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SHORT COMMUNICATION

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Behavioral evidence for host fidelity among populations of the parasitic wasp, *Diachasma alloeum* (Muesebeck)

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Abstract The concept of "host fidelity," where host-specific mating occurs in close proximity to the oviposition site and location of larval development, is thought to impart a pre-mating isolation mechanism for sympatric speciation (*sensu* members of the genus *Rhagoletis*). The apple maggot fly, *Rhagoletis pomonella*, and the blueberry maggot fly, *R. mendax*, are morphologically similar sibling species thought to have speciated in sympatry by divergence of host plant association. Both of these fly species are attacked by the specialist braconid parasitoid, *Diachasma alloeum*. The current study demonstrates that both male and female *D. alloeum* exhibit a behavioral preference for the odor of the fruit of their larval *Rhagoletis* host species. Specifically, those *D. alloeum* emerging from puparia of *R. pomonella* are preferentially attracted to hawthorn fruit and those emerging from puparia of *R. mendax* are preferentially attracted to blueberry fruit. However, male *D. alloeum* reared from either *R. pomonella* or *R. mendax* were equally attracted to females originating from both *Rhagoletis* species. We suggest that the data herein present evidence for "host fidelity," where populations of *D. alloeum* exhibit a greater tendency to mate and reproduce among the host plants of their preferred *Rhagoletis* hosts. Furthermore, host fidelity may have resulted in the evolution of distinct host races of *D. alloeum* tracking the speciation of their larval *Rhagoletis* prey.

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Introduction

Tephritid flies of the *Rhagoletis pomonella* sibling species complex include morphologically similar species, each closely associated with a particular host plant. Host races of the apple maggot fly, *R. pomonella* (Walsh), are attracted to exact blends of volatiles emitted by their specific host fruit (Nojima et al. 2003a, b; Linn et al. 2003). The *Rhagoletis* sibling species complex has served as a long-running model system for sympatric speciation given the unique biology of these animals (Bush 1966; Berlocher 2000). Specifically, the concept of "host fidelity," where host-specific mating occurs in close proximity to the oviposition site and location of larval development (host fruit), imparts pre-mating isolation among diverging and existing species (Feder et al. 1994; Feder 1998). Given that mating occurs exclusively on or near unabscised host fruits directly prior to oviposition (Feder et al. 1997), and that flies of both sexes exhibit strong innate attraction to the volatiles of their host fruit, it has been suggested that host fidelity can potentially impart complete pre-mating isolation between sympatric *Rhagoletis* species (Filchak et al. 2000).

Braconid wasps of the subfamily Opiinae parasitize larval stages of the Tephritidae (Wharton and Marsh 1978). *Diachasma alloeum* (Muesebeck) is a specialist parasitoid and occurs on hawthorn, *Crataegus mollis* Scheele, and highbush blueberry, *Vaccinium corymbosum* L., in the northeastern United States and bordering regions of Canada (Stelinski et al. 2004). It specifically attacks the apple maggot, *R. pomonella* (Glas and Vet 1983) and the blueberry maggot, *R. mendax* Curran (Liburd and Finn 2003; Stelinski et al. 2004). Studies of *D. alloeum* that attack hawthorn-race *R. pomonella* using abscised fruit have suggested that visual cues are important stimuli used by females for locating hawthorn fruit (Glas and Vet 1983). In addition, the vibrations produced by *R. pomonella* larvae feeding within hawthorn fruit appeared to stimulate location of host-infested hawthorns by female wasps (Glas and Vet 1983). However, a more contemporary study focusing on the foraging behavior of feral *D. alloeum* in blueberries suggests that female *D. alloeum* exploit chemical cues emitted by unabscised, host-infested fruit (Stelinski et al. 2004). Specifically, *D. alloeum* were more attracted to and spent more time on blueberry fruit infested with *R. mendax* larvae compared with uninfested blueberry fruit (Stelinski et al. 2004).

D. alloeum may represent a unique and novel model for sympatric speciation in which the parasitoid has tracked the speciation of their *Rhagoletis* fly hosts. The present study suggests that sympatrically occurring populations of *D. alloeum* infesting both *R. pomonella* in hawthorns and *R. mendax* in blueberries exhibit evidence for behavioral host fidelity, yet respond equally to one another's female-produced sex pheromone. Thus, the behavioral association with the fruit of their larval hosts may be creating distinct "host races" of parasitoids, the posited first step in sympatric speciation (Feder et al. 1994).

Materials and methods

Insect source

R. mendax puparia were collected from infested fruit of unsprayed blueberries (var. Jersey) from an abandoned blueberry plantation in Fennville, Mich., described by Stelinski et al. (2004). Puparia of hawthorn-race *R. pomonella* were also obtained in Fennville from infested hawthorn growing within 0.8 km of the above-mentioned blueberry plantation. Although it cannot be ruled out that the 0.8-km separation between these two sites has resulted in allopatric populations, braconid parasitoids are known to disperse up to 1.6 km (Barlow et al. 1998; Nouhuys and Hanski 2002), suggesting that the populations were sympatric. Collected puparia were placed into cold storage for 140 days at 4°C. Pupal development was initiated by removing puparia from cold storage and placing them into an environmental chamber at 24°C, 55–60% RH, under a 16:8 (L:D) photocycle. Three weeks after removal of puparia from 4°C, *D. alloeum* began emerging from ca. 55 and 38% of *R. mendax* and *R. pomonella* puparia, respectively. The parasitoids have been identified by R.A. Wharton (Texas A&M University) and voucher specimens were deposited at Michigan State University. Prior to testing, wasps were maintained in aluminum-screen cages (30 cm×30 cm×30 cm) and supplied with 5% sugar water. Adults were kept at 24°C, 55–60% RH, under a 16:8 (L:D) photocycle.

Y-tube olfactometer studies

Choice tests comparing behavioral responses of *D. alloeum* to various olfactory stimuli were conducted in a horizontal, glass Y-tube olfactometer (stem length=25 cm, arm length=12.5 cm, internal diameter=2.0 cm). Experiments were conducted at 24°C with a light intensity of 800–1,000 lux generated by two fluorescent bulbs mounted 0.5 m above the olfactometer. Assays ran between 1,230 and 1,500 h, a period when *D. alloeum* are highly active in the field (Stelinski et al. 2004). All wasps used in Y-tube tests were 3–4 days post-emergence, unmated, and never exposed to blueberry or hawthorn fruit prior to the assay. The number of female *D. alloeum* orienting to and eventually contacting stimulus sources in each arm of the olfactometer was recorded. All fruit used in behavioral assays was kept free of *Rhagoletis* infestation in the field by applying mesh bags over developing fruit clusters. which prevented fly oviposition but did not interfere with normal fruit development as described in Stelinski et al. (2004). All fruits were dissected following assays to confirm zero infestation.

In experiment 1, male *D. alloeum* originating from *R. pomonella* (hawthorn) or *R. mendax* (blueberry) were presented with virgin female *D. alloeum* originating either from *R. pomonella* or *R. mendax*. Females of each type were randomly selected and inserted individually into one arm of the Y-tube. The control arm of the Y-tube was left empty. Soon after test stimuli were inserted, carbon-filtered and humidified air (100 ml/min) was delivered via Tygon tubing into each arm of the Y-tube. Typically, wasps exhibited random walking limited to the first 1.5 cm of the Y-tube stem during the 30–45 s prior to introductions of odor stimuli. Directly after an introduction of a stimulus, wasps exhibited arrestment, followed by upwind orientation after a short time interval. Treatment pairs tested in the choice test were: (1) *R. pomonella*-origin *D. alloeum* females versus control, and (2) *R. mendax*-origin *D. alloeum* females versus control.

In the second experiment, male and female *D. alloeum* originating from *R. pomonella* or *R. mendax* were presented with freshly picked blueberry or hawthorn fruit. As before, fruit types were randomly selected and inserted individually into one arm of the Y-tube. Glass marbles of similar size and color to each fruit type were used as visual controls. As in experiment 1 above, wasp orientation within the Y-tube did not commence until the odor stimulus was presented with moving air. Treatment pairs tested in the choice test were: (1) hawthorn fruit versus control, and (2) blueberry fruit versus control.

The final experiment directly compared the attractiveness of *R. pomonella*-origin versus *R. mendax*-origin, virgin female *D. alloeum* and hawthorn versus blueberry fruit to virgin *D. alloeum* originating from either *R. pomonella* or *R. mendax*. These direct-choice tests were conducted to determine whether wasps could distinguish natal fruit odors in the presence of non-natal fruit odors and whether males could distinguish the sex-pheromone of females of the same origin in the presence of females originating from the other fruit type. Choice tests were conducted using the Y-tube as described above. Treatment pairs tested in the choice test were: (1) *R. pomonella*-origin *D. alloeum* female versus *R. mendax*-origin *D. alloeum* female, and (2) hawthorn fruit versus blueberry fruit. In each of the above experiments, groups of five *D. alloeum* males or females were inserted into the olfactometer per replicate and ten replicate groups were assayed per treatment. Observations were conducted for 5 min on each group of wasps assayed. During assays, multiple, identical Y-tubes were used in succession. Each Y-tube was thoroughly washed with acetone and air-dried directly after a treatment replicate had been conducted. All data were subjected to a two-way analysis of variance (ANOVA) and differences between means were separated using Tukey's multiple comparisons test (SAS Institute 2000). In all cases, significance level was $\alpha<0.05$.

Results

Experiment 1

Significantly more ($F_{(2,36)}=9.8$; $P<0.01$) *R. mendax*-origin male *D. alloeum* reached either *R. mendax*- or *R. pomonella*-origin female *D. alloeum* compared with the control arm of the Y-tube (Table 1). Statistically equal ($F_{(2,36)}=1.4$; $P=0.8$) proportions of *R. mendax*-origin *D. alloeum* males contacted virgin females originating from *R. mendax* and *R. pomonella* (Table 1). As observed with *R. mendax*-origin male wasps, significantly more ($F_{(2,36)}=14.8$; $P<0.01$) *R. pomonella*-origin males reached either *R. mendax*- or *R. pomonella*-origin female *D. alloeum* com-

Table 1 Percentages (mean±SE) of naïve male *Diachasma alloeum* contacting females in Y-tube olfactometer

Female type	*D. alloeum* males from *R. mendax* contacting source in Y-tube			*D. alloeum* males from *R. pomonella* contacting source in Y-tube		
	Treatment		Control	Treatment		Control
D. alloeum from *R. mendax*	72.0±1.3a[a]	*	10.0±1.1a	64.0±1.4a	*	6.0±1.0a
D. alloeum from *R. pomonella*	67.0±1.4a	*	10.0±1.1a	73.0±1.3a	*	4.0±1.0a

[a] Means in the same column followed by the same letter are not significantly different and paired values within rows marked with an *asterisk* are significantly different (P<0.05).

Table 2 Percentages (mean±SE) of naïve male or female *Diachasma alloeum* contacting fruit in Y-tube olfactometer (NS indicates lack of significance)

Fruit type	*D. alloeum* males/females from *R. mendax* contacting source			*D. alloeum* males/females from *R. pomonella* contacting source		
	Treatment		Control	Treatment		Control
Blueberry	34.0±1.4a[a]/	*	6.0±1.0a/	6.0±1.0b	NS	2.0±1.0a/
	30.0±1.4a	*	4.0±1.0a	4.0±1.0b	NS	0.0±0.0a
Hawthorn	6.0±1.0b/	NS	6.0±1.0a/	44.0±1.5a	*	2.0±1.0a/
	0.0±0.0b	NS	2.0±1.0a	40.0±1.4a	*	4.0±1.4a

[a] Means in the same column (within sex) followed by the same letter are not significantly different and paired values within rows marked with an *asterisk* are significantly different (P<0.05).

Table 3 Percentages (mean±SE) of naïve male or female *Diachasma alloeum* contacting females or fruit in choice tests using Y-tube olfactometer (*NS* indicates lack of significance)

Wasp type	Female odor source			Fruit odor source		
	R. pomonella-origin		*R. mendax*-origin	Hawthorn		Blueberry
D. alloeum males/females from *R. pomonella* contacting source	42.0±1.4a[a]/	NS	38.0±1.3a/	36.0±1.4a/	*	6.0±1.0a/
	–		–	40.0±1.4a	*	4.0±1.0a
D. alloeum males/females from *R. mendax* contacting source	30.0±1.4b/	NS	40.0±1.4a/	2.0±1.0a/	*	44.0±1.4a/
	–		–	0.0±0.0a	*	48.0±1.4a

[a] Means in the same column (within sex) followed by the same letter are not significantly different and paired values within rows marked with an *asterisk* are significantly different (P<0.05).

pared with the control (Table 1). Once again, statistically equal ($F_{(2,36)}$=1.1; P=0.7) proportions of this wasp type contacted virgin females originating from *R. mendax* and *R. pomonella* (Table 1). Courtship and copulations between male and female *D. alloeum* originating from the same *Rhagoletis* host species (*R. pomonella* or *R. mendax*) appeared identical to those that occurred when sexes were from opposite hosts (methods according to Boush and Baerwald 1967, N=30, data not shown).

Experiment 2

Significantly more ($F_{(2,36)}$=12.3 and 21.0, respectively; P<0.01) *R. mendax*-origin male and female *D. alloeum* contacted a fresh blueberry compared with the control (Table 2). However, blueberry fruit did not elicit a significant ($F_{(2,36)}$=0.8 and 1.0, respectively; P>0.3) response from hawthorn-origin *D. alloeum* of either sex (Table 2). In contrast, significantly more ($F_{(2,36)}$=16.7 and 21.2, respectively; P<0.01) hawthorn-origin male and female *D. alloeum* contacted hawthorn fruit compared with the control, while *R. mendax*-origin *D. alloeum* of either sex did not respond ($F_{(2,36)}$=0.8 and 0.5, respectively; P>0.5) to this fruit type (Table 2).

Experiment 3

In direct-choice tests between two stimuli, *R. pomonella*-origin male *D. alloeum* were equally likely ($F_{(2,36)}$=0.4; P=0.6) to contact virgin females originating from the same host as they were to contact *R. mendax*-origin virgin females (Table 3). An identical result was observed for *R. mendax*-origin male *D. alloeum* ($F_{(2,36)}$=1.7; P=0.5) (Table 3). However, when presented with a choice between the fruit type of their natal host and that of the non-natal host, significantly more ($F_{(2,36)}$=23.5 and 18.7, respectively; P<0.01) male and female *R. pomonella*-origin *D. alloeum* chose hawthorn over blueberry fruit while significantly more ($F_{(2,36)}$=17.8 and 14.3, respectively; P<0.01) *R. mendax*-origin *D. alloeum* of both sexes chose blueberries over hawthorns (Table 3).

Discussion

The data presented herein suggest that male and female *D. alloeum* exhibit a behavioral preference for the fruit of their natal *Rhagoletis* host species. In a companion study to be published separately, experience with *R. mendax*-infested blueberry fruit doubled the proportion of *D. al-*

loeum that subsequently responded to volatiles from uninfested blueberry fruit compared with naïve wasps (L.L. Stelinski et al., unpublished data). *D. alloeum* is a host specialist, restricted to the sibling species *R. pomonella* and *R. mendax*. Specialist parasitoids are known to exhibit innate responsiveness to olfactory stimuli associated with their host or host habitat, which increases with experience (Vet 1983).

Courtship and mating in *D. alloeum* occur on host-plant foliage within close proximity of fruit (L.L. Stelinski, personal observation). We have observed adult *D. alloeum* of both sexes hovering within 30 cm of host fruit with mating occurring within minutes thereafter (L.L. Stelinski et al., unpublished data). The behavioral preference of *D. alloeum* for the particular host plant of their fruit-parasitic larval prey may be indicative of host fidelity, where populations of *D. alloeum* exhibit a greater tendency to mate and reproduce among the host plants of their *Rhagoletis* prey species. This suggests that there may exist distinct "host races" of *D. alloeum*, one attacking *R. pomonella* in hawthorn and the other attacking *R. mendax* in blueberries. It has been posited that such host-associated adaptations may eventually isolate host races to an extent that they essentially become distinct species (Feder et al. 1994).

The courtship behavior of *D. alloeum* (reported originally as *Opius alloeus*) is driven by male attraction to a female-produced sex pheromone (Boush and Baerwald 1967). Evolution of traits increasing assortative mating, such as species-specific pheromones, may further isolate host races already separated by host-associated behavioral adaptations. However, the current data demonstrated that male *D. alloeum* reared from either *R. mendax* or *R. pomonella* were equally attracted to females originating from both *Rhagoletis* species, implying that gene flow could exist between these two putative host races. Gene flow between hawthorn and apple-infesting host races of *R. pomonella* has been estimated at ca. 6% per generation (Feder et al. 1994). However, the potential existence of post-zygotic isolating mechanisms cannot be ruled out and must be explored to determine whether these two populations are distinct species.

The next steps in this investigation of the differences between *D. alloeum* populations infesting *R. mendax* and *R. pomonella* will include morphometric and genetic analyses. Furthermore, identification of the specific volatiles attractive to *R. pomonella*- and *R. mendax*-infesting *D. alloeum* will facilitate subsequent behavioral analyses.

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