



Increased temperature alters feeding behavior of a generalist herbivore

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Temperature can regulate a number of important biological processes and species interactions. For example, environmental temperature can alter insect herbivore consumption, growth and survivorship, suggesting that temperature-driven impacts on herbivory could influence plant community composition or nutrient cycling. However, few studies to date have examined whether rising temperature influences herbivore preference and performance among multiple plant species, which often dictates their impact at the community level. Here, we assessed the effects of temperature on the performance and preference of the generalist herbivore *Popillia japonica* among nine plant species. We show that, on average, consumption rates and herbivore performance increased at higher temperatures. However, there was considerable variation among plant species with consumption and performance increasing on some plant species at higher temperatures but decreasing on others. Plant nutritional quality appeared to influence these patterns as beetles increased feeding on high-nitrogen plants with increasing temperature, suggesting stronger nitrogen limitation. In addition to changes in feeding rates, feeding preferences of *P. japonica* shifted among temperatures, a pattern that was largely explained by differential deterrence of plant chemical extracts at different temperatures. In fact, temperature-induced changes in the efficacy of plant chemical extracts led *P. japonica* to reduce its diet breadth at higher temperatures. Our results indicate that rising temperatures will influence herbivore feeding behavior by altering the importance of plant nutritional and chemical traits, suggesting that climate change will alter the strength and sign of plant–insect interactions.

Environmental temperature regulates a number of critical ecological processes through its effects on the physiology of ectothermic organisms (Melillo et al. 2002). Temperature-driven shifts in metabolic demand can alter vital biological rates, including consumption and growth, of many organisms (Lemoine and Burkepile 2012). The effects of temperature on ectothermic metabolism can therefore potentially propagate throughout the entire biological hierarchy (Allen et al. 2005). For example, temperature influences key ecological interactions, such as herbivory (O'Connor 2009), predation (Rall et al. 2010), or competition (Tilman et al. 1981). By influencing such important interactions, environmental temperature can indirectly control food web structure (Chase 1996, Kratina et al. 2012) community composition (de Valpine and Harte 2001), and nutrient cycling (Melillo et al. 2002). However, the role of temperature in regulating herbivore feeding preferences, and therefore how top–down control is allocated among plant species, remains relatively understudied.

Insect herbivores are the most diverse group of metazoa on earth and can have large impacts on plant community composition (Carson and Root 1999, Maron and Crone 2006) and nutrient cycling (Weisser and Siemann 2004) by differentially consuming plant species. Insects are also

ectotherms whose metabolic rates increase exponentially with temperature (Rall et al. 2010). Temperature can strongly affect insect consumption, growth, and developmental rates by changing basic metabolic processes (Kingsolver and Woods 1997, Briscoe et al. 2012). Further, previous studies suggest that temperature-driven increases in metabolic rates can induce stronger nutrient limitation (Elser et al. 2003), which may result in different food preference hierarchies at different temperatures depending on nutritional quality.

Herbivore performance, for example, is often limited by plant nutritional content, such that herbivores preferentially consume plants rich in limiting nutrients to satisfy nutritional demands (Sterner and Elser 2002). Higher temperatures, however, can increase the rate of protein denaturing, thereby necessitating higher rates of production and repair of nitrogen-rich proteins (Angilletta 2009). The synthesis of heat-shock proteins also occurs at temperatures well below an organism's upper thermal limit (Gehring and Wehner 1995). Additionally, high temperatures lead to increased protein turnover rates and significant respiratory and nitrogen-utilization costs (Hoffman and Somero 1995, Hachiya et al. 2007). Thus, increasing temperatures might force herbivores to increase their intake of nitrogen-rich foods to provide sufficient materials to repair damaged proteins and synthe-

size new proteins (e.g. heat-shock proteins). Similarly, rising temperatures can also increase ectotherm growth rates (Gillooly et al. 2001), potentially escalating the demand for phosphorus-rich materials like RNA and ATP, both of which are required for cellular growth (Elser et al. 2003, Wojewodzic et al. 2011). Thus, at higher temperatures herbivores may also shift feeding preferences towards phosphorus-rich plants. In addition to these nutrient-driven changes, temperature might also influence plant–insect interactions that are mediated by plant chemical defenses. For example, herbivores can become more or less sensitive to chemical defense compounds at higher temperatures (Stamp and Yang 1996, Stamp and Osier 1998), depending on the herbivore and plant species. Yet, the influence of both plant nutritional quality and chemical defenses on herbivore preferences among multiple plant species remains untested.

Here, we used the herbivorous beetle *Popillia japonica* to determine how temperature affects herbivore performance and preference among nine plant species. *Popillia japonica*, a known agricultural pest, is considered invasive in North America, and maintains a broad diet of > 300 plant species from 79 families (Potter and Held 2002). Therefore, *P. japonica* served as a model organism to ask three specific questions: 1) how does temperature affect insect herbivore performance among host plants? 2) Do herbivore feeding preferences vary with temperature? And 3) do plant traits (e.g. nutrient content, chemical defenses) explain variation in herbivore preference and performance at different temperatures? To address these questions, we used no-choice and choice feeding assays with nine species of plants at four temperatures (20°, 25°, 30° and 35°C) to determine how *P. japonica* performance and preferences varied among host species. We coupled herbivore performance data with plant nutritional characteristics to assess how plant traits affected herbivore performance across temperatures. Further, we performed feeding assays incorporating chemical extracts of host-plants into artificial diets to assess whether the effectiveness of chemical defenses varied with temperature. We expected that at high temperatures, nitrogen and phosphorus limitation would increase in strength, thereby increasing the preference of *P. japonica* for plants rich in these limiting nutrients, while strengthening the effects of plant chemical defenses.

Methods

All experiments were conducted at the Smithsonian Environmental Research Center (SERC), Edgewater, MD, USA from June–August 2012. Adult *Popillia japonica* (0.102 ± 0.018 g, mean \pm SD) were collected by hand from various host plants in forest and edge habitats, and all individuals were used in experimental trials within two days of capture. While in captivity, individuals were held at room temperature and fed fresh *Platanus occidentalis* leaves daily. Using laboratory feeding assays, we evaluated the performance and preference of *P. japonica* on nine abundant plant species known to host *P. japonica*: *Acer negundo*, *A. rubrum*, *Liquidambar styraciflua*, *Platanus occidentalis*, *Rosa multiflora*, *Rubus allegheniensis*, *R. phoenicolasius*, *Viburnum prunifolium* and *Vitis vulpina* (see Supplementary material Appendix A1 Fig. B1 for

phylogenetic tree). Most of the plant species are native to the United States, except for *R. multiflora* and *R. phoenicolasius* which are introduced plant species with the same native range as *P. japonica* and are confamilial and congeneric, respectively, with North American *R. allegheniensis*. In particular, *P. japonica* strongly prefers plants from the genus *Rosa* as host plants (Held and Potter 2004). Thus, *R. multiflora* represents a preferred host-plant from the native range of *P. japonica*.

Feeding assays

We assessed the performance and feeding preferences of *P. japonica* on each of the nine plant species using both no-choice and choice feeding assays. To standardize leaf quality among replicate assays, fresh, mature leaves were collected from 2–3 neighboring individuals of similar size for each plant species. Thus, all plants from which leaves were harvested within a species were from identical microhabitats and leaves were of similar ages.

In no-choice assays, a single *P. japonica* individual was weighed and placed in a plastic rearing cup with a single, pre-weighed leaf from one of the nine plant species. Each rearing cup was randomly assigned to one of four temperatures (20°, 25°, 30° and 35°C, see Supplementary material Appendix A1 Table A1 for temperature and light data) maintained by growth chambers on a 14:10 light:dark cycle ($n = 8$ per plant species per temperature). Temperatures were selected to represent average temperatures from late spring 20°C (average high temperature in April) to mid-summer months 32°C (average high temperature in July) in the study range, along with a 35°C treatment that represents a conservative 3°C increase beyond the typical peak temperature due to predicted climate change (IPCC 2007). During the month of June, when beetles were collected, daily temperatures in the area averaged 24.25 ± 3.43 °C (NOAA Station APAM2). Thus, 20–30°C falls within two standard deviations of daily temperatures encountered prior to initiating our experiments. Further, *P. japonica* experience normal development and growth between 15–37°C (Ludwig 1928). Thus, our temperature regimes are within the thermal tolerance range of *P. japonica*. Similar experimental designs have been used to evaluate herbivore thermal response curves (Kingsolver and Woods 1997, 1998). Temperatures were held constant, as alternating day:night temperatures has no effect on the growth or development of *P. japonica* as long as the temperatures are within the thermal window of 15–35°C (Ludwig 1928). No individual *P. japonica* was used for more than one feeding assay.

Leaf petioles were placed in water-filled microcentrifuge tubes capped with cotton to prevent desiccation, and we observed no obvious differences in leaf turgor during the assays. After 24 h, beetles and leaves were reweighed to estimate consumption rates and changes in herbivore mass. Feeding assays of this duration have been used to assess herbivore performance and dietary preferences in lepidopterans (Kingsolver and Woods 1997, 1998) and coleopterans (Gange et al. 2012, Kosonen et al. 2012). Furthermore, results from 4–6 h assays testing *P. japonica* feeding preferences among plant species generally mimic survival patterns on each species after 21 days (Held and Potter 2004), and body mass

of adult coleopterans is positively correlated with reproductive fitness (e.g. number of eggs laid, Vamosi 2005). Thus, mass change may represent one possible metric of adult *P. japonica* fitness, although longer-term studies with direct counts of eggs produced would better capture the relationship between feeding behavior and adult fitness.

Control assays with no herbivores accounted for autogenic change in leaf weight over the 24 h period ($n = 5$ per plant species per temperature). Leaves of all plant species except *L. styraciflua* gained mass over 24 h in the absence of herbivores. Larger leaves gained more mass than did smaller leaves except in *R. phoenicolasius*, where mass gain was constant across all leaf sizes. We therefore used species-specific equations to correct for autogenic change based on leaf mass (Supplementary material Appendix A1 Table A2). Mass-specific autogenic changes, whether positive or negative, were added to consumption rates. Negative autogenic changes (i.e. plants lost mass in control assays) would therefore lower estimates of consumption and vice versa.

To gauge how herbivore feeding preferences varied with temperature among multiple host plants offered simultaneously, we conducted choice-feeding assays with 10 adult *P. japonica* placed in clear, 1.6-l plastic containers with a single leaf from all nine species simultaneously. Leaf petioles were placed in water-filled microcentrifuge tubes as described above. After 24 h, leaves were reweighed to estimate daily consumption rates. We replicated choice assays at 25° and 35°C ($n = 8$ per temperature) because these temperatures represent the average high temperatures at the study site in May, when adults first reach high abundances, and 3°C above the average high temperature in July. Consumption rates were corrected for autogenic changes in plant mass as described above.

Foliage used in this study was collected from plants under natural field conditions and not from plants grown at each temperature. Theoretically, rising temperatures could increase plant growth rates (Veteli et al. 2002) and alter nutritional content or concentrations of defensive compounds as plants shuttle more resources into growth (Coley et al. 1985). However, previous studies have found relatively small effects of temperature on plant phytochemistry, particularly % water, % nitrogen, and concentrations of tannins, phenols, and other chemical defenses (Veteli et al. 2002, Richardson et al. 2002, Williams et al. 2003, Zvereva and Kozlov 2006). This holds true for some species used in our study (Williams et al. 2000, 2003). Thus, growing the different plant species to use in feeding assays at the different temperatures described above likely would have had minimal effects on the patterns in our results.

Plant traits

To assess the mechanism by which temperature affects herbivore performance among plant species, we quantified nutritional characteristics of undamaged leaves ($n = 3-5$) of each plant species. Leaf toughness was measured using a force gauge. Prior to all nutrient content analyses, leaves were weighed, dried to a constant weight at 60°C, and re-weighed to estimate water content. Dried leaf material was ground to a fine powder for carbon (C), nitrogen (N), phosphorus (P) and protein analyses. Percent C and N were estimated using an elemental analyzer. Phosphorus content was determined

using dry oxidation–acid hydrolysis extraction followed by colorimetric analysis on a microplate spectrophotometer. Protein content was measured using a modified Lowry kit optimized for a microplate spectrophotometer.

Total crude extracts of plant chemical defenses

In order to examine how plant defensive chemistry affected feeding by *P. japonica* and whether the effectiveness of plant defenses varied among temperatures, we conducted feeding assays with total crude extracts from plant leaves of each species used in the feeding assays described above. All chemical extract methods and feeding assays follow Lind and Parker (2010). Fresh leaves of each plant species (2 g equivalent dry weight) were ground in a coffee grinder and then extracted using both lipophilic and hydrophilic solvents (2:1 v/v dichloromethane (DCM): methanol (MeOH), 1:1 v/v DCM:MeOH, 2:1 v/v MeOH:H₂O) added to freshly chopped leaves to extract secondary chemical compounds. Extraction times were 2 h, 2 h and 12 h for each step, respectively. All plant species were subject to identical extraction times. Extracts were condensed using a vacuum centrifuge. These methods have successfully demonstrated that plant secondary metabolites affect herbivore feeding behavior (Stachowicz and Hay 1996, Cruz-Rivera and Hay 2003, Lind and Parker 2010).

Extracts were resuspended in 5 ml acetone and added to an artificial diet mixture of 1 g wheat germ, 1 g cellulose, and 0.025 g FABCO-1 antifungal agent. Thus, extracts from 2 g of dried leaves were added to 2 g of artificial diet preserving the natural ratio of secondary chemicals to food mass. Twenty ml of boiling water were added to the diet and the mixture stirred until all acetone had evaporated. Agar powder (0.75 g) was added to the mixture to act as a solidifying agent and the mixture immediately poured into a 1.5 cm wide mold. Control foods lacking chemical extracts were prepared in an identical fashion using acetone without chemical extracts. Green food coloring was added to the control diets to mimic the color of the chemically treated foods (Pleau et al. 2002).

For each plant species, 1.5×1 cm strips of control and chemically treated agar foods were weighed to the nearest milligram and placed on opposite sides of an insect rearing cup (350 ml). A single, weighed adult *P. japonica* individual was then placed in the center of each cup. After 24 h, the agar strips were reweighed and mass-corrected consumption calculated as $(\text{Mass}_{\text{final}} - \text{Mass}_{\text{initial}}) / \text{Mass}_{\text{herbivore}}$. We replicated these chemical extract bioassays at 25° and 35°C ($n = 8$ per plant species per temperature).

Statistical analyses

Consumption and herbivore mass change in no-choice assays were analyzed using a factorial ANOVA with plant species and temperature as fixed effects. The assumption of homogeneous variances could not be met, thus we used a weighted model where each observation was weighted by the inverse of the variance within its corresponding plant species \times temperature treatment, allowing us to keep the data on the original, untransformed scale (Zuur et al. 2009). Plots of residuals versus fitted values showed that weighting observations adequately resolved issues with heterogeneous

variances. In the presence of a significant interaction, post hoc comparisons were made using pairwise *t*-tests with non-pooled variances. Since the main purpose of this study was to examine relative herbivore performance among plant species at different temperatures, we only conducted post hoc comparisons among species within a temperature. This reduced the number of unplanned comparisons from 630 (all possible comparisons) to 144. As a Bonferroni adjustment on 144 comparisons results in an extreme correction to the critical *p*-value ($\alpha = 0.0003$) and high likelihood of type II errors, we used the false discovery rate (FDR) correction with 144 unplanned comparisons to assess the significance of post hoc pairwise *t*-tests (García 2004). Choice assays were analyzed using MANOVA, with percent loss of each species as response variables and temperature as the predictor. Use of a MANOVA accounts for non-independence of the response variables in choice assays (Roa 1992). Pairwise *t*-tests with FDR corrections were used for post hoc comparisons of species within each temperature as in the no-choice assays.

We were also interested in examining whether rising temperatures would increase the variance in consumption rates, herbivore mass changes, or feeding preferences among plant species. The assumption was that temperature stress would cause feeding to shift towards relatively fewer, more nutritious species. We thus used one-tailed *F*-tests to determine whether variance among mean consumption and relative growth rates was higher at 35° than at 25°C. To assess higher variance in the choice trials where all food options were offered simultaneously, we calculated 1-Simpson's diversity index (*D*) of consumption for each choice assay as $D = \sum p_i^2$, where p_i is the proportional consumption of each plant species, and 0 represents low diet diversity and 1 represents high diet diversity. We used a *t*-test with unequal variances to compare diversity indices between temperatures to determine whether temperature affected the diet breadth of *P. japonica*.

As some plant traits were highly correlated (% water:% protein $r = -0.75$, % water:%P $r = 0.59$), principle components analysis (PCA) was used to reduce the dimensionality of the plant trait matrix and account for potential collinearity among plant traits. Mean consumption and mass changes of *P. japonica* for each plant-temperature combination were used as the response variable in multiple regressions against the principle components. Each regression model included main effects for temperature, each principle component, and interaction effects for each principle component and temperature. Thus, we used two models, one for consumption and one for mass changes, to assess if the effect of plant traits varied across temperatures. No traits exhibited a significant phylogenetic signal (Supplementary material Appendix A1 Table A3), suggesting that phylogenetically controlled contrasts were unnecessary.

Chemical extract bioassays were analyzed by calculating the difference in consumption between chemically treated food and control food as the response variable. A positive value indicates preference for chemically-treated food, while a negative value indicates avoidance of chemically treated food. A linear model with no intercept term was used to assess whether feeding preferences varied among plant species. Removal of the intercept allowed us to include a dummy variable for each plant species. Thus, *t*-tests of each

coefficient inherent in the regression determined whether the mean response for each species differed from zero. Two separate linear models were run for 25° and 35°C. Variances were heterogeneous among species, therefore we used a weighted model where each observation was weighted by the variance of its corresponding species (Zuur et al. 2009). We used a one-tailed *F*-test to determine whether the variance in mean effects of chemical extracts increased with temperature, as described above.

All statistical analyses were conducted in R ver. 2.15 (R Development Core Team). Weighted regressions were run using the 'nlme' package (Pinheiro et al. 2012).

Results

Feeding assays

In no-choice assays, average consumption rates increased with temperature ($F_{3,248} = 41.66$, $p < 0.001$), but this overall trend masked considerable variability in consumption rates of each plant species across temperatures (plant species \times temperature interaction, $F_{24,248} = 4.18$, $p < 0.001$, Fig. 1A, see Supplementary material Appendix A1 Table A4 for post hoc results). As temperature increased, the rank order of consumption of each plant species changed. At 20°C, *Popillia japonica* consumed more *Rosa multiflora* and *Vitis vulpina* than all other species except *Platanus occidentalis*. At 25°C, *P. japonica* consumed more *R. multiflora* than any other plant. Moreover, the plant species consumed least by *P. japonica* changed with temperature, with *Rubus phoenicolasius* going from one of the least consumed species at 25°C to being consumed at average rates at 35°C. Additionally, the variance of mean consumption rates of each plant species in no-choice assays was over 4 \times higher at 35° than at 20° ($F_{8,8} = 4.029$, $p = 0.033$, Supplementary material Appendix A1 Fig. B2), indicating that rising temperatures increased the discrepancy between the greatest and least consumed plants.

Similar to consumption rates, *P. japonica* gained more mass at higher temperatures than at lower temperatures ($F_{3,248} = 81.77$, $p < 0.001$). However, the effect of temperature on *P. japonica* mass gains was contingent on plant species identity (plant \times temperature interaction, $F_{24,248} = 7.59$, $p < 0.001$, Fig. 1B, see Supplementary material Appendix A1 Table A4 for post hoc results), although individuals gained the most mass on *R. multiflora* at all temperatures. At 20°C, *P. japonica* mass changes were uniformly low on all other plant species. But as temperature increased, *P. occidentalis*, *V. vulpina* and *Rubus* spp., became better host-plants than others, chiefly *Viburnum prunifolium* and *Liquidambar styraciflua*. Moreover, while there was little variation in *P. japonica* mass changes at low temperatures, variance in mass changes among plant species increased rapidly with rising temperatures (Supplementary material Appendix A1 Fig. B3) and was over 3 \times higher at 35° than 20°C ($F_{8,8} = 3.36$, $p = 0.053$). Excluding the uniformly high growth rates on their native host-plant *R. multiflora* strengthened this pattern considerably, as variance in *P. japonica* mass changes among plant species was over 21 \times higher at 35° than 20°C ($F_{8,8} = 21.38$, $p < 0.001$). Thus, the relative

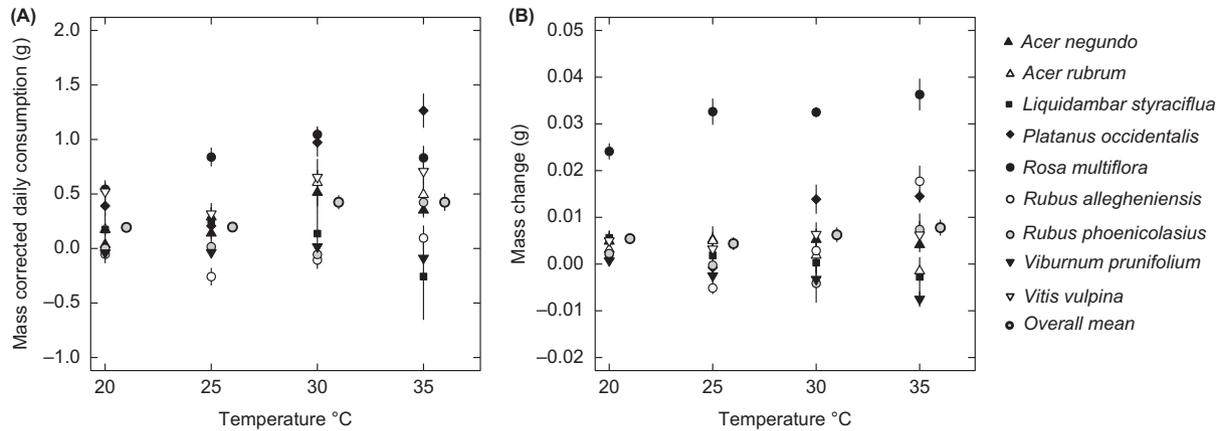


Figure 1. (A) Mass-corrected consumption and (B) mass changes of *P. japonica* on each of the nine plant species at each temperature during the no-choice assays. Overall means at each temperature are displayed adjacent to the species means. Data points represent mean \pm SE.

suitability of plants as host species changed dramatically as temperature increased, with a larger difference between hosts at higher temperatures.

In choice assays, differences in *P. japonica* feeding preferences varied with temperature (Wilk's $\Lambda = 0.153$, $\chi^2 = 17.82$, $p = 0.037$, see Supplementary material Appendix A1 Table A5 for post hoc results). At 25°C, *P. japonica* preferentially consumed more *R. multiflora* than all other species (Fig. 2). At 35°C, *P. japonica* feeding preferences shifted among plant species, preferentially consuming *R. multiflora* and *V. vulpina* over all other species. However, the increase in consumption of *V. vulpina* was offset by decreased consumption of all other plant species, leading to an overall decrease in consumption rates (Fig. 2). Accordingly, *P. japonica* had a significantly less diverse diet at 35°C ($1-D = 0.760 \pm 0.031$) than at 25°C ($1-D = 0.834 \pm 0.004$, $t = 2.96$, $p = 0.021$), indicating a reduction in dietary breadth at higher temperature for the species tested here.

Plant traits

PCA of plant traits isolated three principle components with eigenvalues > 1 (Supplementary material Appendix

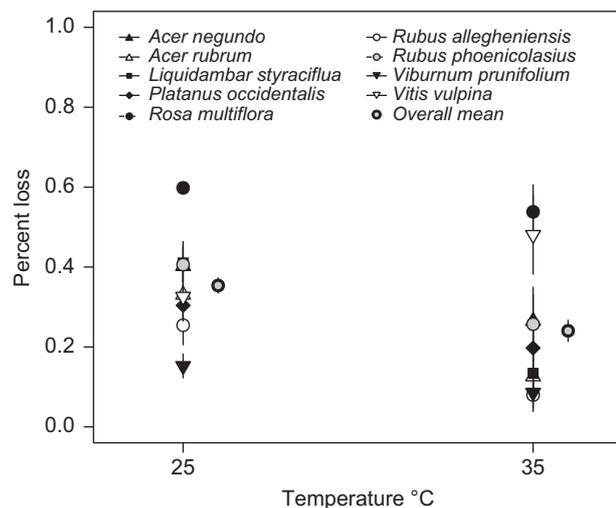


Figure 2. Percent mass loss of all nine plant species at each temperature during the choice assays. Data points represent mean \pm SE.

A1 Table A6). The first axis represented water content, % P, and % protein in plant leaves. The second axis was predominately a % C and toughness axis. The third axis represented leaf %C and % N. Together, these three axes explained 86% of the variation in nutritional characteristics among plant species. Consumption rates were not significantly related to any principle component, nor did temperature interact significantly with any principle component. Although there was some indication of an effect of temperature and an interaction between PC 3 and temperature ($p = 0.073$ for both; Supplementary material Appendix A1 Table A7), low power ($\beta = 0.321$, Monte Carlo power simulation, Bolker 2008) may have inhibited our ability to detect a statistically significant interaction at $\alpha = 0.05$.

Similarly, there was no effect of any principle component on herbivore mass change. However, disproportionately high growth rates on the native host-plant *R. multiflora* at all temperatures appears to have masked the influence of other plant traits in explaining impacts on *P. japonica* growth. Excluding *R. multiflora*, changes in *P. japonica* mass varied with PC 3, although a significant interaction with temperature indicated that the effect varied with temperature ($p = 0.003$, $R^2 = 0.30$, Fig. 3, Supplementary material Appendix A1 Table A7). At 20° and 25°C, there was no relationship between *P. japonica* mass change and PC 3. However, at 30° and 35°C, there were strong negative relationships between *P. japonica* mass change and PC 3. As negative PC 3 values indicate plants with high % N and % C, this suggests that *P. japonica* performed best on plants with high % N and % C and performed poorly on plants with low % N and % C at high temperatures. In contrast, the absence of any PC 3 effect at low temperatures suggests that the measured components of plant nutritional quality were unimportant to *P. japonica* performance at low temperatures. Repeating these analyses excluding both *R. multiflora* and *R. phoenicolasius*, leaving only native plant species that share no evolutionary history with *P. japonica*, led to qualitatively similar results.

Chemical extracts

At 25°C, there was no overall effect of plant crude chemical extracts on *P. japonica* feeding (Supplementary

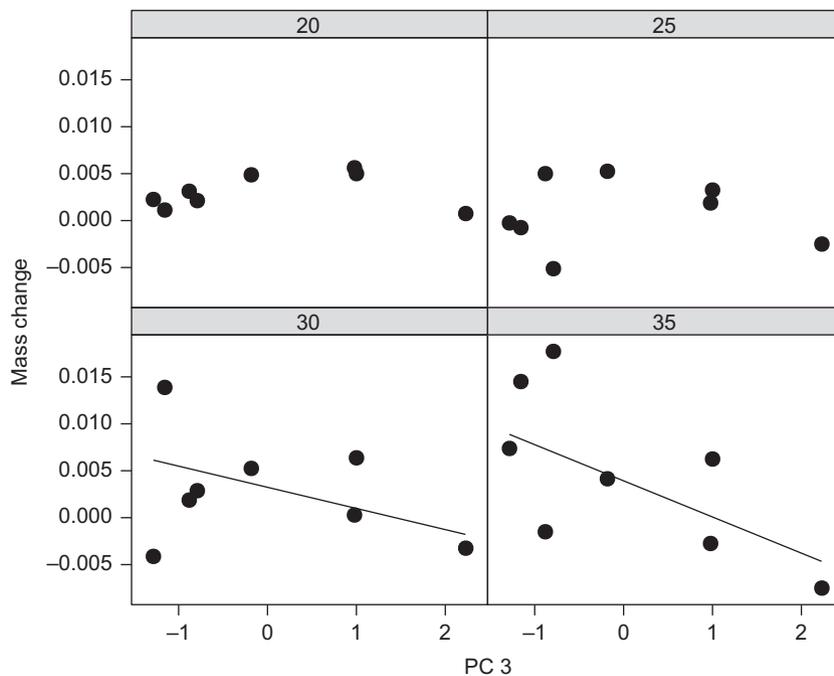


Figure 3. Relationship between *P. japonica* mass change and PC 3. Negative PC 3 values indicate high % N, high % C of leaf material. *Rosa multiflora* not shown, as it was excluded from regression analyses. Regression lines that did not differ from zero not shown.

material Appendix A1 Table A8, Fig. 4), and only extracts from *A. negundo* were deterrent. At 35°C there was still no overall effect of plant crude chemical extracts, although three plant species exhibited shifts in plant chemical deterrence. Extracts of *R. multiflora* and *V. vulpina* became significantly stimulatory, whereas extracts from

R. phoenicolasius became significantly deterrent (Supplementary material Appendix A1 Table A8, Fig. 4). Moreover, variance in the effects of chemical extracts among species increased substantially with temperature and was nearly 17 × higher at 35°C than 25°C ($F_{9,9} = 16.973$, $p < 0.001$).

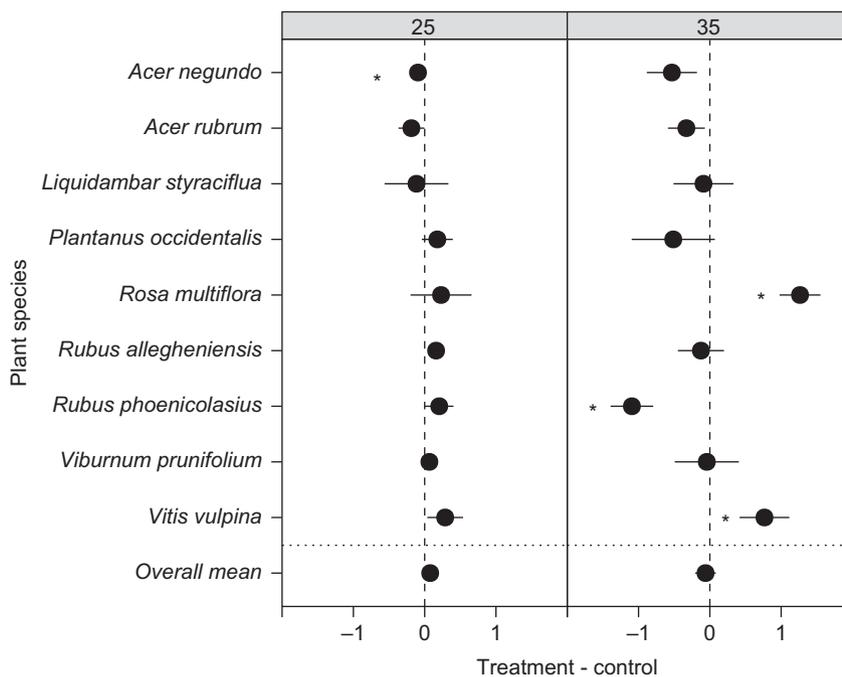


Figure 4. Difference in consumption rates between chemically-treated and control artificial diets. Positive values indicate preferential consumption of chemically-treated foods, negative values indicate avoidance of chemically-treated food. Asterisks denote species that differed significantly from 0. Data points represent mean ± SE.

Discussion

Metabolism of ectothermic organisms increases exponentially with temperature (Gillooly et al. 2001, Clarke 2004), suggesting that temperature can greatly alter individual nutritional requirements for either nitrogen or phosphorus by stimulating growth rates or protein synthesis and repair (Elser et al. 2003, Angilletta 2009, Wojewodzic et al. 2011). Here we demonstrate that higher temperatures increased variation in *Popillia japonica* consumption and growth rates among multiple plant species and that these changes were correlated with plant nutritional content. At low temperatures, herbivores performed equally well on all plant species. However, at higher temperatures, herbivores performed better on plant species with high nitrogen content compared to species with low nitrogen content. Furthermore, higher temperatures lead to changes in *P. japonica* feeding preferences and caused a reduction in diet breadth. Such changes were correlated with changes in the efficacy of plant chemical extracts at higher temperatures. Earlier studies addressing the interaction between diet quality and temperature on generalist herbivore performance have largely used artificial diets (Stamp and Yang 1996, Stamp and Osier 1998, Kingsolver and Woods 1998, Kingsolver et al. 2006) or measured variation in nutritional quality within a single plant species (Himanen et al. 2008). To our knowledge, this is the first study demonstrating that temperature-driven changes in herbivore feeding preferences and performance correlate with natural variation in nutritional quality and secondary chemical composition across a range of plant species.

Averaged across all plant species, consumption rates of *P. japonica* increased with temperature while growth remained relatively constant (Fig. 1). These patterns are consistent with predictions that ectothermic herbivores must increase food intake at higher temperatures to offset increased metabolic or nutritional demands (O'Connor et al. 2011). One possible outcome of increased temperatures is increased repair and synthesis of nitrogen-rich proteins, which can limit growth rates (Somero 2011). Other generalist, aquatic insects show similar rapid increases in growth rate with increasing temperature only on high nitrogen diets (Gresens 1997). A second possible outcome is that thermal stress increases demand for carbon-rich materials such as carbohydrates or lipids, which are important components of insect diets (Raubenheimer and Simpson 2003, Thaler et al. 2012). Grasshoppers exposed to predation risk, for example, increase their demand for carbohydrate-rich foods and feed on plants with higher carbon:nitrogen ratios compared to unstressed grasshoppers (Hawlena and Schmitz 2010). Carbohydrates, particularly sugars, are thought to be an important feeding stimulant for *P. japonica* (Held and Potter 2004), and in our study nitrogen and carbon content were positively correlated (Fig. 3). Thus, the increased feeding on plants of higher nutritional quality that we observed could have been due to higher nitrogen, higher carbon content, or a combination of both.

To compensate for increased nutritional demands, generalist consumers can either increase uptake of low-quality diets (i.e. compensatory feeding) or feed only on high-quality hosts. For example, specialized lepidopteran herbivores often display compensatory consumption of low-quality

diets at intermediate temperatures (Williams et al. 1994). In contrast, the generalist coleopteran herbivore *P. japonica* in our study showed no evidence for compensatory feeding. In fact, *P. japonica* exhibited widely differing thermal response curve shapes depending on host-plant species (Fig. 1). Interestingly, at low temperatures, beetles did not lose mass even on low quality plants. Accordingly, beetles performed worse on low quality plants at high temperatures than at low temperatures. Given that body size of adult coleopteran herbivores can be positively correlated with reproductive effort (Vamosi 2005), including within *P. japonica* (Saeki et al. 2005), these results suggest that temperature-driven impacts on diet choice may influence *P. japonica* fitness.

To date, the effects of temperature on chemically-mediated plant-insect interactions has received much less attention than the impact of plant nutrient content. Previous work has focused on either subsets of specific chemicals within a plant species (i.e. tomato, Stamp and Yang 1996), or the effects of temperature on the expression of chemical defenses within a plant (Zvereva and Kozlov 2006). We know of only one other study that has examined how bulk chemical extracts affect herbivore feeding behavior at multiple temperatures (Sotka and Giddens 2009). Overall, the effect of temperature on chemical defenses appears to be highly variable. Some chemicals become more or less effective (Stamp and Yang 1996, Stamp and Osier 1998, Sotka and Giddens 2009). Our data also support the premise that the efficacy of plant chemical defenses at higher temperatures will be highly variable among plant species. *Popillia japonica* did not select for or against extracts from any plant species at 25°C. But at 35°C, *P. japonica* preferred food incorporated with extracts from *Rosa multiflora* and *Vitis vulpina*, while avoiding foods incorporated with extracts from *Rubus phoenicolasius*. Interestingly, these patterns in chemical extract feeding assays matched patterns in herbivore choice assays. For example, at 25°C *P. japonica* displayed little preference among plant species (Fig. 2). At 35°C, *P. japonica* strongly preferred both *R. multiflora* and *V. vulpina* (Fig. 2) as assays with the chemical extracts would suggest. In our study, this occurs independent of any temperature-mediated changes in plant phytochemistry. These results suggest that temperature can affect herbivore feeding preferences among plant species by altering the efficacy of chemical defenses even without altering plant production of these defenses. The effects of temperature on chemically mediated plant-insect interactions therefore require significantly more research to understand the interactions between temperature, plants and herbivores.

Some patterns in the data were only apparent after excluding *R. multiflora* from the analyses. This might reflect the deep evolutionary relationship between *R. multiflora* and *P. japonica*. *Rosa multiflora* is an invasive plant with the same native host range as *P. japonica*, and *P. japonica* prefers *Rosa* spp. as host-plants (Held and Potter 2004) in both its native and introduced range. Such an evolutionary relationship, coupled with uniformly high growth rates on *R. multiflora*, suggests that *P. japonica* may have developed a physiology attuned to *R. multiflora* chemical compounds independent of plant nutritional quality (Verhoeven et al. 2009). For example, some herbivores grow best when presented with chemicals from plants with strong co-evolutionary

relationships even when nutritional content is held constant (Bowers and Puttick 1988). Likewise, seed beetles performed best on the host to which they have adapted, regardless of temperature and, most likely, nutritional content (Stillwell et al. 2007). In our study, individuals fed *R. multiflora* grew at least twice as fast as individuals fed any other plant at all temperatures, despite the relatively average nutritional value of *R. multiflora* (2.08% N, 0.17% P, 0.13% protein) compared to the other plant species examined here ($2.12 \pm 0.36\%$ N, $0.21 \pm 0.03\%$ P, $0.16 \pm 0.09\%$ protein, mean \pm SD). This may be because *P. japonica* has a close evolutionary history with *R. multiflora* that allows *P. japonica* to circumvent defenses of *R. multiflora* or efficiently digest chemical compounds produced by *R. multiflora*. Alternatively, high growth *P. japonica* on *R. multiflora* may be due to unmeasured nutritional compounds, such as carbohydrates, sugars, or sterols. *Rubus phoenicolasius* is also present in the home range of *P. japonica* and may also have a similar evolutionary history. This may explain why chemical compounds of *R. phoenicolasius* were effective deterrents despite its high nutritional quality. Excluding both *R. multiflora* and *R. phoenicolasius* from analyses did not change our results, suggesting that, in the absence of a shared evolutionary history, plant nutritional quality interacts significantly with temperature to alter herbivore growth patterns.

The exclusion of *R. multiflora* from data analysis therefore resulted in a set of plant species that are not preferred host plants from the native range of *P. japonica*, and, accordingly, consumption and growth rates of *P. japonica* on these plant species was solely a product of plant nutritional content and defenses, rather than a co-evolved interaction. The overall trend was that increasing plant nitrogen and carbon content and decreasing plant toughness had little effect on beetle growth at low temperatures but was a strongly correlated with beetle growth at high temperatures (Fig. 3).

We focused on examining herbivore response to increasing temperature while holding plant phytochemistry constant, but other studies have focused on examining plant response to rising temperatures. For example, plant growth rates increase with rising temperatures (Veteli et al. 2002), which could alter nutritional content or concentrations of defensive compounds as plants shuttle more resources into growth (Coley et al. 1985). However, studies have shown that the effects of temperature on plant secondary chemistry are highly idiosyncratic (Richardson et al. 2002, Veteli et al. 2002, Zvereva and Kozlov 2006). Temperature does not affect any measure of nutritional quality (e.g. water, nitrogen, tannins, phenols, sugars) of two *Acer* species, including *A. rubrum*, a species used in this study (Williams et al. 2000, 2003). Furthermore, although temperature can alter nutritional quality within a species, variation caused by temperature is substantially lower than inherent variation among plant species (Aerts et al. 2009). However, the effects of rising temperature on plant nutritional quality are likely highly idiosyncratic among species (Aerts et al. 2009), and the effects of temperature on plant chemistry must be considered more completely before applying results such as ours in a climate change context. Yet, our study does suggest that rising temperatures with climate change may reorder herbivore feeding preferences which may ultimately alter top-down forcing on plant communities.

Our short-term feeding assays indicate that herbivore feeding behavior and performance vary with temperature and among plant species within a single generation. However, long-term studies, encompassing a full feeding season or multiple generations, may result in different patterns. For example, it is possible that our results are due to short-term temperature changes rather than a response to long-term temperature changes. However, the temperatures chosen for this experiment were within the range of temperatures experienced by *P. japonica* in the weeks preceding the experiment. Yet over longer time periods, physiological plasticity may allow individuals to increase nitrogen uptake efficiency or reduce metabolic demands at high temperatures (Terblanche et al. 2007). However, in some ectothermic species, individuals do not exhibit acclimation over long periods (Watts et al. 2011). Whether *P. japonica* successfully acclimates to high temperatures remains to be tested. Alternatively, developmental plasticity may allow future generations to increase performance at high temperatures (Donelson et al. 2011). Long-term physiological acclimation or adaptation that allows herbivores to increase nitrogen uptake efficiency in thermally stressful environments is a promising avenue of research.

There are few data regarding the role of temperature on plant–insect interactions via direct changes in herbivore physiology (Bouzat and Imeh-Nathaniel 2008). Here, we show that rising temperatures lead herbivores to perform better on plants that are higher in nitrogen, possibly due to altered herbivore physiology as a result of increased temperatures. Moreover, temperature altered the effect of plant chemical defenses, resulting in high variance in herbivore preferences among plant species and reduced diet breadth. In addition to driving changes in plant–insect interactions in a changing climate, such temperature-driven redistributions of herbivory might already be impacting contemporary plant community structure by releasing or subjugating competitively dominant plants to intense herbivory as temperature changes on a daily, seasonal, or yearly basis. To our knowledge this possibility has not been considered. Finally, our results suggest that herbivore feeding preferences and host-plant interactions are highly contingent upon environmental temperature, further supporting the notion that the abiotic template of an environment can dictate herbivore feeding behavior, with potential impacts on plant community structure, trophic dynamics and ultimately ecosystem-level processes.

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Supplementary material (available online as Appendix oik-00457 at <www.oikosoffice.lu.se/appendix>). Appendix A1.