

specific host preferences and perhaps the eventual formation of host races, but current evidence suggests that this has not yet happened.

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