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ORIGINAL PAPER

Leaf shape variation and herbivore consumption and performance: a case study with *Ipomoea hederacea* and three generalists

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Abstract The effect of leaf shape variation on plant-herbivore interactions has primarily been studied from the perspective of host seeking behavior. Yet for leaf shape to affect plant-herbivore coevolution, there must be reciprocal effects of leaf shape variation on herbivore consumption and performance. We investigated whether alternative leaf morphs affected the performance of three generalist insect herbivores by taking advantage of a genetic polymorphism and developmental plasticity in leaf shape in the Ivyleaf morning glory, *Ipomoea hederacea*. Across four experiments, we found variable support for an effect of leaf shape genotype on insects. For cabbage loopers (*Trichoplusia ni*) and corn earworms (*Helicoverpa zea*) we found opposing, non-significant trends: *T. ni* gained more biomass on lobed genotypes, while *H. zea* gained more biomass on heart-shaped genotypes. For army beetworms (*Spodoptera exigua*), the effects of leaf shape genotype differed depending on the age of the plants and photoperiod of growing conditions. Caterpillars feeding on tissue from older plants (95 days) grown under long day photoperiods had significantly greater consumption, dry biomass, and digestive efficiency on lobed genotypes. In contrast, there were no significant differences between heart-shaped and lobed genotypes for caterpillars feeding on tissue from younger plants (50 days) grown under short day photoperiods. For plants grown under short days, we found that *S. exigua* consumed significantly less leaf area when feeding on mature leaves than juvenile leaves, regardless of leaf shape genotype. Taken together, our results suggest that the effects of leaf shape variation on insect performance are likely to vary between insect species, growth conditions of the plant, and the developmental stage and age of leaves sampled.

Keywords Leaf shape polymorphism · Herbivory · Coevolution · *Ipomoea hederacea* · Resistance · Digestive efficiency · *Spodoptera exigua* · *Trichoplusia ni* · *Helicoverpa zea*

Introduction

Coevolution between plants and herbivores is a major driving force of both plant and insect diversity and adaptations (e.g., Ehrlich and Raven 1964; Gilbert 1975; Berenbaum 1983; Futuyma and Slatkin 1983; Becerra 1997, 2003; Farrell 1998; Farrell and Sequeira 2004; Thompson 2005; Zangerl and Berenbaum 2005). For example, there is strong evidence that many plant defenses such as secondary compounds and physical structures (e.g., spines, hairs,

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thorns, etc.) have evolved, at least in part, due to selection by herbivores (Mauricio and Rausher 1997; Berenbaum and Zangerl 1998; Shonle and Bergelson 2000; Rausher 2001; Agrawal 2005; Gómez and Zamora 2000, 2002). In contrast to chemical and physical defenses, the evolution of leaf shape and form is often considered to have been driven by primarily abiotic factors, especially light and water availability (Givnish and Vermeij 1976; Givnish 1979; Smith 1986; Givnish 1990). While environmental conditions cannot be ignored as a selective agent on leaf morphology, several authors have suggested that herbivores may also play an important selective role in the evolution of leaf shape and specialized morphologies such as egg-mimics (Rausher 1978; Gilbert 1979; Williams and Gilbert 1981; Smith 1986; Niemala and Tuomi 1987; Givnish 1990; Brown et al. 1991; Rivero-Lynch et al. 1996; Campitelli et al. 2008).

The role of leaf shape in plant-herbivore coevolution has primarily been studied from the perspective of host seeking behavior in visually searching insects (e.g., Gilbert 1975, 1979; Rausher 1978; Williams and Gilbert 1981; Mackay and Jones 1989; see Rivero-Lynch et al. 1996 for an exception with beetle herbivory). These studies suggest that oviposition preferences by female butterflies can have strong influences on leaf shape. For instance, Gilbert (1975) inferred that the striking variation in leaf shape among *Passiflora* vines is the result of selection by visually searching *Heliconius* butterflies. In addition, Rausher (1978) showed that butterflies are capable of forming search images for leaf shape, and Williams and Gilbert (1981) demonstrated that female butterflies avoid ovipositing on host plants with egg-mimics. Taken together, these studies strongly suggest that insect herbivores are capable of recognizing morphological variation in leaves, and that features of leaf morphology can potentially reduce herbivory.

Despite circumstantial evidence that insect herbivores can impose selection on leaf shape and morphology (*see above*), we still lack evidence on whether alternative leaf shapes and morphologies affect insect consumption and performance (Brown et al. 1991; Rivero-Lynch et al. 1996). However, these are exactly the type of data that are required to evaluate the role of leaf shape variation in plant-herbivore *coevolution*. That is, while herbivores may drive the evolution of leaf shape, does shape variation affect herbivores in any way? Leaf shape variation could affect insect performance in at least three, non-exclusive ways. First, if loci for leaf shape are in linkage disequilibrium with loci for plant defense traits, such as trichome density, this may result in a correlation between the two traits. Second, loci for leaf shape may have pleiotropic effects on secondary compounds or physical defenses. Finally, it is possible that changes to leaf shape, and its attendant changes to vascular architecture (e.g., vein density, or the allocation, type, and amount of support tissues) could alter either the nutritive properties or toughness of the leaf for herbivores apart from any effects on secondary chemistry or traditional physical defenses.

Here we investigate the possibility that the alternate leaf shape morphs of the Ivyleaf morning glory, *Ipomoea hederacea*, affect the performance of three species of generalist insect herbivores. *Ipomoea hederacea* is an ideal study system for this question, as it exhibits both developmental plasticity and a genetic polymorphism in leaf shape (Fig. 1). Mature leaf shape in *I. hederacea* is controlled by a single Mendelian locus (Elmore 1986; Bright 1998), with homozygous individuals expressing either heart-shaped chordate leaves, or three-lobed leaves. Lobed genotypes produce 1–3 heart-shaped juvenile leaves shortly after germination, before transitioning to produce mature, lobed leaves; in contrast, heart-shaped genotypes produce a single leaf shape throughout their life. The Mendelian nature of leaf shape in *I. hederacea*, along with the developmental plasticity in shape expressed by lobed genotypes, allows a simultaneous investigation of the effects of both leaf shape genotype and the physical structure of the leaf (e.g., veination, support structures, etc.) on insect consumption and performance.

Results of past work in *I. hederacea* allow specific predictions about the likely direction of any effects of leaf shape genotype on insect herbivore performance. Previous studies on *I. hederacea* have shown that heart-shaped

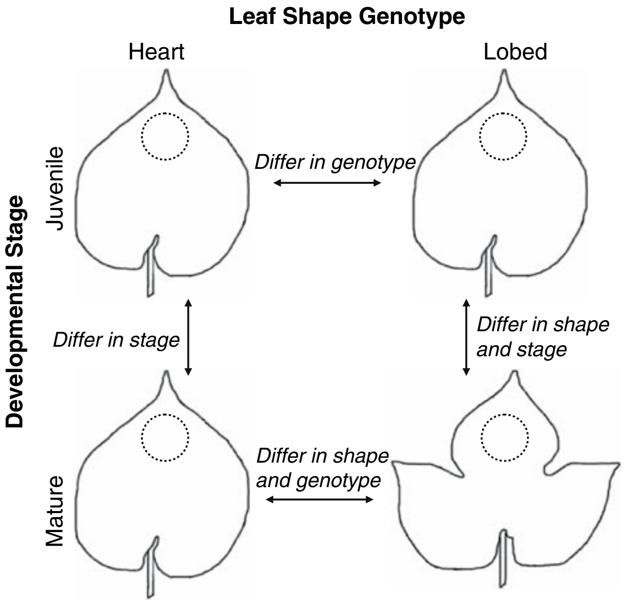

Fig. 1 Illustration of the genetic polymorphism and developmental plasticity of leaf shape in *I. hederacea*. All plants, irrespective of leaf shape genotype, produce 1–3 heart-shaped leaves in the juvenile stage. Lobed genotypes transition to produce divided leaves with 3 lobes, as shown. Circles indicate approximate locations of leaf punches

genotypes suffer significantly more deer damage (Bright 1998), and that there is a negative genetic correlation between resistance to deer and insect herbivory (Stinchcombe and Rausher 2001). These past findings lead to the prediction that heart-shaped genotypes will be more resistant to insect herbivory. In addition, there is significant genetic variation in *Ipomoea hederacea* for how induced responses to simulated mammalian herbivory (intended to mimic deer damage) affect insect herbivores (Simonsen and Stinchcombe 2007), although it is unknown whether heart and lobed genotypes respond equally. Accordingly, we sought to answer the following questions: (1) Does variation in leaf shape genotype affect insect consumption and performance? (2) Does simulated mammalian herbivory alter or modify the effect of leaf shape genotype on insect consumption and performance? (3) Do mature and juvenile leaves differ in their effects on insect herbivore consumption and performance? and (4) Are the effects of leaf shape genotype on insect consumption and performance consistent across insect species, growing conditions of plants, and the age of plant tissues sampled?

Materials and methods

Plant and insect species

Ipomoea hederacea (Convolvulaceae), Ivyleaf morning glory, is an annual weedy vine that is typically found in open or disturbed habitats such as agricultural fields (Stinchcombe and Rausher 2001). Geographically, it ranges throughout the southeastern United States, and is an important agricultural weed to soybean, corn, cotton, and peanuts (Hull-Sanders and Eubanks 2005; Baucom and Mauricio 2008). Following germination, it typically takes 4–6 weeks until flowering and another 4 weeks for seed maturation (Stinchcombe and Rausher 2001). Plants are largely selfing in the field (Ennos 1981; Hull-Sanders et al. 2005), and are attacked by a wide diversity of natural enemies (e.g., Stinchcombe and Rausher 2001; Hull-Sanders and Eubanks 2005; reviewed in Simonsen and Stinchcombe 2007).

A genetic polymorphism for leaf shape in *I. hederacea* produces mature leaves of strikingly different shapes (e.g., Fig. 1). Although both leaf morphs of *I. hederacea* have been referred to as separate sub-species (heart-shaped: *Ipomoea hederacea intergriuscula;* lobed: *Ipomoea hederacea hederacea*), both Elmore (1986) and Bright (1998) have determined that leaf shape behaves as a single Mendelian locus, and as such, we consider them separate genotypes of the same species.

We investigated the responses of three generalist insect herbivores to leaf shape variation: beet armyworms (*Spodoptera exigua*), corn earworms (*Helicoverpa zea*), and cabbage loopers (*Trichopusia ni*). *Spodoptera exigua* is a natural herbivore of *I. hederacea* (Hull-Sanders and Eubanks 2005; Simonsen and Stinchcombe 2007). *Helicoverpa zea* also feeds naturally on *I. hederacea*, and has been shown to impose natural selection on resistance in its congener *I. purpurea* (Rausher and Simms 1989; Simms and Rausher 1989). *Trichoplusia ni* was chosen as a universal generalist insect for comparison. Insects were obtained as eggs from Benzon Research (http://www.benzonresearch.com/; Carlisle, PA, USA). Insect colonies at Benzon research have been maintained on constant diet and rearing conditions, without interbreeding new material from other strains or the field, since 1992 (approx. 225 generations; G. Benzon, pers. comm.). These data suggest that the insect colonies are relatively genetically homogeneous, and the potential for insect genotype * plant genotype interactions (e.g., Weis and Abrahamson 1986; Pilson and Rausher 1995; Tétard-Jones et al. 2007) in our experiments is unlikely.

Experimental design: overview

Our experimental approach was to feed leaf samples to neonate insect herbivores and then compare leaf area consumption, weight gain, and digestive efficiency for insects that had been fed either lobed or heart-shaped leaf material. Because we performed four separate experiments, here we describe features common to all of them; exceptions for individual experiments are noted below. In general, we used 61 inbred lines of *I. hederacea* that had been maintained by self-fertilization for four generations; below, replicates refer to different individuals of the same inbred line. The inbred lines used have similar frequencies of heart-shaped and lobed genotypes (11% and 89%, respectively) to natural populations described by Bright (1998). Our use of multiple, independently derived inbred lines of each leaf shape facilitates comparisons across experiments and mimics the natural mating system of *I. hederacea*, but also means that the leaf shape allele and the rest of the genetic background are confounded. However, for the sake of clarity, we here use the term "leaf shape genotypes" because leaf shape is genetically determined in this species, and the multiple generations of self-fertilization have made the inbred lines homozygous at the leaf shape locus.

Depending on the experiment, we sampled two to six replicates per inbred line for feeding trials. For experiments 1–3, plants were grown in randomized, blocked designs in the greenhouse; experiment 4 used unblocked plants grown in a walk-in growth chamber. We deliberately performed our experiments under a range of growth conditions and sampling regimes to explore the generality of our results and whether any of the observed effects were sensitive to plant growth conditions, insect herbivore species, and the age of the sampled plants.

For each feeding trial, freshly hatched neonates were given uniformly sized and shaped leaf discs to feed on for several days. Our use of uniformly sized and shaped leaf discs eliminated any external cues of shape to the insects, and hence allowed us to focus on the effects of leaf shape genotype per se, and any physical differences in leaf architecture that might exist between lobed and heart-shaped genotypes. Although the use of leaf discs has been criticized because the use of variably sized discs used can affect feeding preferences (presumably because of changes in the concentration of chemicals between the center and the edge; Jones and Coleman 1988), these problems are unlikely to affect our experiments because we used the same leaf disc size within each experiment. As such, for each test of the effects of leaf shape genotype on insect performance, the insects received equal sized discs between heart and lobed genotypes.

For all experiments, leaf discs and neonates were placed in petri plates with moistened filter paper, and sealed with parafilm. Within each experiment, leaf discs were harvested from leaves of comparable developmental stages to account for any ontogenetic shifts in plant defense; the age and developmental stages of leaf tissues used varied between experiments, and are noted below. After feeding trials were over, we estimated the amount of leaf tissue consumed, dry biomass of insects (after 3–4 days drying at 65°C), and digestive efficiency of insects, calculated as dry biomass divided by the amount of leaf tissue consumed. For simplicity, we assumed that initial neonate weights were constant (e.g., Simonsen and Stinchcombe 2007).

Experiment 1: Spodoptera exigua on mature leaves

In this experiment, we used 5 replicates of 57 inbred lines that had been self-fertilized for three generations. More complete methods for this experiment can be found in Simonsen and Stinchcombe (2007). Because previous work suggests that leaf shape genotype, and deer and insect resistance, may be related (Bright 1998; Stinchcombe and Rausher 2001), we included a simulated mammalian herbivory treatment (cutting with licked scissors to provide saliva; Simonsen and Stinchcombe 2007) to examine whether the effects of leaf shape on insect consumption and performance differed between clipped and unclipped plants. Data from this experiment are thus split between a control (two replicates) and clipping treatment (three replicates). The clipping treatment was imposed after 70 days of growth; we subsequently sampled mature leaf tissue from 95 day old plants, and gave insects 5.6 cm^2 of leaf tissue to feed on for 4 days. Plants for this experiment had been grown under long day conditions (16 L:8 D) in a greenhouse. The amount of leaf tissue consumed by insects was estimated with an acetate grid sheet at a resolution of 1 mm.

Experiment 2: Spodoptera exigua on juvenile and mature leaves

In this experiment, we compared insect consumption and performance on juvenile and mature leaves of *I. hederacea*. We gathered leaf discs (4.3 cm^2) from the first true leaf of plants (juvenile leaves), which was heart-shaped in all cases, and from the most recently fully expanded leaf (mature leaves), which was heart-shaped for heart-shaped genotypes and lobed for lobed genotypes. As such, comparisons of insect performance on juvenile leaves of the two leaf shape genotypes test the effects of different leaf shape alleles in the same physical shape background. In like fashion, comparisons between juvenile and mature leaves allow for a test of developmental shifts in resistance. Finally, determining whether the developmental effects on resistance are the same between lobed and heart-shaped genotypes allows for a test of the consequences of changes in shape over development (Fig. 1).

We harvested single leaf discs from both juvenile and mature leaves from 6 replicate individuals of 60 inbred lines of *I. hederacea* that had been self-fertilized for 4 generations ($N = 2 \times 6 \times 60 = 720$ total discs). All plants had been grown under SD conditions (10 L:14 D) in a greenhouse, and leaf discs were harvested after 50 days of growth. Neonates were allowed to feed for 4 days.

After feeding trials were over, we taped leaf discs to overhead transparencies to minimize water loss and leaf disc shrinkage, as well as facilitate scoring damage over a light table. We stored transparency sheets in a refrigerator until damage was scored. For each disc, we estimated leaf consumption by superimposing a transparent grid sheet (resolution 2 mm × 2 mm), and counting the number of squares that had any damage. Prior to scoring damage with the transparency grid, we verified the accuracy of this method with a pilot study that compared it to damage estimates obtained from digital scans and image analysis. Estimates of damage using the two methods were highly correlated ($r = 0.80$, $P < 0.0001$, $N = 110$), and there was no evidence of bias or systematic over- or under-prediction due to the square counting method. Image analysis required substantially more handling time and numerous judgment calls about whether regions of the scan indicated damage or variation in image quality. Because the square counting method using transparency sheets was unbiased, required considerably less handling time, fewer subjective judgments, and facilitated storage of the leaf discs in manner that prevented shrinkage and water loss, we used it for the remaining experiments.

Experiment 3: Trichoplusia ni on mature leaves

Experimental conditions for *T. ni* were identical to experiment 2, with the following exceptions. We harvested a single leaf disc from mature leaves only, and used only four replicate individuals per line. Leaf discs were 3.8 cm^2, and we allowed neonates to feed for 3 days.

Experiment 4: Helicoverpa zea on mature leaves

For *H. zea*, we utilized three replicates of the 60 inbred lines that had been grown in growth room under SD conditions (8 L:16 D). We provided the neonates with 3.8 cm^2 leaf discs from mature leaves (one disc per plant), and allowed them to feed for 3 days. For this experiment, leaf discs were harvested after 57 days of growth.

Analysis of defensive traits and plant chemistry

We measured several plant traits that are known to affect insect performance in 57 of the inbred lines used for experiments 1–4 described above. All measurements of defensive traits and plant chemistry were made from individuals of experiment 1. For each plant, we estimated trichome density, which negatively affects generalist insect herbivore consumption and performance in *I. hederacea* (Simonsen and Stinchcombe 2007). We also estimated specific leaf area (leaf area per unit mass), and total leaf nitrogen and carbon content of the leaves, all of which have been shown previously to affect insect herbivore performance (see e.g., Schadler et al. 2003; Agrawal 2004; Agrawal 2005; Johnson 2008). Leaf nitrogen and carbon content were estimated by microcombustion, using dried ground leaf material and an Elemental Combustion system 4010, CHNS-O analyzer (Costech Analytical Technologies, Valencia, CA, USA).

Statistical analysis

We compared insect consumption, weight gain, and digestive efficiency (weight gain/consumption) between leaf shape genotypes with a series of mixed model ANOVAs (Proc Mixed, SAS v 9.1.3). For all experiments and statistical models, our primary hypothesis was tested by the significance of the leaf shape genotype effect, and any interaction terms containing leaf shape genotype, all of which are fixed effects. For completeness, we also present the random effects which test for genetic variation and $G \times E$ for resistance traits in these inbred lines (e.g., Stinchcombe and Rausher 2001; Stinchcombe and Rausher 2002; Stinchcombe 2002; Simonsen and Stinchcombe 2007). We tested the significance of random effects with likelihood ratio tests, comparing the -2 Log likelihoods of models with and without the random effects with a 1-tailed χ^2 test (because a variance cannot be less than zero; Littell et al. 1996, p. 44). We analyzed $\log(y + 1)$ transformed data to improve the normality of the residuals, but we present untransformed data for clarity. For all figures, we present our results as lsmeans (± 1 s.e) obtained from the models described below.

For experiment 1, fixed effects in the model included block, clipping treatment (clipped/control), leaf shape genotype (heart or lobed), and the leaf shape genotype × clipping treatment interaction. Random effects included line nested within leaf shape and line × clipping treatment nested within leaf shape. We also used this model to test for differences in specific leaf area, trichome density, and total leaf carbon and nitrogen content between leaf shape genotypes.

For experiment 2, we utilized a similar model, with the exception that developmental stage (juvenile/mature leaves) replaced the clipping treatment variable and there was no line * treatment interaction. For experiment 3, the model included only leaf shape genotype and block as fixed effects, and inbred line nested within leaf shape genotype as a random effect. The model for experiment 4 was similar to experiment 3, but without the block effect.

Results

Experiment 1: *Spodoptera exigua* on mature leaves

Using mixed-model ANOVA, we found significant effects of leaf shape genotype on insect dry biomass, leaf consumption, and digestive efficiency ($F > 4.18$, $P < 0.05$ for all three traits; Table 1). For all three variables, the lsmeans showed that heart-shaped genotypes were significantly more resistant to herbivory: they suffered less leaf damage, insects gained less mass and had lower digestive efficiency after feeding on them (Fig. 2a–c). In this experiment, which contained a clipping treatment, there was no evidence of a leaf shape genotype × clipping treatment interaction ($F < 2.27$, $P > 0.14$ for all three response variables) or a main effect of clipping treatment ($P > 0.12$ for all three response variables).

Table 1 Mixed model ANOVAs examining the effects of leaf shape genotype on *S. exigua* leaf consumption, dry biomass, and digestive efficiency from experiment 1

Source	Leaf consumption		*Spodoptera exigua* dry biomass		Digestive efficiency	
	χ^2 or F-statistic	P	χ^2 or F-statistic	P	χ^2 or F-statistic	P
Block	$F_{4,167} = 0.89$	0.70	$F_{4,164} = 0.91$	0.46	$F_{4,164} = 0.27$	0.90
Clipping treatment	$F_{1,55} = 2.49$	0.12	$F_{1,55} = 1.21$	0.28	$F_{1,55} = 0.49$	0.49
Leaf Shape Genotype	**$F_{1,55} = 7.76$**	**0.007**	**$F_{1,55} = 17.03$**	**0.0001**	**$F_{1,55} = 4.18$**	**0.045**
Leaf shape genotype × clipping treatment	$F_{1,55} = 2.27$	0.13	$F_{1,55} = 1.49$	0.22	$F_{1,5} = 0.14$	0.71
Inbred line (leaf shape)	**$\chi^2 = 18.9$**	**<0.0001**	**$\chi^2 = 13.2$**	**0.0001**	**$\chi^2 = 6.2$**	**0.006**
Inbred line (leaf shape) × clipping treatment	**$\chi^2 = 7.2$**	**0.0035**	**$\chi^2 = 4.4$**	**0.018**	$\chi^2 = 0.9$	0.17

All response variables were $\log(y + 1)$ transformed. For random effects we present the χ^2 value from a likelihood ratio test; for fixed effects, we present F-statistics. Significant effects are shown in bold

Experiment 2: *Spodoptera exigua* on juvenile and mature leaves

In contrast to experiment 1, we failed to detect statistically significant differences in leaf consumption by *S. exigua* between the leaf shape genotypes (Table 2, $P = 0.30$). There was a highly significant effect of developmental stage, in that insects ate significantly more leaf tissue out of mature leaves than juvenile leaves (21.49 ± 0.66 vs. 10.54 ± 0.67 squares of damage; Fig. 3). However, these developmental differences in leaf consumption were not affected by leaf shape genotype, as indicated by the non-significant leaf shape genotype × developmental stage interaction ($P = 0.35$).

We did not detect any significant effects of either leaf shape genotype or developmental stage on insect dry biomass accumulation ($P = 0.63$ and 0.65, respectively), and there was no statistical interaction between the two ($P = 0.64$).

In contrast to the results for leaf consumption and dry biomass, we detected marginally significant effects of leaf shape genotype on digestive efficiency ($P = 0.10$), with the evidence pointing towards greater digestive efficiency of insects feeding on heart-shaped genotypes. Furthermore, the effects of leaf shape genotype on digestive efficiency depend marginally on the developmental stage of the plants ($P = 0.058$; Table 2; Fig. 4). We used means contrasts to partition the leaf shape genotype × developmental stage interaction—i.e., we compared leaf shape genotypes at each developmental stage, and we compared developmental stages for each leaf shape genotype—using the "slice" option of Proc Mixed. These analyses suggested that insect digestive efficiency differed significantly between the two leaf shape genotypes for juvenile leaves, with insects having higher digestive efficiency on heart-shaped genotypes ($F_{1,609} = 4.77$, $P = 0.03$), but not for mature leaves ($F_{1,609} = 0.32$, $P = 0.57$). In addition, insect digestive efficiency differed significantly between juvenile and mature leaves, but only for the lobed genotypes. Means contrasts indicated that insects had significantly higher digestive efficiency on mature leaves than juvenile leaves of the lobed genotypes ($F_{1,609} = 7.13$, $P = 0.008$), but showed no significant difference in digestive efficiency between mature and juvenile leaves of the heart genotypes ($F_{1,609} = 0.74$, $P = 0.39$; Fig. 4).

Experiment 3: *Trichoplusia ni* on mature leaves

We failed to detect any significant differences between leaf shape genotypes in total consumption ($F_{1,58} = 0.25$, $P = 0.62$), dry biomass gained ($F_{1,57} = 1.84$, $P = 0.18$), or digestive efficiency ($F_{1,57} < 0.01$, $P = 0.95$) of *T. ni* feeding on mature leaf tissue. Plots of the lsmeans suggests that any trend would be for insects feeding on heart-shaped genotypes to gain less weight (Fig. 5a).

Experiment 4: *Helicoverpa zea* on mature leaves

The two leaf shape genotypes do not differ significantly in the amount leaf tissue consumed by *H. zea* ($F_{1,57} = 0.82$, $P = 0.37$), although there is a non-significant trend for insects to gain more dry biomass on heart-shaped genotypes ($F_{1,58} = 2.61$, $P = 0.11$; Fig. 5b).

Analysis of defensive traits and plant chemistry

Our analysis of defensive traits and plant chemistry failed to detect any significant differences between heart and lobed genotypes for trichomes ($F_{1,55} = 0.37$, $P = 0.55$), specific leaf area ($F_{1,54} = 0.14$, $P = 0.71$), total carbon content of leaves ($F_{1,52} = 0.17$, $P = 0.68$), or total nitrogen content of leaves ($F_{1,55} = 1.35$, $P = 0.25$). Furthermore, for none of these variables was there a

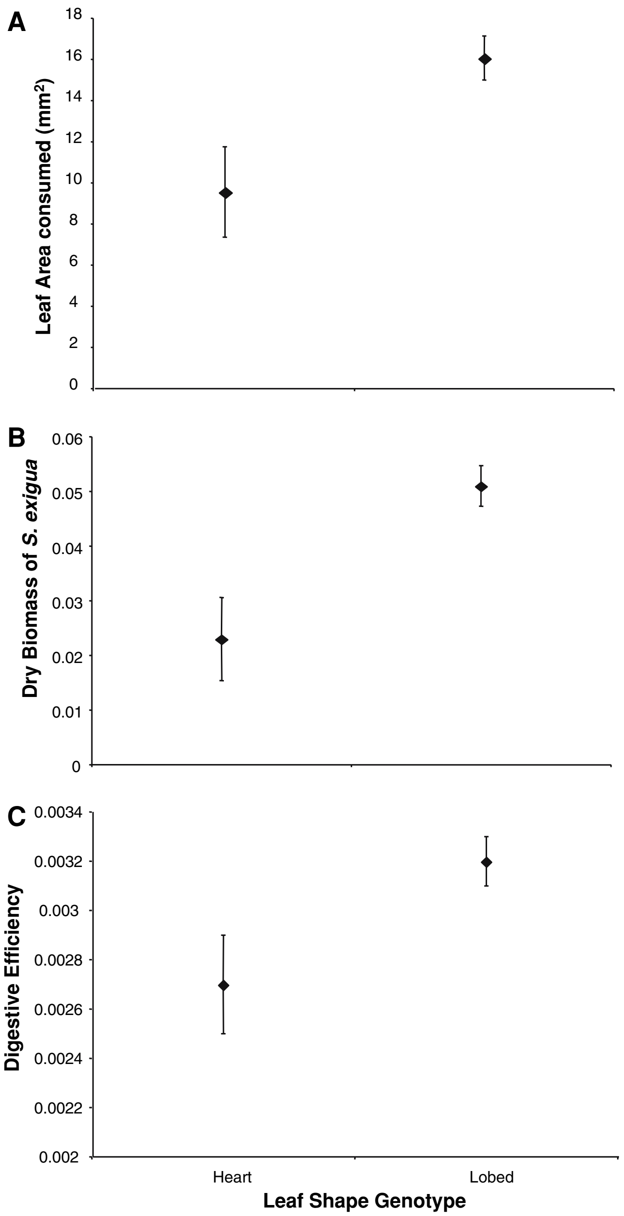

Fig. 2 Effects of the leaf shape genotype on *Spodoptera exigua* consumption (**a**), dry biomass gained (**b**), and digestive efficiency (**c**) in experiment 1. Points are plotted lsmeans ±1 s.e

significant interaction between clipping treatment and leaf shape genotype ($F < 0.55$, $P > 0.46$). These results indicate that any differences between leaf shape genotypes in insect resistance and performance in Experiment 1 are not driven by simple differences between these traits.

Discussion

A pre-requisite for leaf shape to affect plant-herbivore coevolution is for alternate leaf shapes to differentially affect herbivore performance, and direct assessments of this have been lacking. We evaluated this by examining the effects of leaf shape genotype, and its attendant effects on leaf structure and morphology, on insect consumption and performance. Our results suggest that while leaf shape can impact herbivore performance, its effects are likely to vary between insect species, the growth conditions of plants, and the developmental stage of the leaves sampled. Here we discuss the implications of our findings in light of past studies on the role of leaf shape and herbivory, developmental shifts in plant defense, and potential mechanisms underlying the observed differences between experiments.

Leaf shape and plant-herbivore interactions

The potential for leaf shape to be a character that responds to herbivore imposed-selection has been recognized since Gilbert's (1975, 1979) and Rausher's (1978) early studies of host seeking butterflies, along with several follow up studies (e.g., Gilbert 1979; Williams and Gilbert 1981, Rausher 1981; Mackay and Jones 1989). These studies clearly suggest that visually seeking herbivores have the potential to impose selection on leaf shape. To date however, there are remarkably few studies that have examined the role of leaf shape in affecting herbivore performance. Brown et al. (1991) reviewed a handful of circumstantial cases where leaf size and shape variation influenced insect performance, although most of the examples they cited are more related to leaf size than leaf shape per se. In fact, Brown et al. (1991) noted their surprise that more data on the topic are not available, and specifically called for more studies evaluating the performance of insects on single species of plants with variably shaped leaves. As such, evaluating how our results on insect performance fit into a larger context is difficult, because there have been few rigorous examinations of the effects of leaf shape variation on herbivore performance traits.

In our first experiment, we find clear support for an effect of leaf shape on insect consumption and performance: discs from heart-shaped genotypes suffered less damage from *S. exigua*, led to smaller caterpillars, and lower digestive efficiency. Based on Bright's (1998) finding that heart-shaped genotypes are more resistant to deer herbivory, and Stinchcombe and Rausher's (2001) finding of a negative genetic correlation between deer and insect resistance, we had predicted that heart-shaped genotypes would be more resistant to insect damage, and results from experiment 1 support this prediction. Interestingly, although heart-shaped genotypes may be less resistant to deer herbivory (Bright 1998) and possibly more resistant to insect herbivory (experiment 1), we detected no differences between heart and lobed genotypes in their induced response to simulated mammalian herbivory (Table 1).

Table 2 Mixed model ANOVAs examining the effects of leaf shape genotype and developmental stage on *S. exigua* leaf consumption, dry biomass, and digestive efficiency from experiment 2

Source	Leaf consumption		*Spodoptera exigua* dry biomass		Digestive efficiency	
	χ^2 or F-statistic	P	χ^2 or F-statistic	P	χ^2 or F-statistic	P
Block	$\mathbf{F_{2,617} = 4.13}$	**0.02**	$\mathbf{F_{2,614} = 3.24}$	**0.04**	$F_{2,609} = 1.17$	0.31
Developmental stage	$\mathbf{F_{1,617} = 70.66}$	**<0.001**	$F_{1,614} = 0.23$	0.63	$F_{1,609} = 0.05$	0.83
Leaf shape genotype	$F_{1,58} = 1.08$	0.30	$F_{1,58} = 0.21$	0.65	*$F_{1,58} = 2.73$*	*0.104*
Leaf Shape genotype × developmental stage	$F_{1,617} = 0.89$	0.35	$F_{1,614} = 0.22$	0.64	*$F_{1,609} = 3.61$*	*0.058*
Inbred line (leaf shape)	$\chi^2 = 0$	0.99	$\chi^2 = 1$	0.16	$\chi^2 = 0$	0.99
Developmental stage × inbred line (leaf shape)	$\chi^2 = 0.3$	0.29	$\chi^2 = 0$	0.99	$\chi^2 = 0$	0.99

All response variables were $\log(y + 1)$ transformed. For random effects we present the χ^2 value from a likelihood ratio test; for fixed effects, we present F-statistics. Significant effects are shown in bold, marginally significant effects ($0.05 < P < 0.10$) in italics

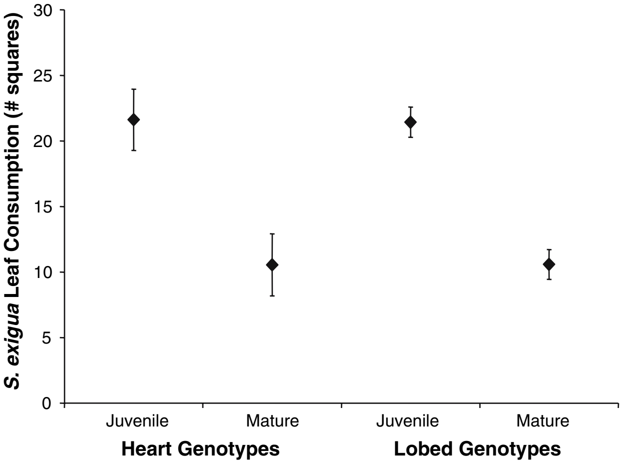

Fig. 3 Effects of leaf shape genotype and developmental stage on *Spodoptera exigua* leaf tissue consumption in experiment 2. Points are plotted lsmeans ±1 s.e

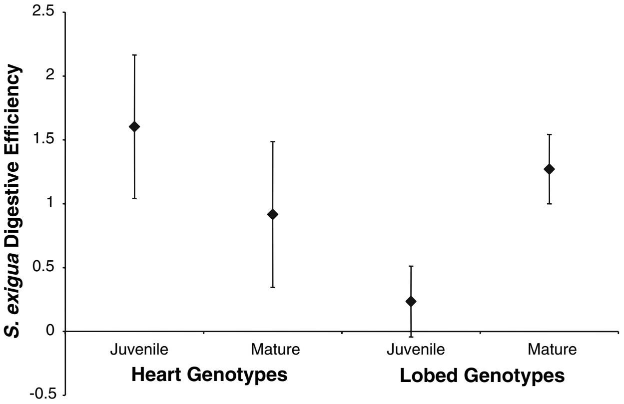

Fig. 4 Effects of leaf shape genotype and developmental stage on *Spodoptera exigua* digestive efficiency in experiment 2. Points are plotted lsmeans ±1 s.e

Our analysis of plant traits that might explain the results of Experiment 1—that is, potential variation in trichome density, specific leaf area, or total C or N concentration—failed to reveal any significant differences between heart and lobed genotypes. As such, the exact mechanisms behind these results remains elusive, although our use of uniformly sized discs eliminates the possibility of differences in visual appearance or edge effects. These findings suggest that either physical differences in leaf structure, toughness, unknown secondary compounds associated with leaf shape, or leaf nutritive properties associated with different leaf shape genotypes beyond total C or N concentration may be responsible.

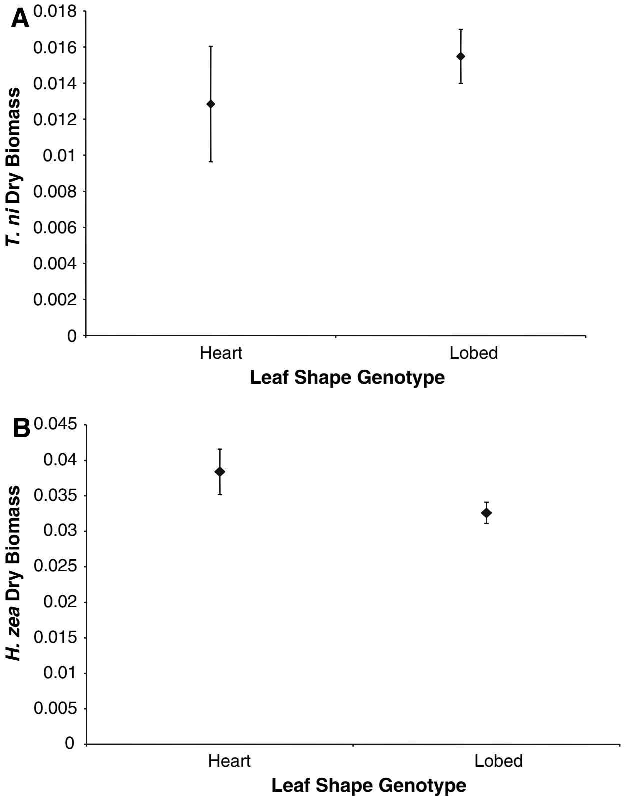

Fig. 5 Effects of leaf shape genotype on dry biomass of *Trichoplusia ni* (**a**) and *Helicoverpa zea* (**b**). Points are plotted lsmeans ±1 s.e

Our second experiment, however, which also used *S. exigua*, failed to detect any significant effects of leaf shape genotype on leaf consumption or dry biomass. In addition, in experiment 2, we found that caterpillars feeding on heart-shaped genotypes had, if anything, *higher* digestive efficiency. Our follow-up experiments with *T. ni* and *H. zea*, while neither revealed significant results, showed trends in opposite directions. Our data suggest that the effects of leaf shape genotype on insect herbivore performance are likely to be affected by plant growth conditions, the age of the plants, and the tissue sampled—all features that typically vary both in natural populations and in experimental protocols for studying plant-herbivore interactions. In addition, while unlikely for our experimental design, evidence from other systems suggests that plant genotype × insect genotype interactions may exist (Moran 1981; Weis and Abrahamson 1986; Pilson and Rausher 1995; Tétard-Jones et al. 2007), suggesting that leaf shape genotype × insect genotype interactions may be possible in natural populations.

Most previous empirical studies on the role of leaf shape in plant-herbivore interactions have focused on host-seeking in a single insect order (Lepidoptera), with the exception of Rivero-Lynch et al. (1996). Rivero-Lynch and colleagues (1996) found that adult flea beetles (*Phyllotreta* sp.) caused more damage to deeply lobed leaves than to undivided leaves of Shepherd's purse (*Capsella bursa-pastoris*) in both field sampled plants and a common garden experiment but not in laboratory feeding trials. They also failed to detect significant differences in feeding preferences in laboratory feeding trials with armyworms (*Spodoptera exigua*), although adult vine weevils (*Otiorhynchus sulcatus*) showed a significant preference for undivided leaves. Although Rivero-Lynch et al. interpret these data as, on the whole, rejecting Brown et al.'s (1991) hypothesis of reduced foraging efficiency of insect herbivores on divided leaves, two important aspects of their results stand out.

First, Rivero-Lynch et al. (1996) detected significant effects of leaf shape on insect consumption patterns—for the case of flea beetle damage, driven by the correlated effects of leaf size in their interpretation—and in opposite directions for flea beetles and vine-weevils. Although Brown et al.'s (1991) foraging efficiency hypothesis is one potential mechanism for differences between leaf shapes, other mechanisms exist, and there is no a priori reason to expect them to operate in the same direction for different insect species. For instance, the effects of changing the physical structure of a leaf (i.e., arrangement of vascular and support tissues) may not affect flea beetles and vine weevils in the same manner. In addition, Rivero-Lynch et al. (1996) detected significant effects of leaf size/shape in field experiments, but not the lab for flea beetles. As such, the heterogeneity of results we have described—both within individual insect species and between species—appears to be consistent with limited data available in the literature, at least for patterns of overall consumption.

Second, as noted by Rivero-Lynch et al. (1996), divided and undivided leaves of *Capsella bursa-pastoris* appear at different times in development, are of different sizes, and possibly differ in essential nutrients for insects. As such, Rivero-Lynch et al. (1996) note that controlling for or reducing these confounding factors would enhance the ability to evaluate the role of insect herbivores in the evolution of leaf shape. The nature of the developmental plasticity and genetic polymorphism in leaf shape in *I. hederacea*, coupled with our design, helped eliminate some of these confounding factors. Nevertheless, differences in age of leaves sampled may have contributed to the contrasting results we detected between experiments 1 and 2 (*see below*).

Possible mechanisms for differences between experiments

The contrasting results between experiments 1 and 2 are puzzling: we used largely the same inbred lines of plants (56 inbred lines in common between the experiments) and the same insect species (and supplier). Restricting the data to inbred lines represented in both experiments has no effect on the results of either experiment individually, or the differences between them (results not shown), suggesting the minor differences in the inbred lines used are not responsible. One clear difference between the two experiments is the presence of a clipping treatment in the first experiment and its absence in the second experiment. However, we failed to detect any effect of the clipping treatment on the variables we analyzed (Table 1), and there was no evidence of a clipping treatment * leaf shape genotype interaction. Accordingly, the evidence suggests that the differences between these two experiments are not driven by the clipping treatment. Our suspicion is that the differences between the two experiments, namely the photoperiod (LD vs. SD) and the age of plants when leaves were sampled (95 vs. 50 days old) sampled are likely to be key.

For either the photoperiod or age of sampling explanations to account for the differences between experiments 1 and 2, they must act differentially between the two leaf shape genotypes. One hypothesis is that there are differences in developmental rates between heart and lobed genotypes. Consider the possibility that leaves produced later in development are better defended (e.g., juvenile and mature leaves of the heart-shaped genotypes in Fig. 3). If heart-shaped genotypes develop at a faster rate than lobed genotypes, the differences detected in experiment 1 could be reflections of accelerated plant development leading to better defended leaves in heart-shaped genotypes at the time of sampling (95 days) in experiment 1, but insufficient time for this effect to have manifested itself in experiment 2 (at 50 days). Unfortunately, quantitative data on developmental rates (e.g., time to flowering, flowering duration, leaf production rates, time to senescence) between heart and lobed genotypes are lacking. However, the interpretation of heart-shaped genotypes as juvenilized forms of the lobed genotypes (i.e., they have lost the transition to producing lobed leaves) with faster development is consistent with this possibility.

Developmental differences in plant defense

Our results indicate clear developmental differences in plant defense: discs taken from juvenile leaves receive significantly more damage than discs taken from mature leaves (Fig. 3). In a similar fashion, insects feeding on mature leaf discs have lower digestive efficiency when feeding on heart-shaped genotypes (Fig. 4). These results are broadly consistent with optimal defense theory, in which younger, nutrient rich leaves are expected to be better defended than older leaves (McKey 1974; Ohnmeiss and Baldwin 2000; Strauss et al. 2004). (We note that juvenile leaves—the first leaves produced by a plant—will be *older leaves* at any given sampling point, and vice-versa for mature leaves under our sampling regime).

In contrast, for lobed genotypes we found a different pattern: insects feeding on juvenile leaves, which are heart-shaped, have significantly *lower* digestive efficiency than those feeding on mature leaves (which are lobed). Digestive efficiency of caterpillars on mature leaves did not differ between heart and lobed genotypes, suggesting that this trend is driven by a reduction in digestive efficiency of neonates feeding on juvenile leaves. The proximate mechanisms for these developmental shifts in plant defense, and why they might differ between heart and lobed genotypes for digestive efficiency, remain unknown in *I. hederacea*, although developmental shifts in plant resistance and secondary chemistry are not uncommon (e.g., Ikonen 2002).

Conclusions and future directions

While our results suggest that leaf shape genotype, and possibly the physical structure of a leaf, can significantly affect the consumption and performance of insect herbivores, other factors clearly play a role. Two of the more immediate possibilities are that differences in photoperiod the plants are exposed to, and the age at which leaves are sampled, could mediate the effects of leaf shape on herbivore consumption and performance. We also note that our study was performed with three generalist insect species, and that the effects we detected could differ if we had used specialists (e.g., tortoise beetles). More generally, however, the simple Mendelian nature of leaf shape in *I. hederacea* allows crosses to be performed to isolate the effects of leaf shape alleles against the remainder of the genetic background (with the exception of loci in tight linkage), and these crosses are currently underway. By combining crosses and experimental manipulation of environmental factors, a more complete examination of the effects of leaf shape variation on herbivore performance should be possible.

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