

# Host–plant quality alters herbivore responses to temperature: a case study using the generalist *Hyphantria cunea*

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## Abstract

The nutritional quality of host plants is an important determinant of fitness in insect herbivores. However, it remains an open question whether the ingestion of a particular plant will have the same effects on an herbivore under differing thermal conditions. We measured the performance of the generalist-feeding caterpillars of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) raised on one of five natural host plants to determine their nutritional quality: *Platanus occidentalis* L. (Platanaceae), *Sophora japonica* (L.) Schott (Fabaceae), *Prunus × yedoensis* Matsum. (Rosaceae), *Cornus kousa* Hance (Cornaceae), or *Betula platyphylla* Sukaczew (Betulaceae). Caterpillars performed well on *P. occidentalis*, *S. japonica*, and *P. × yedoensis*, but poorly on *C. kousa* and *B. platyphylla*. The nutritional phenotype of caterpillars varied among host–plant groups, with the proportion of lipid-free body mass to lipid content being higher for caterpillars raised on *P. occidentalis* and *S. japonica* (3.8–4.2:1) than for caterpillars raised on *P. × yedoensis* (1.6–2.1:1). A multi-factorial experimental design was employed to investigate the interactive effects of host–plant quality and temperature on the performance of *H. cunea* caterpillars raised on either *P. occidentalis* or *P. × yedoensis* at three rearing temperatures (20, 25, or 30 °C). Caterpillars raised on *P. occidentalis* displayed a monotonic decrease in development time with increasing temperature, but the development time of those on *P. × yedoensis* decreased rapidly as temperature rose from 20 to 25 °C and then stayed unchanged despite further increase in temperature. The rate at which body size increased with decreasing temperature was much steeper for caterpillars raised on *P. occidentalis* than for those on *P. × yedoensis*. Collectively, these results indicate that host plant can alter the thermal reaction norms for the key life-history traits of herbivores. This study has implications for understanding the impacts of climate change on herbivore–plant interactions.

## Introduction

Selecting a food plant that supports maximal performance is an ultimate goal of foraging insect herbivores (Slansky, 1993; Simpson et al., 2004). Whether a particular plant is a suitable food for herbivores is determined by a number of plant characteristics (e.g., plant structure, toughness, and chemical defense), but nutrition has always been a central factor defining the trophic linkage between plants

and insect herbivores (Bernays & Chapman, 1994; Awmack & Leather, 2002). Traditionally, the nutritional quality of plants has been considered to be limited by a single, predominant factor (e.g., nitrogen: Mattson, 1980; Slansky, 1993), but it is becoming increasingly clear that the balance of multiple nutrient currencies (e.g., protein and carbohydrate) is a more reliable predictor of host–plant quality (Raubenheimer & Simpson, 1997; Simpson et al., 2004; Simpson & Raubenheimer, 2012). Many studies have documented inter- and intraspecific variations in host–plant quality with respect to their effects on the major fitness components of herbivores (Hunter & McNeil, 1997; Awmack & Leather, 2002; Agosta, 2008). However, studies investigating the host–plant effects on

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the nutritional phenotype of herbivores are rare. Further, it remains an open question whether the nutritional quality of a particular host plant will have the same effects on herbivores if it is consumed under different thermal conditions. This question is particularly relevant for those herbivores living in temperate regions where diurnal and seasonal variation in environmental temperature is high.

Temperature has profound effects on nearly all aspects of biology in ectotherms (Angilletta, 2009), and its interactions with diet have considerable consequences for foraging and performance of ectotherms (Lindroth et al., 1997; Kingsolver & Woods, 1998; Petersen et al., 2000; Kingsolver et al., 2006; Stillwell et al., 2007; Lee & Roh, 2010). Increased temperature can change the pattern of host-plant use by herbivores by altering their nutritional requirements and their sensitivity to plant secondary compounds (Stamp, 1993; Stamp & Yang, 1996; Lemoine et al., 2013).

An inverse relationship between developmental temperature and adult body size is the most widespread pattern of phenotypic plasticity in ectotherms, a phenomenon known as the temperature-size rule (Atkinson, 1994; Angilletta, 2009). However, recent studies have indicated that the effects of temperature on final body size vary considerably depending on foods in herbivores (Diamond & Kingsolver, 2010; Clissold et al., 2013). For example, Diamond & Kingsolver (2010) found that the temperature-size rule was followed by tobacco hornworm, *Manduca sexta* L., a specialist herbivore feeding on a preferred, high-quality host plant (tobacco, *Nicotiana tabacum* L.), but a reversal of the rule was observed when feeding on a less preferred, low-quality host plant [devil's claw, *Proboscidea louisianica* (Mill.) Thell.]. This result raises the possibility that the quality of the host plant can modulate the magnitude and direction of thermal reaction norms for fitness components (Stamp, 1993; Kingsolver et al., 2006; Lee & Roh, 2010). However, further research is needed to dissect the role of nutrition in modulating the thermal responses of herbivores.

Given this background, our aim in this study was to examine whether the nutritional quality of host plant affects the thermal reaction norms for key fitness components in a generalist insect herbivore, *Hyphantria cunea* Drury (Lepidoptera: Arctiidae). In the field, *H. cunea* caterpillars are likely to encounter a wide range of nutritional and thermal environments due to their extremely polyphagous feeding habit and the high level of thermal variability they experience in the temperate zone. In this study, we first examined the nutritional quality of the host plants consumed by *H. cunea* caterpillars in the field. This was done by comparing the life history and nutritional responses of caterpillars feeding on each of five host plants.

We then investigated whether there were significant interactions between host plant and temperature for key fitness components and the nutritional phenotype. To do so, we raised caterpillars on one of two nutritionally diverging host plants under three rearing temperatures.

Until recently, several studies have investigated biological aspects of *H. cunea*, including life-history and voltinism (Gomi & Takeda, 1996; Gomi, 2007; Gomi et al., 2007, 2009), host-plant preferences and performance (Morris, 1967; Greenblatt et al., 1978; Williams & Myers, 1984), and diapause (Gomi, 1997; Li et al., 2001; Chen et al., 2014). To the best of our knowledge, this is the first work that investigates how the thermal responses of this generalist caterpillar are altered by the nutritional quality of host plant.

## Materials and methods

### Study insect

*Hyphantria cunea* is a moth native to North America and is one of the most widely distributed invasive pests of deciduous trees in the Palearctic region, ranging from Western Europe to the Far East (Yang et al., 2008). The larvae of *H. cunea* are extremely polyphagous, attacking more than 636 documented species of trees and shrubs worldwide (Warren & Tadić, 1970). In Korea, this species was first observed from field sites near Seoul (37°1'N, 126°28'E) in 1958 (Woo, 1961) and since then it has spread rapidly, reaching the southern coastlines of the Korean Peninsula by the late 1970s. In native North America black- and red-headed forms of *H. cunea* caterpillars occur (Oliver, 1964; Jaenike & Selander, 1980), but in Korea only the black-headed form is found (Kim & Kil, 2012). The Korean populations of *H. cunea* generally complete two generations per year, but some populations in the southern region of the country may complete three generations per year. The number of generations per year varies with latitude – for example, in Japan, the populations north of 36° N are bivoltine whereas those south of 36° N are trivoltine (Gomi & Takeda, 1996; Gomi, 1997, 2007).

The laboratory culture of *H. cunea* was established from hundreds of early-instar, wild caterpillars collected from multiple field sites near Pusan (35°05'N, 129°00'E) in May 2013. Field-collected insects were raised to adulthood and allowed to reproduce under laboratory conditions at Seoul National University. The first-generation progeny of these field-collected larvae were utilized for this study. Cohorts of 50–60 field-collected individuals were housed in rectangular plastic rearing cages (50 × 50 × 50 cm) where they were given ad libitum access to the fresh leaves of Yoshio

cherry, *Prunus* × *yedoensis* Matsum. (Rosaceae). These plastic rearing cages were placed in a room where temperature was not controlled but ranged between 22 and 27 °C under natural light conditions. Pupae were collected and sexed by inspecting the last abdominal segment under a dissecting microscope (Loewy et al., 2013). Each sex was separated and then incubated until adult emergence in an environmental chamber (Jeio-tech, Seoul, Korea) set at 25 °C and L16:D8 photoperiod. Four newly enclosed adults of each sex were transferred to each rectangular plastic mating chamber (25 × 25 × 25 cm) at room temperature under natural light conditions. In each mating chamber, adult moths were provided with a cotton ball soaked in a 10% (wt/vol) sucrose solution as energy source and the leaves of *P.* × *yedoensis* as oviposition substrate. Clusters of eggs laid on the leaves were cut out and placed in Petri dishes (9 cm diameter). Newly hatched neonates received fresh leaves of *P.* × *yedoensis* every alternate day until reaching the third larval stage. Throughout the pre-experimental rearing stages, caterpillars were reared at a density of 20–30 individuals per dish in an environmental chamber set at 25 °C and L16:D8 photoperiod. This photoperiodic condition was chosen to prevent caterpillars from entering diapause regardless of the experimental temperature they were exposed to (Gomi, 1997; Takeda, 2005; Chen et al., 2014).

#### Host plants

In this study, we used five abundant species of deciduous woody plants regularly consumed by *H. cunea* as experimental diets: *Platanus* [American sycamore, *Platanus occidentalis* L. (Platanaceae)], *Prunus* (*P.* × *yedoensis*), *Sophora* [Pagoda tree, *Sophora japonica* (L.) Schott (Fabaceae)], *Cornus* [Kousa dogwood, *Cornus kousa* Hance (Cornaceae)], and *Betula* [Asian white birch, *Betula platyphylla* Sukaczew var. *japonica* (Betulaceae)]. To minimize undesirable intraspecific variation in host-plant quality owing to differences in microhabitats and collection time, for each species we harvested mature leaves from more than five neighboring individual trees on the campus of Seoul National University at the same time of the day (16:00–17:00 hours). Collected leaves were soaked in 1.2% sodium hypochloride solution for 5 min and then washed with distilled water to remove any pathogens on the leaf surface. For each host-plant treatment, washed leaves from different individual trees were mixed at random, put together into self-sealing plastic bags with moisture and stored overnight in a cold laboratory chamber at 4 °C before they were presented to caterpillars the next morning. This procedure continued daily until all caterpillars had ceased to feed in the prepupal stage.

#### Experimental design and protocol

In the first experiment, *H. cunea* caterpillars were randomly assigned to one of the five plant treatments at 25 °C and L16:D8 photoperiod, whereas in the second experiment, caterpillars were assigned to one of six combinations of two plants (*Platanus* or *Prunus*) and three developmental temperatures (20, 25, or 30 °C), all at L16:D8 photoperiod.

Both experiments started concurrently when the caterpillars had ecdysed into the third instar. These newly molted caterpillars were collected from the maintenance culture (see above) and randomly divided over experimental treatments. Each replicate consisted of three caterpillars housed in a Petri dish (9 cm diameter), with 10 replicate dishes being used to yield a total of 30 caterpillars per treatment. Fresh leaves were provided to each dish on a daily basis and any frass was removed from the dishes every alternate day. For each individual caterpillar, we measured the following life-history and body composition parameters: survival, development time to pupation, mass at pupation, pupal lipid content, and lipid-free pupal mass. Survival was measured as a fraction of caterpillars surviving until pupation for each treatment group. Development time was determined as the number of days between the start of the third instar and pupation. Pupae were sexed and killed by freezing at –20 °C. After thawing, dead carcasses were dried in an oven set at 50 °C for 4 days and weighed to the nearest 0.1 mg using an Ohaus microbalance (Parsippany, NJ, USA). To extract lipids from carcasses, dried pupae were individually placed in a 10-ml glass vial where they were soaked in chloroform and gently swayed on a flat-bed shaker (Vision, Seoul, Korea) for 72 h. During the lipid-extraction period, the chloroform was refreshed every 24 h. After 72 h, the chloroform was removed from the vials and lipid-extracted pupae were re-dried and re-weighed following the same method as described above. Lipid content was estimated as the mass change after lipid extraction.

#### Statistical analysis

Analyses on life-history parameters (development time and pupal mass) were performed using a factorial analysis of variance (ANOVA) with diet (host plant), sex, and temperature as the fixed factors using Proc GLM in SAS v. 9.1 (SAS Institute, Cary, NC, USA). The effects of these fixed factors on the bivariate measure of body composition (lipid content and lipid-free pupal mass) were tested using an equivalent multivariate analysis of variance (MANOVA) with Pillai's trace statistic. Whether data conformed to the assumptions of normality and homoscedasticity was checked by Kolmogorov–Smirnov and Bartlett's test, respectively, before performing these parametric tests.

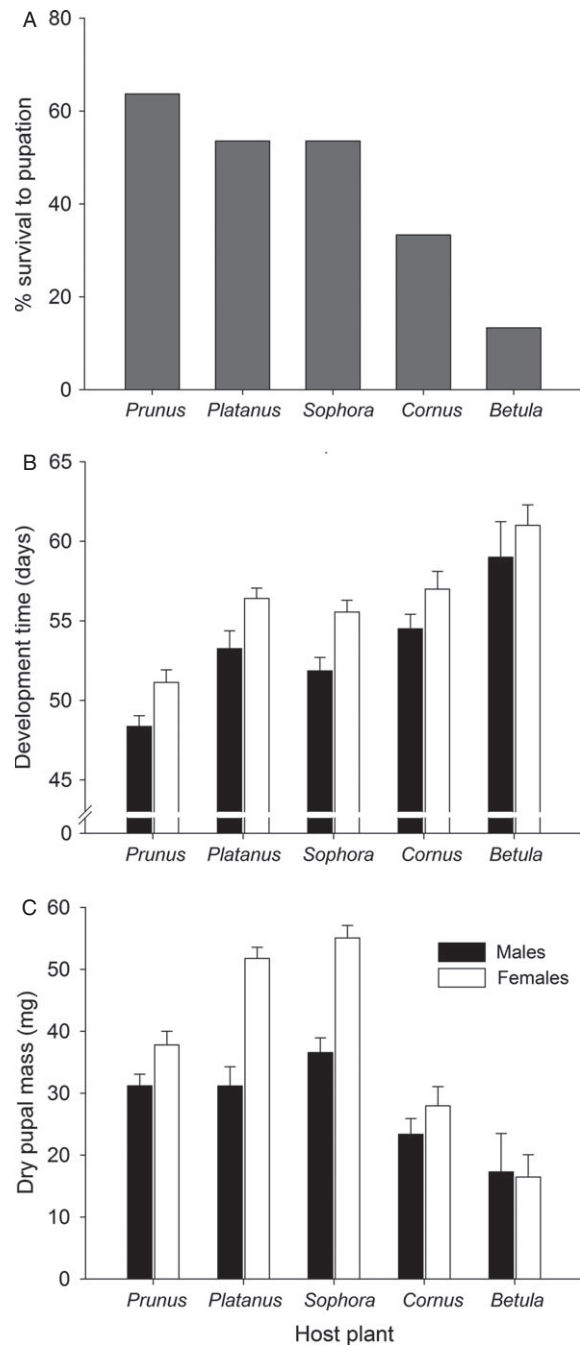
Holm-Sidak multiple comparison tests were used to detect significant differences between treatment groups after obtaining significant ANOVA results. When multivariate contrasts were performed as post-hoc tests following MANOVA, the Bonferroni method was applied for adjusting the significance level. Any effect of treatment on survival was tested by linear response function implemented in Proc CATMOD in SAS v. 9.1.

## Results

### Experiment 1: Host-plant quality

Host plant significantly affected the survival of *H. cunea* caterpillars ( $\chi^2 = 30.05$ , d.f. = 4,  $P < 0.001$ ). Survival was highest on *Prunus* (64%) and lowest on *Betula* (13%; Figure 1A). Development time was also significantly affected by host plant: caterpillars developed fastest on *Prunus* and slowest on *Betula* (Table 1, Figure 1B). Males reached the pupal stage earlier than females on all host plants, as indicated by a significant sex effect and non-significant sex\*host-plant interaction (Table 1, Figure 1B). Caterpillars on *Sophora* and *Platanus* attained heavier pupae than those on *Cornus* and *Betula* for both sexes (see host-plant effect in Table 1, Figure 1C). As indicated by a significant sex\*host-plant interaction (Table 1), females were heavier than males on *Prunus* (by 21%; Holm-Sidak multiple comparison:  $P = 0.026$ ), *Platanus* (67%;  $P < 0.001$ ), and *Sophora* (51%;  $P < 0.001$ ), but no detectable sex difference in pupal mass was found for those on *Cornus* ( $P = 0.26$ ) and *Betula* ( $P = 0.91$ ).

Because complex sex\*host-plant interaction was detected for the bivariate patterns of body composition (MANOVA:  $F_{8,110} = 2.23$ ,  $P < 0.001$ ), we examined the effect of host plant on body composition separately for each sex (Figure 2). The body composition of both sexes was affected by host plant (MANOVA: male:  $F_{8,48} = 15.37$ ; female:  $F_{8,62} = 13.53$ , both  $P < 0.001$ ). Post-hoc multivariate contrasts revealed that the final body composition of insects raised on *Prunus* was different from that of those on either *Platanus* (male:  $F_{2,23} = 46.97$ ; female:  $F_{2,30} = 18.81$ ) or *Sophora* (male:  $F_{2,23} = 95.42$ ; female:  $F_{2,30} = 22.14$ , all  $P < 0.001$ ), whereas the body composition of insects raised on *Platanus* and *Sophora* did not differ from one another (male:  $F_{2,23} = 2.49$ ,  $P = 0.11$ ; female:  $F_{2,30} = 0.61$ ,  $P = 0.55$ ). Insects raised on *Platanus* or *Sophora* contained more lipid-free pupal mass than those raised on *Prunus*. As such, the body composition ratio of lipid-free pupal mass to lipid content was higher on *Platanus* (male: 4.2:1; female: 4.0:1) and *Sophora* (male: 3.9:1; female: 4.2:1) than on *Prunus* (male: 1.6:1; female: 2.1:1) (male:  $F_{2,19} = 21.76$ ; female:  $F_{2,26} = 13.78$ , both  $P < 0.001$ ; Figure 2).



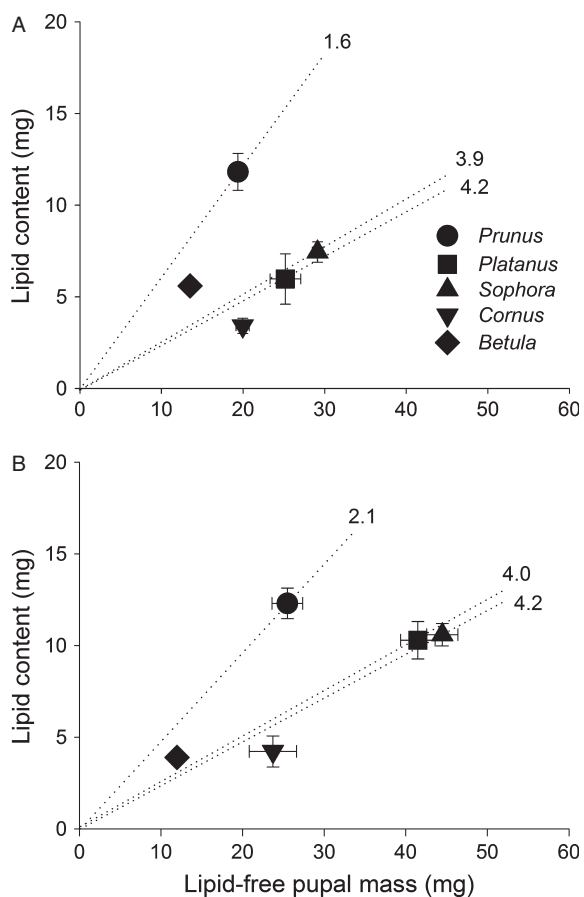
**Figure 1** Juvenile (A) survival (% surviving to pupation) and mean (+ SEM) (B) development time (no. days between the start of the third instar and pupation), and (C) dry pupal mass of *Hyphantria cunea* raised on one of five host plants (*Prunus*, *Platanus*, *Sophora*, *Cornus*, or *Betula*; see text for details).

### Experiment 2: Host-plant effects on thermal reaction norms

Survival to pupation was lowest at 30 °C for caterpillars raised on *Prunus* and *Platanus*, as indicated by a significant

**Table 1** Two-way ANOVA table for the effect of host plant (diet) and sex on development time (i.e., no. days between the start of the third instar and pupation) and dry pupal mass of *Hyphantria cunea* raised on one of five host plants

Response	Source	d.f.	MS	F	P
Development time	Diet	4	114.3059	22.97	<0.001
	Sex	1	78.1590	15.71	<0.001
	Diet*sex	4	0.9038	0.18	0.95
	Error	55			
Pupal mass	Diet	4	1005.1071	26.00	<0.001
	Sex	1	958.3883	24.79	<0.001
	Diet*sex	4	194.9961	5.04	0.002
	Error	55			

**Figure 2** Body composition plots demonstrating the bivariate mean ( $\pm$  SEM) lipid content and lipid-free pupal mass for *Hyphantria cunea* (A) males and (B) females raised on one of five host plants (*Prunus*, *Platanus*, *Sophora*, *Cornus*, or *Betula*; see text for details). The dotted lines and the numbers at the end represent the ratio of lipid-free pupal mass to lipid content for insects raised on three high-ranking host plants (*Prunus*, *Platanus*, and *Sophora*).

temperature effect on survival ( $\chi^2 = 16.96$ , d.f. = 2,  $P < 0.001$ ; Figure 3A). Caterpillars tended to survive better on *Prunus* than on *Platanus* at the two low rearing temperatures (20 and 25 °C), but survival was neither significantly affected by host plant ( $\chi^2 = 3.10$ , d.f. = 1,  $P = 0.078$ ) nor by temperature\*host-plant interaction ( $\chi^2 = 2.52$ , d.f. = 2,  $P = 0.28$ ). Development time decreased with increasing temperature (Table 2, Figure 3B), but the effect of temperature on development time was contingent on host plant, as indicated by a significant temperature\*host-plant interaction (Table 2). When raised on *Platanus*, caterpillars exhibited a monotonic decrease in development time as temperature rose from 20 to 30 °C (Figure 3B). For caterpillars on *Prunus*, however, the relationship between temperature and development time was biphasic: the time to pupation dropped rapidly as temperature rose from 20 to 25 °C and then leveled off over the temperature range of 25–30 °C (Figure 3B). Males developed more quickly than females regardless of host plant and temperature, as indicated by a significant sex effect and non-significant sex\*host-plant, sex\*temperature, and sex\*temperature\*host-plant interactions (Table 2). Dry pupal mass increased with decreasing temperatures and was consistently higher on *Platanus* than on *Prunus* (temperature and host-plant effects in Table 2, Figure 3C). A significant temperature\*host-plant interaction for pupal mass (Table 2) indicated that the rate at which pupal mass increased with decreasing rearing temperatures was much greater on *Platanus* (linear regression slope  $\pm$  SEM, male:  $-2.260 \pm 0.302$ ; female:  $-3.192 \pm 0.427$ ) than on *Prunus* (male:  $-1.289 \pm 0.214$ ; female:  $-1.542 \pm 0.491$ ). When pupal mass was compared between the two sexes, females were heavier than males (see sex effect in Table 2, Figure 3C), but this trend was more pronounced on *Platanus* than on *Prunus*, as indicated by a significant sex\*host-plant interaction for pupal mass (Table 2, Figure 3C).

**Table 2** Three-way ANOVA table for the effect of host plant (diet), temperature (temp), and sex on development time (i.e., no. days between the start of the third instar and pupation), and dry pupal mass of *Hyphantria cunea* allocated to six treatments [two host-plant groups (*Platanus* or *Prunus*)  $\times$  three temperature groups (20, 25, or 30 °C)]

Response	Source	d.f.	MS	F	P
Development time	Diet	1	1.6194	0.29	0.60
	Sex	1	86.1281	15.17	<0.001
	Temp	2	333.8267	58.80	<0.001
	Diet*sex	1	4.4220	0.78	0.38
	Diet*temp	2	30.4757	5.37	0.006
	Sex*temp	2	0.5444	0.10	0.91
	Diet*sex*temp	2	4.4128	0.78	0.46
	Error	91			
Pupal mass	Diet	1	1384.4500	30.73	<0.001
	Sex	1	2484.8981	55.15	<0.001
	Temp	2	3218.9188	71.44	<0.001
	Diet*sex	1	802.5286	17.81	<0.001
	Diet*temp	2	301.2782	6.69	0.002
	Sex*temp	2	117.6728	2.61	0.079
	Diet*sex*temp	2	18.7139	0.42	0.66
	Error	91			

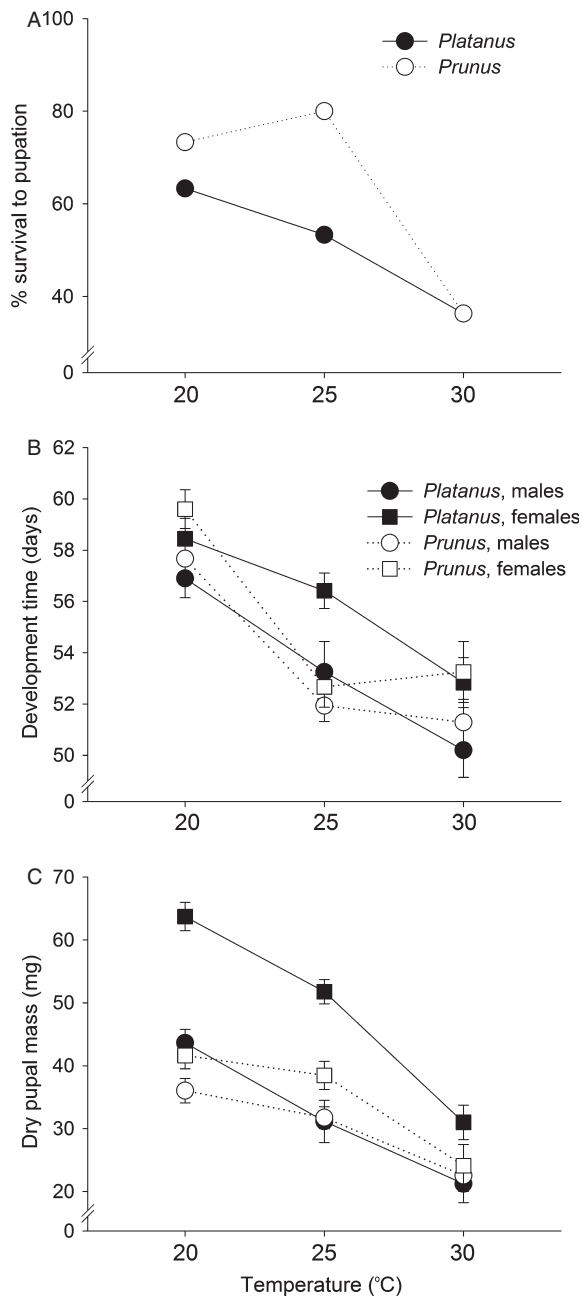
The bivariate patterns of body composition were affected by host plant, temperature, and sex (Table 3). Lipid-free pupal mass was higher on *Platanus* than on *Prunus*, and higher in females than in males of the two host-plant groups (Figure 4). Body lipid content tended to be lower for males raised on *Platanus* than on *Prunus*, but was similar between females in the two host-plant groups, as indicated by a significant sex\*host-plant interaction (Table 3). A significant temperature\*host-plant interaction for the bivariate patterns of body composition (Table 3) indicated that the way that body composition was related to temperature (i.e., the bivariate thermal reaction norm for body composition) depended on host-plant identity (Figure 4). When temperature decreased from 30 to 20 °C, both lipid-free pupal mass and lipid content increased significantly, but the extent to which lipid-free pupal mass increased with declining temperature was much greater on *Platanus* than on *Prunus* (male: 17.12 vs. 7.87 mg; female: 24.79 vs. 11.07 mg). The amount of body lipid that increased in response to decreasing temperature was similar between the two host-plant groups (*Platanus* vs. *Prunus*, male: 5.28 vs. 5.61 mg; female: 7.93 vs. 6.46 mg).

## Discussion

The relative suitability of host plants in this study was determined based on the expression of the key fitness components of caterpillars raised on various host plants. Under a constant temperature of 25 °C, caterpillars raised on

*Prunus*, *Platanus*, or *Sophora* (high-quality plants) exhibited higher survival rates, faster growth, and larger bodies than those on *Cornus* and *Betula* (low-quality plants). Among three high-quality host plants, the rank of their relative suitability differed depending on the herbivore trait of interest. For example, caterpillars of both sexes survived better and developed more quickly on *Prunus* than on *Sophora* or *Platanus*, indicating that in terms of survival and growth *Prunus* is a higher ranking host plant. However, when the final body mass at pupation was considered, the ranking order was reversed, with female caterpillars feeding on *Sophora* or *Platanus* pupating at much higher mass than when feeding on *Prunus*. This mismatch raises the possibility that the preferential ingestion of one of these high-quality plants enhances some fitness components at the expense of others. For example, eating *Prunus* is likely to increase herbivore survival by reducing the time-dependent mortality risks associated with predation and parasitism (Loader & Damman, 1991; Benrey & Denno, 1997), but may result in reduced potential fecundity owing to smaller final body size (Honek, 1993).

Factors affecting the nutritional quality of host plant include the quantity and mixture of nutrients and plant secondary compounds (Bernays & Chapman, 1994; Awmack & Leather, 2002). Instead of measuring the detailed chemical profiles of host plants, we inferred their nutritional quality by comparing the pupal body composition of caterpillars raised on different host plants. We are convinced that this provides a simple but robust representation of host-plant nutritional quality because the final



**Figure 3** Thermal reaction norms for juvenile (A) survival (% surviving to pupation) and mean ( $\pm$  SEM) (B) development time (no. days between the start of the third instar and pupation), and (C) dry pupal mass of *Hyphantria cunea* raised on one of two host plants (*Platanus* or *Prunus*) at 20, 25, or 30 °C.

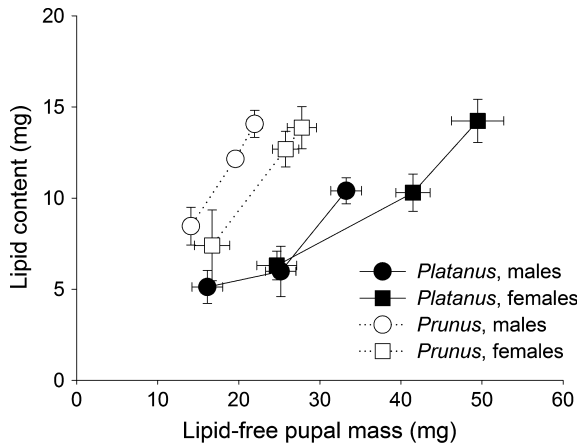
body composition of herbivorous insects is the end product of ingestion, assimilation, and allocation of nutrients supplied from larval host plants. Extensive research using synthetic diets has demonstrated that the nutrient

**Table 3** Three-way MANOVA table for the effect of host plant (diet), temperature (temp), and sex on the bivariate body composition (lipid content and lipid-free pupal mass) of *Hyphantria cunea* allocated to six treatments [two host-plant groups (*Platanus* or *Prunus*)  $\times$  three temperature groups (20, 25, or 30 °C)]

Source	d.f.	Pillai's statistic	F	P
Diet	2,90	0.5914	65.12	<0.001
Sex	2,90	0.4093	31.17	<0.001
Temp	4,182	0.6264	20.75	<0.001
Diet*sex	2,90	0.1641	8.83	<0.001
Diet*temp	4,182	0.1891	4.75	0.001
Sex*temp	4,182	0.0559	1.31	0.27
Diet*sex*temp	4,182	0.0091	0.21	0.93

content of herbivore body strongly reflects that of diets (Lee et al., 2002; Lee, 2010; Lee & Roh, 2010; Simpson & Raubenheimer, 2012). For example, in *Spodoptera littoralis* Boisduval pupae, the ratio of lipid-free body mass to lipid content was shown to increase from 1.3:1 to 4.6:1 as the ratio of protein to digestible carbohydrate in the diets they ate during the larval stage rose from 1:5 to 5:1 (Lee et al., 2002). Our data showed that the ratio of lipid-free body mass to lipid content in *H. cunea* pupae ranged between 2:1 and 4:1 and was ca. 2 $\times$  higher in insects raised on *Sophora* or *Platanus* (ca. 4:1) than in insects raised on *Prunus* (ca. 2:1). This strongly indicates that the macronutrient ratio of protein to digestible carbohydrate in the leaves of *Sophora* and *Platanus* may be higher than in *Prunus* leaves, although data obtained from natural diets are not directly comparable to those from synthetic diets. Such a high variability in body composition ratio observed from caterpillars ingesting field-collected plants is striking, because it has been well documented that herbivores use a variety of post-ingestive regulatory processes to stabilize their body nutrient composition despite having eaten nutrients in imbalanced quantities and ratios (Raubenheimer & Simpson, 1997; Lee et al., 2002; Simpson & Raubenheimer, 2012).

From a viewpoint of resource allocation (Boggs, 2009), a high level of body lipids resulting from feeding on relatively carbohydrate-rich diet, such as *Prunus*, is likely to enhance dispersal potential, starvation resistance, and the probability of survival during diapause in this insect because lipids are the major substrate for energy storage (Stockhoff, 1991; Zera & Denno, 1997; Hahn & Denlinger, 2007). In contrast, ingesting diets that are rich in protein relative to carbohydrate, such as *Sophora* or *Platanus*, may improve the reproductive success of female insects through the allocation of more protein to egg production (Wheeler, 1996; Lee et al., 2008). These results indicate that *Sophora* and *Platanus* are a more suitable diet for



**Figure 4** Bivariate thermal reaction norms for mean ( $\pm$  SEM) lipid content and lipid-free pupal mass for *Hyphantria cunea* raised on one of two host plants (*Platanus* or *Prunus*) at 20, 25, or 30 °C. For male and female insects on each host plant, three symbols are connected representing the body composition measured at 20 (right), 25 (middle), and 30 °C (left).

females than *Prunus*. A recent study using synthetic diets demonstrated that female *Spodoptera litura* Fabricius caterpillars preferred a diet containing a significantly higher ratio of protein to carbohydrate than males (Lee, 2010), but whether caterpillars will prefer a specific plant based on their sex-specific nutrient requirement remains to be elucidated under natural conditions. Collectively, the discovery that different components of fitness are maximized on nutritionally different host plants raises the possibility that the macronutrient balance of diets may mediate the trade-off between key fitness components in this caterpillar as was demonstrated previously in *Drosophila* flies (Lee et al., 2008).

Protandry was evident for *H. cunea* caterpillars, with males reaching the pupal stage earlier than females. Females pupated at a larger size than males, probably owing to this sex-specific difference in development time (Blanckenhorn et al., 2007). Interestingly, the expression of this female-biased sexual size dimorphism (SSD) was strongly host-plant specific, being more pronounced for caterpillars raised on *Platanus* or *Sophora* than for those raised on *Prunus*, *Cornus*, or *Betula*. This pattern of SSD was mainly due to the fact that females accumulated substantially more protein-derived lean body matter than males when both were fed on *Platanus* or *Sophora*. Sex difference in lean body mass on the other plants was not as apparent as on *Platanus* and *Sophora*.

After determining the nutritional quality of host plants in Experiment 1, we used the two high-quality host plants that had contrasting effects on the nutritional phenotype of caterpillars (*Prunus* and *Platanus*) to test whether host

plant can alter the pattern of thermal reaction norms for a variety of caterpillar fitness components in Experiment 2. Previous studies examining thermal effects on ectotherm performance have shown that ectotherms developed more quickly and matured at a smaller body size when rearing temperature was higher (Atkinson, 1994; Angilletta, 2009). High incidence of mortality or developmental failure was observed in many caterpillars growing at their maximal rates under high ambient temperatures (Petersen et al., 2000; Lee & Roh, 2010). The present data were fully consistent with these previous results, but further demonstrated that such herbivore responses to temperature are highly host-plant specific. As expected, development was accelerated with increasing temperature, but the shape of the thermal reaction norm for development time differed qualitatively between *Prunus*- and *Platanus*-raised caterpillars. The development time of caterpillars feeding on *Platanus* decreased at a fixed rate as temperature rose from 20 to 30 °C, thus demonstrating a negative linear thermal reaction norm. For those feeding on *Prunus*, however, the relationship between development time and temperature was biphasic, with development time falling rapidly as temperature increased from 20 to 25 °C and then remaining insensitive to further temperature increase. Whereas physiological processes underlying the complex interactions between host plant and temperature for development time remain to be addressed, the observed plant-induced plasticity in herbivore thermal sensitivity is likely to have critical consequences for the host-plant preference and performance of herbivores facing climate change (Lemoine et al., 2013). For example, development time was longer on *Prunus* than on *Platanus* at 20 °C, but the pattern was reversed at 25 °C, suggesting that temperature can reorder the rank of host-plant suitability (Bauerfeind & Fischer, 2013a). Given the associations between prolonged larval development and the mortality costs of predation (Loader & Damman, 1991; Benrey & Denno, 1997), the relative fitness consequences of ingesting a particular host plant may change depending on environmental temperature.

Caterpillars feeding on *Platanus* or *Prunus* followed the temperature-size rule, but the negative thermal reaction norm for final size also varied as a function of host plant. Especially for females, the rate at which the mass at pupation increased with decreasing temperature was much steeper when they were raised on *Platanus* than on *Prunus*. As such, the extent to which *Platanus*-raised females were heavier than their *Prunus*-raised counterparts increased from 28 to 53% as temperature decreased from 30 to 20 °C. As large body size at maturity is often positively correlated with potential fecundity in Lepidoptera (Honek, 1993), the reproductive benefits of ingesting



*Platanus* in preference to *Prunus* are expected to be greater at lower than at higher temperatures, suggesting that temperature can modify the effects of host plant on herbivore performance. To understand what caused the slope of the thermal reaction norms for final size to differ between the two host-plant groups, we compared how the bivariate patterns of body composition changed in response to temperature for caterpillars on *Prunus* and *Platanus*. We found that the quantity of lipid-free pupal mass that increased in response to decreasing temperature was substantially higher for caterpillars raised on *Platanus* than for caterpillars raised on *Prunus*. However, the extent to which body lipid content increased as temperature fell was similar for the two groups. This result suggests that host plant-specific difference in protein acquisition can be the main factor causing the differences in the thermal reaction norm between the two groups because lipid-free pupal mass is composed predominantly of protein. A previous study indicated that lycaenid caterpillars grew larger in cooler environments by ingesting more food and utilizing ingested nutrients more efficiently (Karl & Fischer, 2008). Given that protein is a primary constituent of the animal body, it is plausible that low protein content expected for *Prunus* relative to *Platanus* may have constrained caterpillars from acquiring sufficient protein required for increasing their body size at low temperatures, thus making the slope of the thermal reaction norm for body size less steep (Petersen et al., 2000).

This study has provided compelling evidence that the pattern of thermal reaction norms for the major fitness components of herbivores can be altered by ingesting host plants with differing nutritional quality, highlighting the importance of nutrition as a factor modulating the thermal responses of ectotherms. In addition, the discovery that temperature causes a shift in the host-plant suitability raises an intriguing possibility that high levels of variations in the thermal environment in the temperate zone may contribute to the maintenance of an extremely broad diet of this caterpillar. This study has focused on examining the direct effects of temperature on herbivores, but temperature is known to also exert indirect impact on herbivore performance through influencing host-plant quality (Zvereva & Kozlov, 2006; Bauerfeind & Fischer, 2013b), adding further complexity to the role of temperature in affecting host-plant selection and fitness of herbivores. The present results have implications for predicting the effects of climate change on herbivore-plant interactions and the spread of invasive species (Braschler & Hill, 2007; Kingsolver et al., 2011; Audusseau et al., 2013; Bauerfeind & Fischer, 2013b), with broader ecological consequences for herbivore populations, plant communities, and nutrient cycling.

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