

# Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host

Margaret S. Keeler · Frances S. Chew

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**Abstract** Exotic plants may act as population sinks or evolutionary traps for native herbivores. The native butterfly *Pieris oleracea* lays eggs on garlic mustard, *Alliaria petiolata*, but larvae develop very poorly on this exotic invasive plant. We examined oviposition preference of individual females and larval performance of their offspring for individuals from one area where garlic mustard is well established and one where it is absent. These data were used to assess whether garlic mustard is being incorporated into or excluded from the diet. Females from the area without garlic mustard showed a wide range of preference, families had low larval survival on garlic mustard, and larval survivorship showed no correlation with mothers' preferences. Females from the area with garlic mustard preferred it to the native host, and larval survivorship on garlic mustard was positively correlated with the mother's preference. Individuals surviving on garlic mustard took longer to pupate and weighed >30% less compared to pupae reared on normal hosts. Our results suggest that where garlic mustard is well established *P. oleracea* may be adapting to this plant by both improved larval performance and increased adult female oviposition preference for it.

**Keywords** *Pieris* · *Alliaria petiolata* · Oviposition · Diet breadth · Population sink

## Introduction

Herbivorous insects encounter novel plant species when an insect colonizes a new plant community or when an exotic plant invades its range (e.g., Cuda et al. 2002; Louda et al. 2005; Mattila and Otis 2003). Because a new ecological context may alter the consequences of normal behavior for a native insect, such encounters offer an opportunity to observe the evolutionary trajectory of the interaction between insect and novel plant. We consider here responses of a native insect to an exotic invasive plant that presents an evolutionary trap (Schlaepfer et al. 2005) because it is highly attractive to the insect, but the insect develops extremely poorly on this food resource (Keeler et al. 2006).

In holometabolous insects with relatively immobile juveniles, such as butterflies, females choose host plants for oviposition, but the consequences of those behavioral choices are borne by their larval offspring. Therefore, the relationship between adult oviposition preference and larval survival and growth (hereafter, performance) is fundamental to understanding many aspects of host plant use including host specificity (e.g., Berdegue et al. 1998; Bossart and Scriber 1999; Joshi and Thompson 1995), and host shifts (e.g., Gross et al. 2004; Sezer and Butlin 1998).

From an insect viewpoint, encounters with a novel host may have several outcomes depending on whether the insect lays eggs (or not) on the novel plant and whether the plant supports larval development (or not). First, if adults fail to recognize the novel plant as a potential host, there may be no direct impact on the native insect [e.g., *Colias philodice* on crown vetch (*Coronilla varia*), Karowe 1990; *Danaus plexippus* on swallow-wort (*Vincetoxicum* spp.), DiTommaso and Losey 2003; but see contrasting results of field-collected individuals in a different geographic region, Casagrande and Dacey 2007]. However, even if not

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M. S. Keeler · F. S. Chew (✉)  
Department of Biology, Tufts University,  
Medford, MA 02155, USA  
e-mail: fchew@tufts.edu

recognized as a potential host, the exotic plant may interfere with host finding by altering the apparency of the native plant or repelling the insect (reviewed by Hämbäck and Beckerman 2003).

Second, if females lay eggs on the novel plant because it is related to native hosts or has similar chemistry, behavioral and physiological pre-adaptation of larvae may permit its incorporation into the diet [e.g., *Euphydryas editha* on *Collinsia*, Singer 1971; *Colias philodice eriphyle* on alfalfa (*Medicago sativa*), Tabashnik 1983; *E. editha* on *Plantago lanceolata*, Thomas et al. 1987]. Some consequences may include competitive release of the herbivore population [e.g., *Rhagoletis pomonella* on apples (*Pyrus malus*), Feder et al. 1995] or herbivore exploitation of an enemy-free space [e.g., *Papilio machaon aliaska* on *Asteraceae*, Murphy 2004; *Phthorimaea operculella* on tomato (*Lycopersicon esculentum*), Mulatu et al. 2004]. The broader diet may permit the herbivore to expand its range or extend its flight season [e.g., *Pieris napi microstriata* on watercress (*Rorippa nasturtium-aquaticum*), Shapiro 1975; *Papilio zelicaon* on sweet fennel (*Foeniculum vulgare*), Sims 1980].

Third, adult females may lay eggs on the novel plant but larval offspring may be unable to develop on it (e.g., *Pieris virginiensis* on garlic mustard, Bowden 1971; Courant et al. 1994; Porter 1994; *Pieris napi macdunnoughii* and *Pieris occidentalis* on pennycress (*Thlaspi arvense*), Chew 1975, 1977; *Anthanassa ardis* and *Anthanassa tulcis* on *Hypoestes* spp., Feldman and Haber 1998; *D. plexippus* on *Vincetoxicum* spp., Casagrande and Dacey 2007). If natural selection occurs, the evolutionary trajectory may depend on whether oviposition preference or larval performance evolves more rapidly (Chew 1977; Karowe 1990). Where adult females lay eggs on a plant that does not support larval development, we might expect natural selection to favor either adult females with less or no preference for the exotic plant, or larvae with better performance on the novel plant.

We investigate the potential outcome of encounter between exotic invasive garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara & Grande (Brassicaceae) and *Pieris napi oleracea* Harris, a bivoltine butterfly native to eastern North America. [*P. oleracea* is a member of the Holarctic species complex *P. napi* L.; see Chew and Watt 2006 and references therein for discussion of relationships within *Pieris*.] Garlic mustard was introduced from Europe in 1868 (Nuzzo 1993), and has since spread to 36 American states and four Canadian provinces (Blossey et al. 2001; Welk et al. 2002; IPANE 2003), including much of *P. oleracea*'s range (Klots 1951; Opler and Krizek 1984). *P. oleracea* is currently more abundant where garlic mustard is absent compared to where it is well established (Benson et al. 2003; Nuzzo 1993), perhaps because *P. oleracea* oviposits on *A. petiolata* (Courant et al. 1994;

Huang et al. 1995) but larvae rarely survive on this plant (Bowden 1971; Courant 1996). The plant is thus a population sink (Casagrande and Dacey 2007) or an evolutionary trap (Schlaepfer et al. 2005). Displacement (after competition) or replacement (after disturbance) of native hosts by garlic mustard could reduce butterfly populations, potentially endangering the butterfly locally (Blossey et al. 2001; Keeler et al. 2006). Larval adaptation could allow a larger population to remain after loss of the native host (Courant et al. 1994; Porter 1994).

Oviposition on garlic mustard is attributed to its glucosinolate similarity to native hosts (Huang et al. 1995; Renwick 2002), whereas larval feeding is partially deterred by at least two compounds, isovitexin-6'- $\beta$ -glucopyranoside and alliarinoside (Haribal et al. 2001; Renwick et al. 2001), and cyanide produced by garlic mustard (Cipollini and Gruner 2007) may affect larval growth. Although glucosinolate profiles of potential host plant species were closely correlated with oviposition behavior in a native *Pieris* in another community (*Pieris napi macdunnoughii* in the Rocky Mountains, Colorado, Rodman and Chew 1980), we determined that in this case, glucosinolate profiles from exemplars of abundant potential host plants were not correlated with insect behavior (Agerbirk et al., unpublished data).

In a common garden experiment, we compared oviposition preference of individual females and larval performance of their offspring for females from one area where garlic mustard is well established (GM+) and one where it is absent (GM-) to test the hypothesis that individuals in the GM+ area would tolerate GM as larvae or avoid it as egg-laying adults compared to individuals from the GM- area. We predicted that females associated with garlic mustard (GM+) would avoid ovipositing on it, while those without contact (GM-) would not discriminate. Alternatively, progeny of GM+ females would perform better on garlic mustard compared to progeny of GM- females.

## Materials and methods

### Study sites and experimental organisms

Oviposition preference and larval performance were evaluated in a common garden experiment for individuals of *P. oleracea* from a GM+ site near Lenox, Massachusetts (42°21'N, 73°17'W) and individuals combined from three GM- localities in Vermont [Craftsbury (44°38'N, 72°22'W), Wolcott (44°33'N, 72°28'W), and Texas Falls (43°56'N, 72°5'W)]. The three GM- localities (treated here as a single area) are separated by 135–240 km straight line distance from the GM+ site, and 60–80 km straight

line distance from the nearest recorded garlic mustard stand, recently reported near Burlington, Vermont (IPANE 2003). First-generation adults were collected near Lenox in May 2004 and 2005. At this GM+ site, garlic mustard and the native host toothwort (*Cardamine diphylla* Michx.) are abundant. Two other Brassicaceae are relatively abundant: introduced watercress (*R. nasturtium-aquaticum* L.) and native circumboreal cuckoo-flower (*Cardamine pratensis* L.). Rosettes of biennial garlic mustard (Cavers et al. 1979) occur March–November and bolting plants occur from early May until mid July. Because spring ephemeral *C. diphylla* senesces by mid July (Courant et al. 1994), second-generation *P. oleracea* probably use watercress and crucifers in fields and along forest edges (Chew 1981; Van Driesche et al. 2004). In 2004, we obtained second-generation females from reared pupae.

We collected first- and second-generation adult females at GM– localities in Vermont: Craftsbury in 2004 and 2005, and Wolcott and Texas Falls in 2005. Garlic mustard is absent but *C. diphylla* is abundant at these GM– sites. *C. diphylla* is the only abundant, suitable host plant available to *P. oleracea* during the first generation in Vermont (Chew 1981); canola (*Brassica rapa* L.), sometimes planted as a cover crop, is the most abundant host plant during the second generation.

Garlic mustard became established at the GM+ site near Lenox, possibly as early as the 1950s (Nuzzo 1993) and was well established by the 1970s (R. W. Pease, in litt.). A mark–recapture study of *P. oleracea* near Craftsbury found that of 236 *P. oleracea* recaptured from a marked sample of ~1,850, four individuals moved 2–6 km within several days; the remainder were re-caught at their original capture sites (Chew 1981). Butterflies in the GM– area are therefore thought to be naive in relation to garlic mustard, while those in the GM+ site have been associated with this plant for perhaps 60–100 generations.

#### Oviposition preference assays

Females were maintained in flight cages at ambient temperature during assays and were otherwise stored in individual glass jars at 4°C. We fed adults twice daily with a ~20% aqueous honey-sucrose solution and provided them with this solution and water during preference assays.

Oviposition preference was assessed in two-way choice trials, comparing garlic mustard and normal host plants. In addition to garlic mustard and a normal host plant, we used non-host parsley [*Petroselinum crispum* (Miller) Mansf. (Apiaceae)] as a negative control, to ensure that females chose a host rather than indiscriminately laid eggs on any available plant (cf. larval preference assay, Berube 1972; Chew 1980). No eggs were laid on this negative control. Normal host plants included *C. diphylla* and watercress for

the first generation and *C. diphylla*, watercress, and cabbage (*Brassica oleracea* L.) for second-generation adults. Individuals were placed in 33 × 25 × 32-cm mesh cages, each containing parsley (the negative control), one of the normal host plants, and garlic mustard (from bolting stalks for first-generation females; rosettes for second generation). We matched leaf samples for each trial by height and approximate foliage area, and placed them in water to maintain turgor. Plant positions in each cage and individual cage position relative to the light source were randomized daily. Assays were run daily for at least four hours, between 0900 and 1600 hours. Because cool temperature and low solar radiation limit oviposition in pierids (Gossard and Jones 1977; Yamamoto and Ohtani 1979), we conducted the assays at 22–32°C under heat lamps in the laboratory, under semi-natural light conditions (sodium vapor lights and sunlight) in a greenhouse, or in natural sunlight in a garden. Eggs were collected at the end of each day. Preference trials for most females lasted 5 days. Only females that laid ≥20 eggs were used in data analysis. Because only a small number of females in oviposition assays using watercress or cabbage met this criterion due to the limited number of females tested ( $n = 6$  watercress,  $n = 4$  cabbage), we restrict our statistical analysis of oviposition preference to females tested with *C. diphylla* as a normal host ( $n = 24$ ), but present results with watercress and cabbage for comparison.

Female preference was calculated as the proportion of total eggs laid on garlic mustard and expressed as an oviposition preference index (OPI) (Huang et al. 1995):

$$\text{OPI} = 100 \times \left( \frac{\text{no. of eggs laid on garlic mustard} - \text{no. laid on normal host}}{\text{total eggs laid}} \right).$$

OPI ranges from –100 (complete aversion to GM) to 0 (equal, or no preference) to +100 (exclusive use of GM).

#### Larval performance assays

Larval assays compared performance on garlic mustard versus normal hosts [including *C. diphylla*, watercress, cabbage (*B. oleracea* L. cv. Primax), canola (*B. rapa*), arugula (*Eruca sativa* L.), radish (*Raphanus sativus* L.), white mustard (*Sinapis alba* L. cv. Salvo), and a few combinations of these plants]. We collected *C. diphylla* near butterfly sites and garlic mustard near these sites and near Medford, Massachusetts (42°26'N, 71°7'W) and stored the plants at 4°C. All other plants were grown from seed (Johnny's Seeds, Maine; except for white mustard, Advanta Seeds, The Netherlands) in a greenhouse or purchased locally (parsley, watercress).

We measured larval performance using survival rate, development time, and pupal mass. Larval development

time (days from egg hatch and pupation) is important because slower developing larvae may die from non-host-related predation and parasitism (e.g., Haggstrom and Larsson 1995; Loader and Damman 1991). Pupal mass is linearly, positively correlated with adult fecundity (e.g., *P. rapae*, Jones et al. 1982; Gilbert 1984). Each performance measure was assessed on garlic mustard versus normal host plants using a split-brood experimental design. Newly hatched first instar larvae from each mother were assigned randomly to garlic mustard or a normal host plant. Sibling groups were divided among multiple transparent plastic boxes (15.2 × 10.2 × 5.1 cm) or plastic petri dishes (150 × 25 mm or 100 × 15 mm) lined with moist absorbent paper. Positions of these multiple boxes were randomized daily. Light cycle was 16:8 h light:dark and average temperature varied asynchronously by 5.3°C (mean maximum temperature = 24.2°C, SD = 8.68; mean minimum = 18.9°C, SD = 7.03). Larvae were tended daily: accumulated frass removed, absorbent liners replaced, and fresh cut leaves supplied. Upon pupation, days to pupation was recorded; individuals were sexed and weighed within 24 h to determine pupal mass. Larval survival for each family was calculated as the percentage that pupated on garlic mustard or normal hosts.

For data analysis, only family–treatment combinations that included eight or more larvae or three of more pupae were included in analyses of larval survival, or days to pupation and pupal mass, respectively. Pupal mass is sexually dimorphic in many Lepidoptera (e.g., Haukioja and Neuvonen 1985; Nitao et al. 1991), and for *P. oleracea* in our study ( $t = 6.77$ ,  $df = 676$ ,  $P < 0.0001$ ). Because not all family–treatment combinations contained both male and female offspring, we scaled male pupal mass to female mass by regressing family means for male and female masses for all family–treatment combinations with three of more pupae of both sexes (e.g., Bossart 2003). The resulting relationship ( $r^2 = 0.739$ ;  $df = 32$ ;  $P < 0.0001$ ) is:

$$\text{mass}_{\text{female}} = 0.8474 \times \text{mass}_{\text{male}} + 6.895.$$

All normal host plant treatments were grouped as a single treatment and contrasted with the novel host (garlic mustard) treatment. While there were significant differences in days to pupation (but not pupal mass) among normal host plants, there was no significant difference in how insects from the two areas responded to normal hosts (two-way ANOVA for generation 1, family means of days to pupation with host plant and site as fixed effects—host plant,  $F_{[8,39]} = 2.72$ ,  $P = 0.0175$ ; site,  $F_{[1,39]} = 1.27$ ,  $P = 0.2662$ ; two-way ANOVA for generation 1 family means of pupal mass with host plant and site as fixed effects—host plant,  $F_{[8,39]} = 1.26$ ,  $P = 0.2933$ ; site,  $F_{[1,39]} = 0.27$ ,  $P = 0.6033$ ). Variation

among these several normal hosts is potentially significant biologically but it is dwarfed by the contrast between the group of normal hosts and the novel plant garlic mustard.

We used SAS 9.1 (SAS Institute, North Carolina, 2002) for all statistical analyses. Larval survival was analyzed using repeated measures two-way ANOVA by generation (PROC MIXED). Measures of family means of development time and pupal mass were analyzed using a two-way ANOVA with site and host plant as fixed effects (PROC MIXED). Correlation between larval survival on garlic mustard and maternal oviposition preference (OPI) and site was analyzed for first-generation offspring using a separate intercept logistic regression model (PROC LOGISTIC). Second-generation survival was not analyzed because only one individual pupated on garlic mustard.

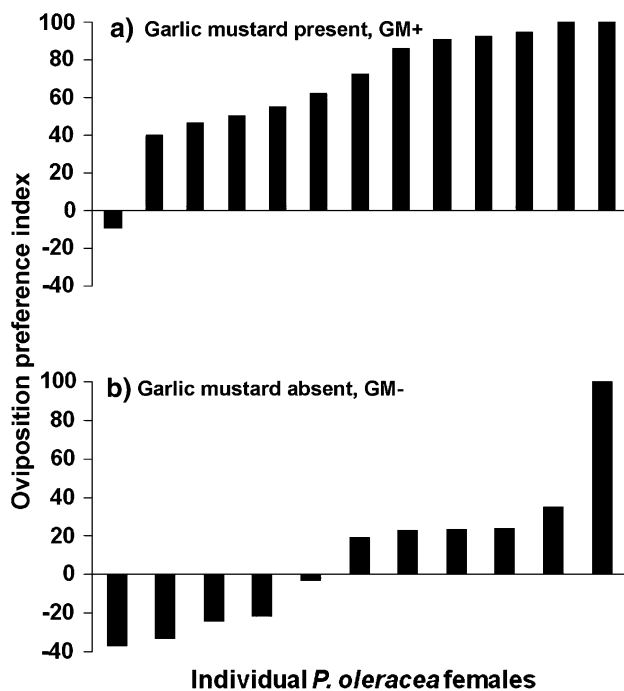
## Results

### Oviposition preference

Preference for garlic mustard (OPI) ranged from avoidance (OPI = −33) to complete preference (OPI = 100) (Fig. 1). GM+ females laid significantly more eggs on garlic mustard than on *C. diphylla* (mean OPI = 48.8, SD = 31.5), compared to GM− females, which showed a wide range in their acceptance of garlic mustard (mean OPI = −17.1 SD = 39.7) ( $t$ -test,  $t = 4.01$ ,  $df = 22$ ,  $P < 0.001$ ). These results were not an artifact of the OPI; analysis of the arcsine square root-transformed proportion of eggs laid on garlic mustard was also significantly different between GM+ females and GM− females ( $t$ -test,  $t = 3.38$ ,  $df = 22$ ,  $P < 0.005$ ). Acceptance of garlic mustard may depend on the alternative. The few females that laid  $\geq 20$  eggs in assays with watercress ( $n = 6$ ) tended to prefer watercress over garlic mustard (mean OPI = −34.2, SD = 57.6), whereas females offered cabbage ( $n = 4$ ) as an alternative showed no obvious preference (mean OPI = 28.2, SD = 74.2).

### Larval survival

Larvae from both GM+ and GM− sites had significantly higher pupation rates on normal hosts compared to garlic mustard (Table 1). On average, 17% of the GM+ larvae and 7% of GM− larvae reared on garlic mustard during the first generation survived to pupation compared to 81 and 90% on normal hosts, respectively. Only one second-generation larva survived on garlic mustard compared to an average of 60% GM+ and 30% GM− larvae reared on a normal host. There was a significant effect of site and a host plant by site interaction on larval survival during the second generation, but only a marginally significant host



**Fig. 1** Oviposition preference index (OPI) (see text for calculations) for 24 *Pieris oleracea* females from areas where garlic mustard (*Alliaria petiolata*) is **a** present (GM+) and **b** absent (GM-). A positive OPI indicates that the female preferred the exotic plant GM over the native *Cardamine diphylla*, zero means equal or no preference, and a negative OPI means that *C. diphylla* was preferred

plant by site interaction effect during the first generation (Table 1). During the second generation, GM+ larvae survived significantly better on normal host plants than did GM- larvae (ANOVA,  $F_{[2,7]} = 14.51$ ,  $P = 0.003$ ); there was no difference in survival on garlic mustard (ANOVA,  $F_{[2,7]} = 0.87$ ,  $P = 0.46$ ).

#### Larval development time and pupal mass

Despite asynchronous butterfly phenology at GM+ and GM- sites, development time of larvae from GM+ and GM- areas did not differ significantly on the normal host plants (Table 2). Larvae from both GM+ and GM- sites had significantly higher pupal mass, and developed more quickly, on normal hosts compared to garlic mustard (Table 2). Larvae took on average 9 days longer to pupate on garlic mustard (>50% longer than days to pupation on normal hosts) and weighed 34% less than larvae reared on normal hosts. Site had no significant effect on pupal mass of larvae reared on garlic mustard (Table 2). However, families from GM+ sites took significantly longer to pupate on garlic mustard (mean = 27.1 days; SD = 3.5,  $n = 7$ ) than did GM- larvae (mean = 19.6 days; SD = 0,  $n = 1$ ) (ANOVA,  $F_{[1,36]} = 9.6$ ,  $P = 0.0032$ ); this difference was driven by a single fast-developing family whose mother

came from Wolcott. Because only one second-generation larva survived on garlic mustard, we did not examine the effect of generation in analyses of larval development time or pupal mass.

#### Correlations between preference and performance

Larval survival on garlic mustard during the first generation was positively associated with maternal oviposition preference (Wald  $\chi^2 = 160.51$ ,  $n = 583$ ,  $P < 0.0001$ ) and presence of GM (Wald  $\chi^2 = 41.39$ ,  $n = 583$ ,  $P < 0.0001$ ) (Fig. 2a). Survival also increased with increasing preference for garlic mustard during oviposition (odds ratio estimate = 1.035). However, maternal oviposition preference for garlic mustard (high OPI values) was also correlated with longer larval developmental times [by correlation analysis (PROC GLM);  $r^2 = 0.74$ ,  $df = 7$ ,  $P = 0.006$ ] (Fig. 2b). Larvae from GM+ mothers took longer to develop than did the progeny of the one surviving GM- family whose mother was collected in Wolcott. Female oviposition preference was not associated with pupal mass in families from either GM+ or GM- areas ( $r^2 = 0.28$ ,  $df = 7$ ,  $P = 0.18$ ) (Fig. 2c).

#### Discussion

To successfully respond to garlic mustard invasion, *P. oleracea* individuals would need to avoid it during oviposition or exhibit enhanced larval performance. We predicted that females from the GM+ site would avoid laying eggs on garlic mustard compared to their naive GM- counterparts, or that GM+ larvae would tolerate garlic mustard better than GM- larvae. We found evidence for both outcomes. Differences between the two areas, other than presence or absence of garlic mustard, might account for our observation that responses of GM+ and GM- females and their larval progeny to garlic mustard are strikingly different. Although larval responses to normal hosts vary (significant variation for larval development time but not pupal mass, see Materials and methods), there was no significant difference by site for these larval responses to normal hosts, suggesting that garlic mustard is a key difference between the two areas. Significant variation among normal hosts in larval development time but not in pupal mass has been reported for *P. oleracea* reared on other normal hosts and may be due to possible weight-gated larval development in an insect with non-overlapping generations (Van der Reijden and Chew 1992).

We now consider hypotheses for why GM+ females showed, on average, stronger preference for garlic mustard than GM- females, and why the differences in measures of larval performance are not consistently in the direction

**Table 1** Effects of host plant on larval survival in *Pieris oleracea* from sites where garlic mustard is well established (GM+) and sites where it is absent (GM–). Individual larvae from families were randomly assigned to split broods reared on garlic mustard or normal

brassicaceous hosts. Generations were analyzed separately using a repeated-measures two-way ANOVA with host plant and site as fixed effects and families as random effects

Host plant	GM+		GM–			
	Generation 1 (n = 15)	Generation 2 (n = 5)	Generation 1 (n = 7)	Generation 2 (n = 5)		
Larval survival (mean ± 1 SE)						
Garlic mustard	16.6 ± 5.15	0	6.66 ± 5.91	1.11 ± 1.11		
Normal host	81.18 ± 2.85	59.65 ± 11.11	90.26 ± 3.98	25.92 ± 12.57		
Effect	Generation 1			Generation 2		
	F	df	P	F	df	P
Results of repeated-measures two-way ANOVA						
Host plant	189.42	1, 42	<0.0001	25.40	1, 14	0.049
Site	0.07	1, 42	0.789	4.64	1, 14	0.0002
Host plant × site	3.79	1, 42	0.058	5.23	1, 14	0.038

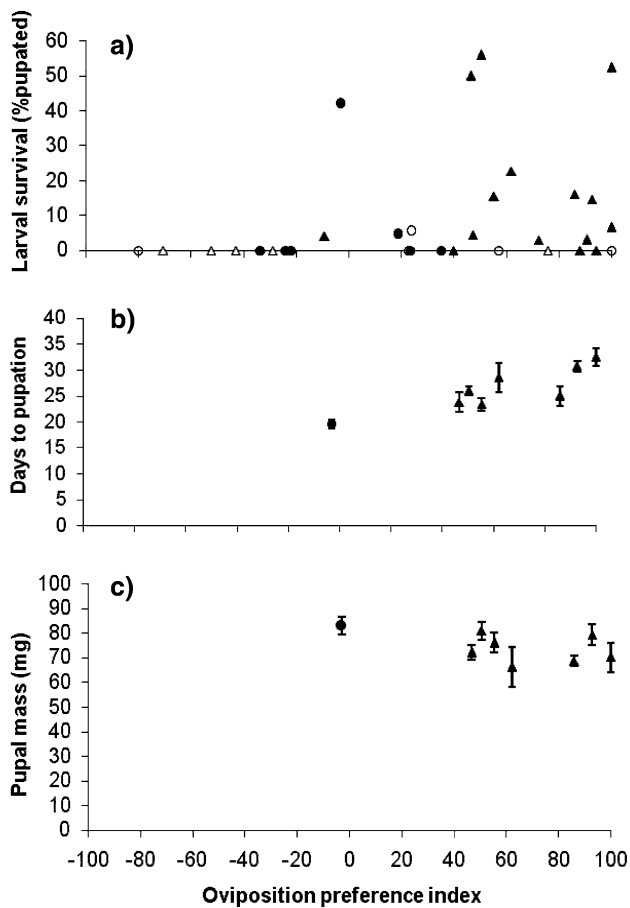
**Table 2** Effects of host plant on mean days to pupation and pupal mass of *P. oleracea* from GM+ and GM– sites. Individual larvae from families were randomly assigned to split broods reared on garlic mustard or normal brassicaceous hosts. Because only one larva

survived on garlic mustard in generation 2, only generation 1 family means for larval development time and pupal mass were analyzed using a two-way ANOVA with host plant and site as fixed effects

Host plant	GM+		GM–			
	Days to pupation (mean ± 1SE)		Days to pupation (mean ± 1SE)			
Larval development: days to pupation						
Garlic mustard	27.14 ± 1.34	n = 7	19.58 ± 0	n = 1		
Normal host	14.42 ± 0.42	n = 21	13.67 ± 0.35	n = 10		
Host plant	GM+		GM–			
	Pupal mass (mean ± 1SE)		Pupal mass (mean ± 1SE)			
Larval development: pupal mass						
Garlic mustard	73.46 ± 2.09	n = 7	82.97 ± 0	n = 1		
Normal host	99.62 ± 3.03	n = 21	101.26 ± 3.43	n = 10		
Effect	Mean days to pupation			Mean pupal mass		
	F	df	P	F	df	P
Results of two-way ANOVA						
Host plant	53.05	1, 36	<0.0001	5.68	1, 36	0.0226
Site	9.96	1, 36	0.0032	0.14	1, 36	0.7901
Host plant × site	8.27	1, 36	0.0067	0.59	1, 36	0.4467

predicted. First, differences in oviposition preference between GM+ and GM– females might be due to differential exposure of larvae or adults to specific plants. The Hopkins host-selection principle states that a female's oviposition preference is induced by encounters with host plants during the larval stage (Dethier 1954; Hopkins 1917). Hovanitz and Chang (1964, 1965) purportedly

induced oviposition preference in *P. rapae* by larval exposure to novel hosts. Most research, however, has shown host preference is determined by early adult contact with the larval host (e.g., Caubet and Jaisson 1991; Rausher 1978; Rojas and Wyatt 1999; Stanton 1982; reviewed by Davis and Stamps 2004). Because most females tested were wild caught, GM+ females may have been



**Fig. 2** Correlation between maternal oviposition preference for garlic mustard and **a** larval survival, **b** days to pupation (mean  $\pm$  1 SE), and **c** pupal mass (mean  $\pm$  1 SE) on garlic mustard for *P. oleracea* from areas where garlic mustard is present (filled triangle, triangle) and absent (filled circle, circle). Each symbol represents one female and her offspring. Only families of eight or more larvae or three or more pupae were included in each analysis. Closed symbols indicate first-generation individuals and open symbols indicate second-generation individuals (the latter not analyzed statistically)

conditioned by prior experience with garlic mustard. Generation 2 (lab-reared) females from the GM+ area showed a wide range of OPI values (Fig. 2a), but our sample is too small to interpret these results.

Second, the differences between GM+ and GM– females and their families may result from adaptation via natural selection. We suggest that *P. oleracea* at least partially meets the criteria for potential natural selection: (1) phenotypic variation occurs in oviposition preference and larval performance; (2) these traits are heritable in other butterflies (e.g., oviposition preference, Forister 2005; Janz 1998; Singer et al. 1988; Thompson 1988b; *P. napi macdunnoughii*, Boggs et al., in review); larval performance, *P. napi napi*  $\times$  *P. oleracea* hybrids reared on garlic mustard (Bowden 1971); Janz 2003; Thompson et al. 1990; C. Boggs and C. Wiklund, unpublished data on

*P. napi macdunnoughii*); (3) our data show strong correlation between an individual's oviposition preference and the survival of her progeny. However, the correlation with other measures of larval performance was not in the direction predicted. Garlic mustard significantly reduced *P. oleracea* pupal mass compared to normal hosts, but there was no correlation between oviposition preference and pupal mass. Garlic mustard also increased the time required for larval development. However, the relationship between oviposition preference and development time was unexpected: larval development time increased with increasing preference for garlic mustard during oviposition. Possibly this relationship represents evidence for incipient larval adaptation to garlic mustard because the higher survival rate in GM+ larvae includes individuals that grow very slowly. Slow growth may reflect physiological costs of detoxification: e.g., cytochrome P450-mediated detoxification is maintained in *Depressaria pastinacella* at the expense of growth when dietary protein is in short supply (Berenbaum and Zangerl 1994). These trends confound the preference–performance relationship and conflict with optimal oviposition theory, which predicts that females should prefer host plants that maximize their fitness (Jaenike 1978). Although strong, positive preference–performance correlations have been found in many species of Lepidoptera, there are numerous examples of lack of concordance (reviewed by Mayhew 1997, 2001; Thompson 1988a). Factors invoked to explain these apparently imperfect relationships include host plant availability (Thompson and Pellmyr 1991; Wiklund 1984), insufficient time to adapt to a novel host (Thompson 1988a), natural enemies (Bernays and Graham 1988), physiological limitations (Bernays 1999; Fox and Lalonde 1993), and genetic constraints (Futuyma 1995; Zangerl et al. 2002). Both physiological limitations and insufficient time for adaptation have been cited as possible causes of poor correlation between oviposition preference and larval performance in closely related *P. napi macdunnoughii* (Chew 1975, 1977). Additionally, competitors or natural enemies might alter the balance between larval performance and host plant selection in wild populations of *P. oleracea* (cf. Ohsaki and Sato 1994 in *Pieris* ssp.; Benson et al. 2003 and Van Driesche et al. 2004 in *P. oleracea*).

Third, the unexpected patterns in larval mass and development time might indicate that oviposition preference has evolved before larval adaptation to a new host plant (e.g., Bowers et al. 1992; Dethier 1954; Thomas et al. 1987). Charlesworth et al. (1987) argued that genes on the X chromosome can evolve faster than those on autosomes, causing differential rates of evolution in oviposition preference [an X-linked trait (*Papilio oregonius* and *P. zelicaon*, Thompson 1988b; *P. napi macdunnoughii*, Boggs et al., in review)] and larval performance [an

autosomal trait (*Polygonia c-album*, Janz 1998; Nylin et al. 2005; *P. oregonius* and *P. zelicaon*, Thompson et al. 1990; C. Boggs and C. Wiklund, unpublished data on *P. napi macdunnoughii*).

Fourth, the imperfect relationship between measures of larval performance and oviposition preference occurs because of a single GM– family from Wolcott, whose mother showed no oviposition preference for garlic mustard (OPI = –3.09) and whose larval progeny developed faster on garlic mustard than any GM+ families (pupae  $n = 12$ ; Table 2). This inconsistency might be due to previous exposure of GM– *P. oleracea* to garlic mustard if it occurred in low, undetected amounts. Although we are fairly confident that garlic mustard has yet to colonize our GM– areas, this invasive plant is slowly spreading throughout the region. Recently it has been found in northern Vermont in the Burlington area (IPANE 2003), 60–80 km from the nearest GM– site where we collected butterflies. However, during this study we noted a small stand of garlic mustard along the road approximately 8 km east of one of our GM– sites at Texas Falls.

Fifth, gene flow between *P. oleracea* at GM+ and GM– sites could account for this family's relatively good larval performance on garlic mustard (e.g., Bergstrom et al. 2004; Bossart and Scriber 1995). Although we have no direct evidence of gene flow among these areas, *P. oleracea* has an open population structure and can occasionally disperse several kilometers (Chew 1981). If genetic exchange occurs, butterflies in GM– areas may acquire some adaptation to garlic mustard without having direct contact with the plant.

Finally, our results suggest that *P. oleracea* may be incorporating garlic mustard into their diet through both improved larval performance and increased oviposition preference. If similar results are found in other populations of *P. oleracea* where garlic mustard has become established, we speculate that this native insect may escape this evolutionary trap. How rapidly this may occur, whether it will involve local adaptation in a geographic mosaic or spread from one area to another, and whether it will occur quickly enough to prevent local extinction by a combination of bottom-up effects (garlic mustard) and top-down effects (e.g., parasites, Benson et al. 2003; Van Driesche et al. 2004), remains to be seen (Keeler et al. 2006).

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