The effects of plant quality on caterpillar growth and defense against natural enemies

P. D. Coley, M. L. Bateman and T. A. Kursar

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A survey of 85 species of Lepidoptera feeding on 40 hosts on Barro Colorado Island, Panama showed that growth and defensive traits of caterpillars were correlated with the nutritional and defensive traits of their hosts. Growth rates were faster on young than mature leaves, reflecting the higher nitrogen and water content of the former. Growth was also positively correlated with leaf expansion rate, partially because of higher nitrogen and water contents of fast-expanding young leaves. Specialists grew faster than generalists, but both responded positively to nutritional quality. There was no effect of lepidopteran family on growth. In analyses where the effects of nitrogen and water were removed, the residuals for growth rate were greater for young than for mature leaves and were positively correlated with expansion rates of young leaves. This suggests that traits other than nutrition were also important. As young, expanding leaves cannot use toughness as a defense, one possible explanation for the differences in growth is differences in chemical defenses. Growth rate residuals for both specialists and generalists were higher for the more poorly defended fast-expanders, but the effect was greatest for generalists, perhaps because generalists were more sensitive to secondary metabolites. We predicted that slow growth for caterpillars would increase their risk to natural enemies and would select for higher defenses. Generalists had more defensive traits than specialists and were less preferred in feeding trials with ants. Similarly, species feeding on mature leaves were the most defended and those feeding on fast-expanding young leaves were the least defended and most preferred by ants. Thus the effects of plant secondary metabolites and nutrients dictate herbivore growth rates, which in turn influence their susceptibility to the third trophic level and the importance of defenses.

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Herbivores face many challenges with respect to diet selection as leaves have an array of chemical and physical defenses (Rosenthal and Berenbaum 1991) and are very low in protein relative to seeds or fruits (Mattson 1980). The mechanisms by which herbivores address the challenges of toxic secondary metabolites, high toughness and low nutritional value will affect their growth, and, in turn, their fitness. For lepidopteran herbivores, fast growth can lead to larger body size at pupation, and an increase in the quality and quantity of egg production (Haukioja and Neuvonen 1985, Ohmart et al. 1985, Awmack and Leather 2002). The length of the larval period should also influence the risk of predation. For example, more slowly growing larvae may be under stronger selection for defense against natural enemies (Feeny 1976, Benrey and Denno 1997, Loader and Damman 1991). It has been argued that plant traits that slow larval growth function as plant defenses by increasing the larva's risk to predation or parasitism. However, the slow-growth high-mortality hypothesis

(Benrey and Denno 1997) has received mixed support (Häggström and Larsson 1995, Lill and Marquis 2001, Medina et al. 2005, Kursar et al., in press). In this study we examine how diet quality may constrain an herbivore's ability to maximize growth and to minimize vulnerability to the third trophic level. We examine this in a tropical forest where both the extent of plant defenses (Coley and Aide 1991) and the impact of the third trophic level (Dyer and Coley 2002) may be higher than in the temperate zone.

For an insect herbivore, two of the most important nutritional components of leaves are nitrogen and water. Both have been shown to affect growth and diet choice in laboratory and field studies with herbivorous insects (Mattson 1980, Rausher 1981, Scriber and Slansky 1981, Raupp and Denno 1983, Osier and Lindroth 2001, Holton et al. 2003). Typically, herbivores prefer young expanding leaves, because they have higher nutritional value and lower toughness than mature leaves (Marquis and Braker 1994, Coley and Barone 1996). This preference is particularly marked in tropical rainforests, where more than 75% of the lifetime damage for a leaf occurs during the short window when it is expanding (Coley and Aide 1991).

Although young leaves generally have higher herbivory than mature, there is a 6-fold difference among species in rates of damage to young leaves (Kursar and Coley 2003). Species differ substantially with respect to the rate of young leaf expansion, nutrients, and secondary metabolites (Coley and Kursar 1996), all traits which influence herbivory on young leaves. In tropical species, the most rapidly expanding leaves double in area every day (Kursar and Coley 1992). Due to the physiological constraint of allocating resources to growth, rapidly expanding leaves tend to be high in nutrients and low in defensive compounds (Orians and Janzen 1974, Kursar and Coley 1991). Rapid expansion should reduce the leaves' period of vulnerability to herbivores thereby reducing damage (Feeny 1976, McKey 1979, Aide and Londoño 1989). However, because of the higher nitrogen and lower chemical defenses, fast-expanders actually suffer high rates of herbivory. In contrast, plants with slow-expanding young leaves have less nitrogen and their leaves are chemically well defended (Kursar and Coley 2003). In summary, the rate of expansion of young leaves may correlate with diet quality, including the combined effects of nutrition and defense, and consequently with caterpillar growth rate.

Rates of predation and parasitism can be high in the tropics (Jeanne 1979, Cornell and Hawkins 1995, Hawkins et al. 1997, Stireman et al. 2005) and caterpillars have evolved a variety of defensive strategies including rapid growth, accumulation of toxic chemicals, construction of shelters, spines and hairs, cryptic coloration and defensive or evasive behaviors (Eisner 1970, Stamp and Casey 1993, Gross 1993, Gentry and Dyer

2002). Is the great variation among caterpillar species in defenses related to the differences among food quality of plant hosts (Barbosa 1988, Gauld and Gaston 1994, Dyer 1995)?

We predicted that both caterpillar growth rates and defense against natural enemies would be correlated with diet quality. In general, caterpillars feeding on fast-expanding leaves should benefit from the combination of high nutrients and low chemical defenses. These caterpillars should grow the fastest. Because fast-growing caterpillars have a short window of vulnerability to natural enemies, they should also invest the least in defenses. Caterpillars feeding on slow-expanding young leaves will encounter intermediate levels of nutrients and higher levels of toxic compounds. Thus we predict that they should have intermediate growth rates and defense. Mature leaf-feeders will have the lowest nutrients and also must overcome high toughness and secondary metabolites. These caterpillars should grow the slowest and invest the most in anti-predator defenses. These predictions apply to both specialist and generalist caterpillars, but they may be more pronounced for generalist caterpillars. Growth rates of specialist caterpillars should be most influenced by the nutritional quality of leaves, as they are presumably adapted to the secondary metabolites of their hosts. Generalists should also be affected by nutrition, but might have a more marked negative response to plant chemical defenses.

To determine how host plant defense and nutrition affect herbivore growth rates and defenses against natural enemies, we examined a community of caterpillars and their host plants from a tropical moist forest in Panama. We compared the growth rates and defensive traits of 85 species of caterpillar feeding on 40 species of plants in 24 families that differed in the nutritional quality, defenses and expansion rates of their leaves.

Methods

Study site

We conducted our study in a moist tropical lowland forest on Barro Colorado Island (BCI), Panama (9°N, 80°W). The island is maintained and protected by the Smithsonian Tropical Research Institute and is part of a larger forested corridor that extends from the Atlantic to the Pacific coasts. BCI experiences a marked dry season that is usually four months long, and the vegetation is classified as tropical, moist forest (Holdridge et al. 1971, Croat 1978, Leigh 1999).

Plant species

We opportunistically collected caterpillars from 40 woody plant species of the island's diverse understory

of shade-tolerant plants (Appendix 1). These relatively common plant species represent a variety of life histories. The plant species included shrubs, juvenile lianas and immature trees with growth strategies that differed widely, even within plant genera. Leaf development times ranged from very rapid to very slow. While many of our species produced leaves continuously, others flushed synchronously.

For each species, we analyzed several characteristics that are relevant to leaf development and herbivore attack: synchrony of leaf production, expansion rates of young leaves, as well as nitrogen, and water contents of both young and mature leaves. Not all data were collected on all plant species. To measure the young leaf expansion rate, we marked freshly emerged leaves and measured their area with plastic grids every 48 h until the leaves stopped expanding. We calculated the daily percent increase in size from 15–80% of full size during the expansion phase using the following equation:

Expansion rate as percent per day

$$= 100 \times [e^{(\ln (area2/area1)/time)} - 1]$$

where "area1" and "area2" are leaf areas at two different measurements and "time" equals the number of days between measurements. Values of 100% per day indicate that the leaves doubled in size daily. We categorized a plant species as a slow expander if its leaf expansion rate was less than 28% per day and as a fast expander if its leaf expansion rate was more than 28% per day.

Nitrogen and water content are highest in young leaves and then diminish as leaves age (Coley 1983, Kursar and Coley 1991, 1992). To measure these parameters, we collected young leaves that were between 10% and 20% expanded and mature leaves that were fully expanded and fully toughened. Three replicates were run for each species and age. Leaves were dried at 55°C to obtain the dry weight. Nitrogen content of the dried ground leaves was determined with an isotope ratio mass spectrometer (delta S, Finnigan MAT, San Jose, California, USA).

Lepidopteran collection and rearing

We reared a total of 400 individuals from 85 species of caterpillars from October 1996 to June 1999 (Appendix 2). Our collection periods included both the dry and wet seasons. For each caterpillar, we recorded the plant species and leaf age on which it was feeding, and photographed specimens. Using larvae and adults, we identified the lepidopterans to the lowest taxon possible: most to family and some to species. Voucher specimens are stored on BCI, and some duplicate specimens are with experts for identification. Using our >10 years of field notes we classified the caterpillars as specialists or generalists. Thus this classification is primarily based on local diet breadth. When possible, we corroborated our classifications using published caterpillar databases and field guides. Following previous workers, we called a caterpillar a generalist if it fed on more than two families of plants (Janzen 1984, DeVries 1987, Marquis 1991, Barone 1998). The specialist caterpillars usually fed on plants from one family, and most restricted their feeding to a single genus.

We reared all caterpillars individually in closed plastic containers at ambient temperature in a screened and shaded porch. Thus, temperature conditions were similar for all growth measurements. We fed them leaves of the same species and age as those on which they were initially found. Each generalist species was collected from a range of host plants, but individuals were reared on leaves of the same species on which the caterpillar was found. Leaves were replaced with fresh ones at least every other day. A moistened paper towel in each cup helped keep leaves fresh. Caterpillars were collected opportunistically, and were generally found while in the early instars.

To calculate relative growth rates, we weighed caterpillars every 24–48 h. For each healthy individual, we averaged its weight change between measurements and divided it by the midpoint weight to get an individual relative growth rate ($g\ g^{-1}\ d^{-1}$). Depending on the developmental time, we obtained from 3 to 10 measurements per caterpillar before it pupated. The negative weight gains just before pupation were dropped. We found no effect of initial weight on subsequent growth rates ($r^2 = 0.000$, $p = 0.3$, $n = 145$; Kursar et al., unpubl.). We averaged relative growth rates for all the individuals of the same caterpillar species feeding on the same leaf age of the same species of plant to get a mean relative growth rate (RGR) for each caterpillar/host combination.

Caterpillar defenses

Caterpillars were scored for traits that could serve as defenses against natural enemies as has been done in other studies (Gentry and Dyer 2002). We scored the 85 species of caterpillars that were reared for growth measurements, plus an additional 11 morphospecies that were not reared. Caterpillars were scored from 0 to 2 for "color", with scores increasing from cryptic species, to colorful to warningly colored. "Gregariousness" was scored as 0 for solitary feeders and 1 for gregarious feeders. These two traits may indicate greater chemical defense. Values for "hairs" and "spines" were 0 for none, 1 for sparse and 2 for dense. If caterpillars made shelters from cut, webbed or folded leaves, they received a score of 1 for the "shelter" category. "Behavior" was scored as 0 if caterpillars hid when not feeding or as 1 if they remained conspicuous. This

was evaluated by watching each caterpillar in the field. Other behavioral defenses, such as evasive tactics when attacked, were not measured.

Statistics

We analyzed our data with SAS (SAS Institute Inc., 2000). Growth rates were compared among leaf classes and among specialist and generalist herbivores with ANOVA (Proc GLM). Simple and multiple regressions were used to examine the effects of expansion, nitrogen and water on growth (Proc GLM and Proc REG). A principle components analysis (Proc PRINCOMP) and a discriminant analysis (Proc DISCRIM) were used to separate caterpillar species based on water, nitrogen and expansion rates of the host. ANCOVA was used to examine the growth residuals for different leaf classes after the effects of water and nitrogen had been removed (Proc GLM). The relationship between expansion and growth was examined in a partial regression with water and nitrogen removed (Proc REG). Differences between anti-predator defenses of specialists and generalists were compared with a t-test, corrected for multiple comparisons. Defense differences for caterpillars feeding on different leaf classes were compared with an ANOVA, also corrected for multiple comparisons.

Results

Leaf nutritional quality for young and mature leaves

We compared the nutritional quality of young and mature leaves of 37 species of plants. Young leaves had approximately 80% more nitrogen than mature leaves (3.6% vs 1.95%; $p < 0.001$). Young leaf nitrogen content varied among species by a factor of 4.3 (from 1.73% to 7.38% of dry weight), while mature leaf nitrogen content ranged from 1.10% to 3.75%. For all species except one, young leaves had higher nitrogen content than the mature leaves. Young leaves also had a higher water content than mature leaves (77.1% of fresh weight vs 62.5%, $p < 0.0001$). Young leaf water content ranged from 64% to 92% of fresh weight while mature leaf water content ranged from 44% to 84%. Nitrogen content was positively correlated with water content for both young and mature leaves considered together ($r^2 = 0.40$, $p < 0.001$), mature only ($r^2 = 0.22$, $p < 0.005$, $n = 37$), and young only ($r^2 = 0.20$, $p = 0.005$, $n = 38$).

Caterpillar growth rates on young and mature leaves

Do the large differences in nutritional quality between young and mature leaves influence caterpillar growth rates? Caterpillar growth rates ranged by 14-fold in our

Table 1. ANOVA for effects of herbivore family, leaf age (young vs mature) and diet breadth (specialist vs generalist) on relative growth rates of caterpillars g g^{-1} d^{-1}.

Variables	DF	Mean square	F value	p value
Diet	1	0.201	12.5	<0.001
Leaf age	1	0.368	22.9	<0.001
Family	18	0.018	1.1	0.373
Error	63	1.902		

community survey of 85 lepidopteran species (0.05 to 0.72 to g g^{-1} d^{-1}). There was no effect of caterpillar family, but significant effects of both leaf age and diet breadth (Table 1). Growth was significantly faster on young leaves for both generalists and specialists (Fig. 1; ANOVA $F_{3,94} = 25.4$, $p < 0.001$). Eleven species of caterpillar were grown on both young and mature leaves, and in 10 cases, growth was faster on young leaves (paired t-test, $t = 2.2$ $p < 0.05$, $n = 11$), with average growth on young leaves being 2.2 times higher.

The extent of diet specialization also influenced growth rates (Fig. 1; ANOVA $F_{3,94} = 9.58$, $p = 0.0026$). Specialists grew ten times faster than generalists on mature leaves and nearly two times faster than generalists on young leaves. These differences between specialists and generalists are not explained by differences in traits of their host plants. In a principle components analysis separating caterpillars species according to the water, nitrogen and expansion rates of their hosts, the first two axes explained 87% of the variance yet failed to separate generalists and specialists. In a discriminant analysis using the same host traits, only 44% of the generalists were correctly classified and 86% of the specialists.

The differences in caterpillar growth rates on young and mature leaves are partly explained by differences in nitrogen and water. For specialists, there was a moderate, but significant, positive relationship between nitrogen
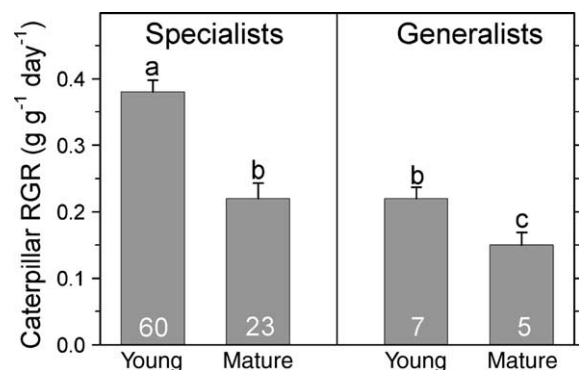

Fig. 1. Relative growth rates (g g^{-1} d^{-1}) of specialist and generalist lepidopteran caterpillars feeding on young and mature leaves of 37 different plant species. The number of morphospecies in each category is presented, values with different letters are significantly different at $p < 0.05$ (ANOVA $F_{3,94} = 13.3$, $p < 0.001$).

content of the host leaves and RGR (Fig. 2a; $r^2 = 0.17$, $p = 0.0003$, $n = 69$). There was a similar positive relationship for water ($r^2 = 0.08$, $p = 0.0052$, $n = 82$). For generalists, the effects of both nitrogen and water on growth were larger (Fig. 2b; nitrogen: $r^2 = 0.68$, $p = 0.007$, $n = 8$; water: $r^2 = 0.73$, $n = 11$, $p < 0.0005$). A multiple regression using both water and nitrogen was significant for both specialists and generalists ($r^2 = 0.16$, $p = 0.001$, $n = 68$, $r^2 = 0.96$, $p < 0.001$, $n = 7$, respectively).

Caterpillar growth rates on young leaves with different leaf expansion rates

Expansion rates of young leaves differed substantially among species, with leaf expansion rates of 106% per day (leaves doubling in size in less than a day) for the fastest species (*Talisia princeps*) and expansion rates of 3.6% per day (doubling time of 20 days) for the slowest species (*Psychotria limonensis*). Expansion rate was positively correlated with leaf nitrogen content (Fig. 3; $r^2 = 0.42$, $p < 0.001$, $n = 24$) but not with water. Caterpillar growth rates varied 10-fold among young-leaf feeders and were positively correlated with expansion rates of young leaves. For specialists, this relationship was significant ($r^2 = 0.13$, $p = 0.003$, $n = 60$ caterpillars) and for generalists it was not ($r^2 = 0.11$, $p = 0.24$, $n = 7$). In a multiple regression including water, nitrogen and expansion rate,
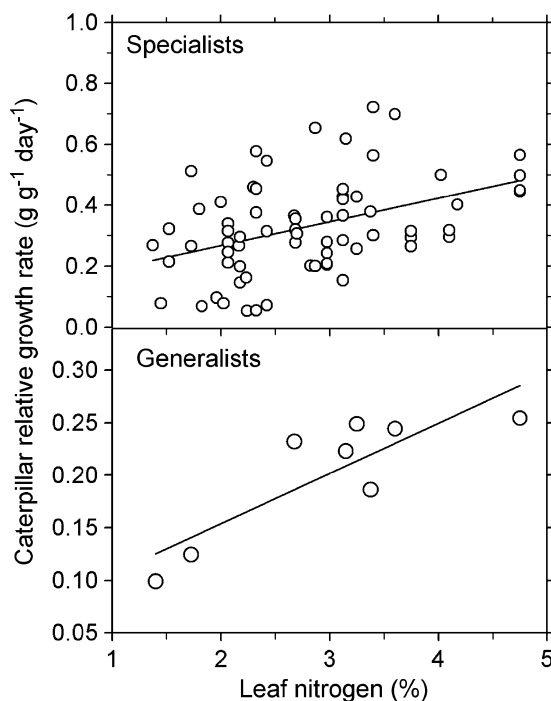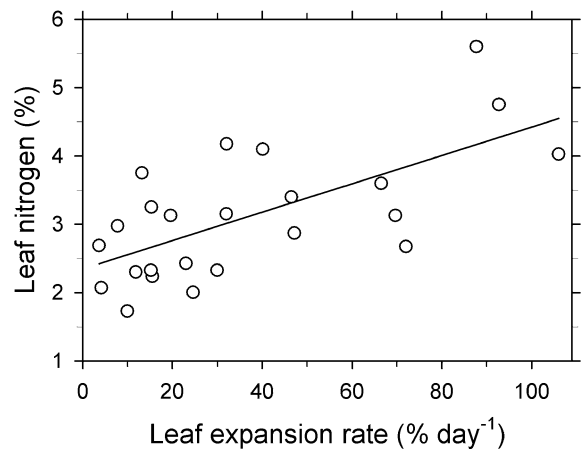

Fig. 3. Correlation between expansion rate of young leaves (% increase in size per day) and nitrogen content (% dry weight) for 24 species ($r^2 = 0.44$, $p < 0.001$, slope $= 0.021$).

the standardized partial coefficients were 0.02, 0.10 and 0.12 respectively, showing that nitrogen and expansion explained most of the variance in growth ($r^2 = 0.20$, $p < 0.0001$).

Effects of nutrients and defenses on growth

The differences in leaf traits between mature, fast- and slow-expanding leaves is reflected in the growth rates of caterpillars (Fig. 4a). The differences in growth for larvae feeding on different leaf functional groups is significant for both specialists (ANOVA, $F_{2,82} = 18.3$, $p < 0.001$; Fig. 4a) and generalists (ANOVA, $F_{2,11} = 4.38$, $p = 0.047$, not shown). When we removed the effects of nitrogen and water, the residual growth rates of specialists were still highest on fast-expanding young leaves and slowest on mature leaves (Fig. 4b, ANOVA $F_{2,78} = 8.39$, $p < 0.001$). For generalists, the trend was the same, but the differences were not significant (ANOVA $F_{2,8} = 1.61$, $p = 0.28$, not shown).

In the case of young leaves, when the effects of nitrogen and water were removed, the growth residuals were also still positively correlated with expansion rates of leaves. For specialists the explanatory power was modest ($r^2 = 0.10$, $p < 0.01$, $n = 60$), but for generalists, there was a strong relationship ($r^2 = 0.52$, $p < 0.05$, $n = 7$).

Are plant traits correlated with herbivore defenses against natural enemies?

Because generalists grow more slowly than specialists (Fig. 1), we hypothesized that their extended larval period should select for greater defenses against natural enemies (Table 2). Three of the comparisons were
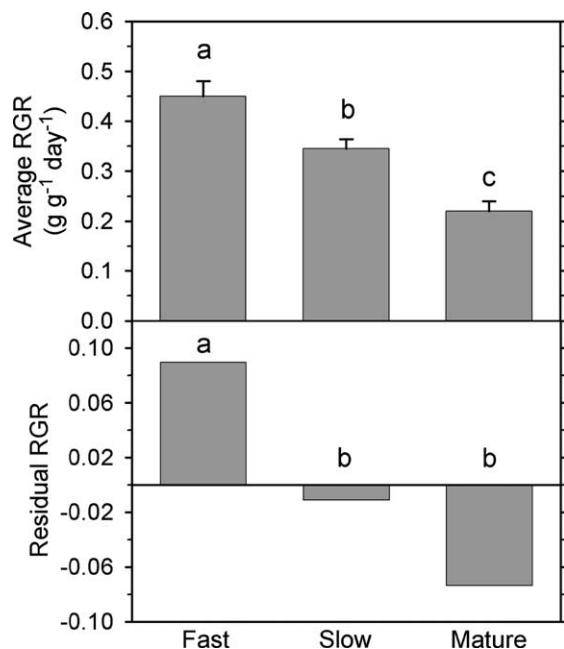

Fig. 2. Effects of leaf nitrogen content on relative growth rates (g g^{-1} d^{-1}) of lepidopteran caterpillars. For specialist caterpillars $r^2 = 0.18$, $p < 0.001$, $n = 69$ morphospecies and for generalist caterpillars $r^2 = 0.68$, $p < 0.007$, $n = 8$ morphospecies.

Fig. 4. (A) Relative growth rates (g g^{-1} d^{-1}) of specialist caterpillar species on fast-expanding young leaves (n =21), slow-expanding young leaves (n =39) and mature leaves (n = 23; ANOVA, F$_{2,82}$ =18.3, p <0.0001). Different letters indicate that values are significantly different at p <0.05. (B) Residuals for relative growth rate with the effects of water and nitrogen content removed (ANCOVA, F$_{2,78}$ =8.4, p <0.005, fast n =21, slow n =38, mature n =20).

significant at the level appropriate for repeated analyses (p <0.01), and trends are evident for the other two. Generalists were more brightly or warningly colored and were more often gregarious, two correlates of effective chemical defense (Table 2A). They also had more hairs and spines. However, they never made protective shelters and did not hide when resting, perhaps because they have effective physical and chemical defenses and have to feed for such an extended period.

A similar pattern for more defense in slow-growing caterpillars was seen when comparing species that fed on different leaf classes: mature leaves, slow-expanding or fast-expanding young leaves. For specialists, warning color, conspicuous behavior, gregariousness and hairs were high for species feeding on mature leaves, intermediate on slow-expanding young leaves and lowest on fast-expanding young leaves (Table 2B). Although mature leaf feeders rarely build houses, species feeding on slow-expanding leaves were more likely to live in shelters than those feeding on fast-expanding leaves. Generalists were not analyzed because of the small sample size.

We tested palatability of caterpillars to ants in feeding trials using *Ectatomma ruidum*, the most common insect predator found on plants on BCI (Coley et al. 2005). *Ectatomma* more frequently rejected caterpillars that fed on mature leaves and more readily carried off species that fed on fast-expanding young leaves (Fig. 5).

Discussion

Does plant nutrient content affect caterpillar growth?

There were large differences in caterpillar growth rates across hosts, with RGR differing 14-fold among species. In general, growth rates on young leaves were double those on mature leaves, and within young leaves, an increase in leaf expansion rate was correlated with an increase in caterpillar growth rate. These differences in growth are partially explained by nutrition, as nitrogen content, and to a lesser extent water content, were positively correlated with growth. Thus, despite the presence of physical and chemical defenses, the signature of nutrition is readily evident in caterpillar growth rates.

Table 2. Traits of caterpillars that could serve as defenses against natural enemies. For "color", scores increase from cryptic species, to colorful to warningly colored (0–2). For "gregariousness" solitary feeders received a score of 0 and gregarious feeders a score of 1. Values for "hairs" and "spines" ranged from 0 to 2. If caterpillars made shelters, they received a score of 1 for the "shelter" category. Behavior received a 0 if it was hiding when not feeding, versus a 1 if it was resting in an obvious position. Generalist and specialist scores were compared with a t-test, scores for caterpillar species feeding on different leaf classes were compared with an ANOVA. "N" is the number of morphospecies of caterpillars, values are the mean scores with standard errors in parentheses. To account for multiple comparisons, p values should be <0.01.

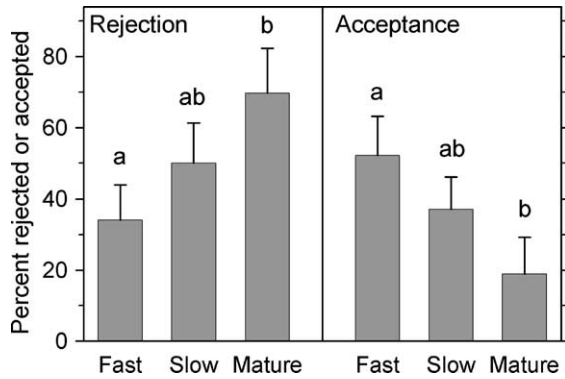|	N	Color	Gregarious	Hairs	Spines	Shelter	Behavior
A. Diet specialization							
Generalists	20	1.19 (0.13)	0.29 (0.10)	1.24 (0.22)	0.33 (0.14)	0.00 (0.00)	0.91 (0.07)
Specialists	86	0.38 (0.07)	0.08 (0.03)	0.17 (0.06)	0.13 (0.04)	0.39 (0.05)	0.29 (0.05)
t-test (p value)		<0.001	0.032	<0.001	0.09	–	<0.001
B. Leaf classes (specialists only)							
Mature leaves	25	0.65 (0.14)	0.15 (0.07)	0.35 (0.13)	0.27 (0.12)	0.19 (0.08)	0.58 (0.10)
Slow-expanders	39	0.28 (0.10)	0.08 (0.04)	0.10 (0.06)	0.05 (0.04)	0.51 (0.08)	0.18 (0.06)
Fast-expanders	22	0.23 (0.11)	0.00 (0.00)	0.09 (0.09)	0.09 (0.06)	0.41 (0.11)	0.14 (0.08)
ANOVA (p value)		0.03	0.15	0.12	0.08	0.03	<0.001

Fig. 5. Rejection and acceptance rates by the predatory ant, *Ectatomma ruidum* for caterpillars specialized on different leaf classes: fast-expanding young leaves, slow-expanding young leaves and mature leaves. Ant behaviors were scored as rejection when they contacted the caterpillar but immediately rejected it and as acceptance if they carried it to the nest. For each of the 36 caterpillar morphospecies tested (n = 12 for fast, n = 12 for slow and n = 12 for mature), approximately 9 individuals were presented to ants and the proportion rejected or accepted was calculated. Rejection and acceptance values do not add to 100% because there were other classes of behaviors (such as biting or carrying the caterpillar for a short distance). For comparisons within rejection or acceptance rates, values with different letters are significantly different at p <0.05 using Duncan's multiple range test (ANOVA, reject $F_{2,36}$ =2.61, p =0.09, accept $F_{2,36}$ = 2.72, p =0.08).

Do plant defenses affect caterpillar growth?

We did not measure plant defenses directly, but indirect evidence suggests that both toughness and secondary metabolites affect caterpillar growth. In a multiple regression with water, nitrogen and expansion rates, nitrogen and expansion contributed most to growth differences. When the effects of nitrogen and water were removed, there were still correlations of larval growth with leaf age classes and with leaf expansion rates, suggesting that other traits must be influencing growth. One possibility is other nutritional factors that we did not measure, such as phosphorous, minor minerals or non-structural carbohydrates. These traits vary among species and tissues and can affect host use and performance (Awmack and Leather 2002, Perkins et al. 2004). Alternative explanations for growth differences among leaf classes are physical and chemical defenses.

Mature leaves are very tough, and toughness is one of the most effective defenses (Coley 1983). The slowest residual growth rates were associated with mature leaves, mostly a result of high toughness. Young leaves are not tough, so differences in caterpillar growth rates among species with different expansion rates more likely reflect differences in secondary metabolites (Coley and Barone 1996, Kursar and Coley 2003, Coley et al. 2005). When the effects of nitrogen and water were removed, the residuals for growth were smallest on

species with slowly expanding leaves, indicating that slow leaf expansion is associated with a plant trait that slows caterpillar growth. Previous studies of 24 species demonstrated that extracts from slow-expanding young leaves were less preferred by insect herbivores in choice tests and reduced growth more in fungal pathogens than extracts from that fast-expanders (Kursar and Coley 2003). These bioassays indicated that slow-expanding species have more deterrent or more toxic secondary metabolites. The present study, based on herbivore growth rates, is an independent test of these ideas and provides additional evidence consistent with more effective secondary metabolites in species with slow-expanding young leaves. Even though these correlations are informative, much variation in caterpillar growth rate is unexplained. Ultimately, a good understanding of the role of plant chemistry will require direct analysis of plant chemical defenses and herbivore responses.

How do specialist and generalist caterpillars differ?

Specialist herbivores grew significantly faster than generalists. We found no evidence that specialists choose a subset of plants which would shape their growth rates. Using three plant traits indicative of diet quality (water, nitrogen and leaf expansion rate), PCA and discriminant analyses were unable to separate specialists and generalists. The growth differences were also apparently not due to phylogenetic biases as herbivore family had no significant effects (Table 1). Furthermore, the generalist species were from six different families, and all but one (Saturniidae) had specialist representatives. We suggest that specialists grew faster because they were less sensitive to host-plant defenses or could make more efficient use of host nutrients. Thus, rather than being shaped by phylogeny or the diet quality provided by the host, the slow growth of generalists may be due to a trade off with the herbivores' broad diet breadth.

Both specialists and generalists showed positive responses to increased nitrogen and water, demonstrating the importance of nutritional traits. However, we predicted that generalists would be more sensitive to secondary metabolites as they might lack specialized detoxification or sequestration mechanisms (Futuyma and Moreno 1988, Berenbaum and Zangerl 1994, Mackenzie 1996, Cornell and Hawkins 2003). This is best examined in young leaf-feeders as confounding effects of toughness are avoided. For young leaves, a partial regression removing the effects of water and nitrogen showed a much stronger relationship between caterpillar growth and leaf expansion rate for generalists (r^2 =0.52, p <0.05, n =7) than for specialists (r^2 =0.10, p <0.01, n =60). Although we cannot

eliminate effects of other unmeasured nutritional differences, we interpret this result to indicate that secondary metabolites present a greater challenge to generalists and may have a bigger negative effect on their growth. However, even among specialists, the growth rate residuals were significantly correlated with leaf expansion rate, suggesting better defended plant species present a bigger cost of detoxification for all Lepidoptera. Thus bottom-up effects of plant quality due to nutritional and defensive differences were evident for both specialist and generalist herbivores. These results also indicate that the more responsive, and therefore more appropriate, Lepidoptera for bioassays of plant chemical activity would be the generalists.

Are plant traits correlated with herbivore defenses against natural enemies?

We find that the differences in plant nutrition and defense place predictable constraints on the life history traits of herbivores feeding on them, including herbivore growth and defense against natural enemies. The nutritional value of the host plant is correlated with the growth rate of the herbivores. Differences in larval growth rates influence the amount of time a larva is at risk of predation, so slow-growing caterpillars should be under strong selection to evolve defenses against natural enemies (Price et al. 1980, Loader and Damman 1991, Benrey and Denno 1997). Our results support this hypothesis, with much higher defense scores for generalists than specialists and for mature versus young leaf feeders. The differences in defensive traits were also confirmed in feeding trials with ants. The generalists were from eight different families, so it is unlikely that defense differences were driven by phylogenetic biases.

Although our data indicate that slow larval growth rates of generalists are correlated with greater defense, these results conflict with other studies in which specialists were less palatable to predators (Bernays and Cornelius 1989, Dyer 1995). In Dyer's work with tropical lepidopterans, specialists were more able to sequester host plant chemicals and had reduced predation (Dyer and Floyd 1993, Dyer 1997, Gentry and Dyer 2002). While we noted that some specialist caterpillars had aposomatic coloring or spines, suggesting effective defense, we also found 14 species of specialists in 11 families that were green and constructed shelters. In our study, the generalists were better defended by all the measured traits, including indicators of chemical defense, however these could be produced autogenously rather than sequestered from the host. More extensive studies of the physical and chemical defenses of generalists would shed light on whether sequestration determines host choice.

What is the relative importance of top-down vs bottom-up effects on caterpillar traits?

These results could be viewed as supporting bottom-up effects, with diet quality shaping herbivore traits and hence their susceptibility to the third trophic level. However, an alternative explanation is that the evolution of caterpillar defenses influences host use. For example, a caterpillar with effective intrinsic defenses, such as spines, hairs or chemicals it synthesizes would be less constrained in diet than a caterpillar that relies on sequestering host secondary metabolites. Hence, the evolution of intrinsic defenses could predispose the evolution of generalist feeding. In either case, the greater investment in defensive traits for species with longer larval developmental times suggests that predation is a strong selective factor and that top-down effects could be important in the ecology and evolution of tropical lepidopteran larvae (Dyer and Coley 2002).

The relative importance of top-down versus bottom-up effects should determine herbivore populations. The low abundance of mature leaf feeders and generalists is a notable characteristic of tropical rainforests despite the obvious abundance of potential food (Barone 1998, Novotny et al. 2002, 2004). We hypothesize that the very slow growth rates of these herbivores result in strong top-down effects, which may severely limit their success (Hairston et al. 1960, Price et al. 1980). Thus, for mature leaf feeders, bottom-up effects of poor food quality interact with predators and parasitoids to determine herbivore populations. In contrast, the fast-growing herbivores that feed on young, expanding leaves, grow rapidly and are more abundant, suggesting the primacy of bottom-up effects. These herbivores may be more analogous to temperate species found on spring flushes. These temperate and tropical young leaf feeders are not only confronted with plant nutrition and defense, but they must also be adapted to plant phenological traits that result in a very short window of food availability.

It should be noted that the results in this study are from a single site. Our field observations in Central African Republic and the Republic of Congo indicate that the caterpillar community has relatively few young leaf specialists (pers. obs.). Instead, the forest is dominated by caterpillars that are well defended species and fairly abundant. Hence the lepidopteran community composition can best be understood by considering how diet quality, an herbivore's susceptibility to enemies, and herbivore defenses play out in different ecosystems (Singer and Stireman 2005).

Conclusions

This study shows that growth and defensive traits of lepidopteran herbivores are constrained by the nutritional and defensive traits of their hosts. This is mediated by the effect of leaf toughness, secondary metabolites and nutrients on herbivore growth rates which in turn influences their susceptibility to the third trophic level. Thus the combination of bottom up factors such as nutritional quality and defenses of plants, and top-down effects of natural enemies all interact to shape diet breadth and abundance of caterpillars.

References

Aide, T. M. and Londoño, E. C. 1989. The effects of rapid leaf expansion on the growth and survivorship of a lepidopteran herbivore. – Oikos 55: 66–70.

Awmack, C. S. and Leather, S. R. 2002. Host plant quality and fecundity in herbivorous insects.–Annu. Rev. – Entomol. 47: 817–844.

Barbosa, P. 1988. Natural enemies and herbivore–plant interactions: influence of plant allelochemicals and host specificity. – In: Barbosa, P. and Letourneau, D. K. (eds), Novel aspects of insect-plant interactions. John Wiley and Sons, pp. 201–229.

Barone, J. A. 1998. Host-specificity of folivorous insects in a moist tropical forest. – J. Anim. Ecol. 67: 400–409.

Benrey, B. and Denno, R. F. 1997. The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. – Ecology 78: 987–999.

Berenbaum, M. R. and Zangerl, A. R. 1994. Costs of inducible defense: protein limitation, growth and detoxification in parsnip webworms. – Ecology 75: 2311–2317.

Bernays, E. A. and Cornelius, M. L. 1989. Generalist caterpillar prey are more palatable than specialists for the generalist predator *Iridomyrmex humilis*. – Oecologia 79: 427–430.

Coley, P. D. 1983. Herbivory and defense characteristics of tree species in a lowland tropical forest. – Ecol. Monogr. 53: 209–233.

Coley, P. D. and Aide, T. M. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. – In: Price, P. W., Lewinsohn, T. M., Fernandes, W. W. et al. (eds), Plant–animal interactions: evolutionary ecology in tropical and temperate regions. John Wiley and Sons, pp. 25–49.

Coley, P. D. and Barone, J. A. 1996. Herbivory and plant defenses in tropical forests. – Annu. Rev. Ecol. Syst. 27: 305–335.

Coley, P. D. and Kursar, T. A. 1996. Anti-herbivore defenses of young tropical leaves: Physiological constraints and ecological tradeoffs. – In: Mulkey, S. S., Chazdon, R. and Smith, A. P. (eds), Tropical forest plant ecophysiology. Chapman and Hall, pp. 305–336.

Coley, P. D., Lokvam, J., Rudolph, K. et al. 2005. Divergent defensive strategies of young leaves in two species of *Inga*. – Ecology 86: 2633–2643.

Cornell, H. V. and Hawkins, B. A. 1995. Survival patterns and mortality sources of herbivorous insects: some demographic trends. – Am. Nat. 145: 563–593.

Cornell, H. V. and Hawkins, B. A. 2003. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. – Am. Nat. 161: 507–522.

Croat, T. B. 1978. The flora of Barro Colorado Island. – Stanford Univ. Press.

DeVries, P. J. 1987. The butterflies of Costa Rica and their natural history: Papillionidae, Pieridae, Nymphalidae. – Princeton Univ. Press.

Dyer, L. A. 1995. Tasty generalists and nasty specialists? A comparative study of antipredator mechanisms in tropical lepidopteran larvae. – Ecology 76: 1483–1496.

Dyer, L. A. 1997. Effectiveness of caterpillar defenses against three species of invertebrate predators. – J. Res. Lepidoptera 34: 48–68.

Dyer, L. A. and Floyd., T. 1993. Determinants of predation on phytophagous insects: the importance of diet breadth. – Oecologia 96: 575–582.

Dyer, L. A. and Coley, P. D. 2002. Tritrophic interactions in tropical and temperate communities. – In: Tscharntke, T. and Hawkins, B. (eds), Multitrophic level interactions. Cambridge Univ. Press, pp. 67–88.

Eisner, T. 1970. Chemical defense against predation in arthropods. – In: Sondeheimer, E. and Simeone, J. B. (eds), Chemical ecology. Academic Press, pp. 157–217.

Feeny, P. P. 1976. Plant apparency and chemical defense. – Recent Adv. Phytochem. 10: 1–40.

Futuyma, D. and Moreno, G. 1988. The evolution of ecological specialization. – Annu. Rev. Ecol. Syst. 19: 207–233.

Gauld, I. and Gaston, K. 1994. The taste of enemy-free space: parasitoids and nasty hosts. – In: Hawkins, B. and Sheehan, W. (eds), Parasitoid community ecology. Oxford Univ. Press, pp. 279–299.

Gentry, G. L. and Dyer, L. A. 2002. On the conditional nature of Neotropical caterpillar defenses against their natural enemies. – Ecology 83: 3108–3119.

Gross, P. 1993. Insect behavioral and morphological defenses against parasitoids. – Annu. Rev. Entomol. 38: 251–273.

Häggström, H. and Larsson, S. 1995. Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. – Oecologia 104: 308–315.

Hairston, N. G., Smith, F. E. and Slobodkin, L. B. 1960. Community structure, population control, and competition. – Am. Nat. 94: 421–425.

Haukioja, E. and Neuvonen, S. 1985. The relationship between size and reproductive potential in male and female *Epirrita autumnata* (Lep., Geometridae). – Ecol. Entomol. 10: 267–270.

Hawkins, B., Cornell, H. and Hochberg, M. 1997. Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. – Ecology 78: 2145–2152.

Holdridge, L., Grenke, W., Hatheway, W. et al. 1971. Forest environments in tropical life zones. – Pergamon Press.

Holton, M. K., Lindroth, R. L. and Nordheim, E. V. 2003. Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype. – Oecologia 137: 233–244.

Janzen, D. 1984. Two ways to be a tropical big moth: Santa Rosa saturniids and sphingids. – Oxford Surv. Evol. Biol. 1: 85–140.

Jeanne, R. L. 1979. A latitudinal gradient in rates of ant predation. – Ecology 60: 1211–1224.

Kursar, T. A. and Coley, P. D. 1991. Nitrogen content and expansion rate of young leaves of rainforest species: implications for herbivory. – Biotropica 123: 141–150.

Kursar, T. A. and Coley, P. D. 1992. Delayed greening in tropical leaves: an anti-herbivore defense? – Biotropica 24: 256–262.

Kursar, T. A. and Coley, P. D. 2003. Convergence in defense syndromes of young leaves in tropical rainforests. – Biochem. Syst. Ecol. 21: 929–949.

Kursar, T. A., Wolfe, B. T., Epps, M. J. et al. 2006. The effects of plant quality, food availability, competition and parasitism on performance and diet electivity in tropical Lepidoptera feeding on Inga (Fabaceae). – Ecology (in press).

Leigh, E. G. 1999. Tropical forest ecology: a view from Barro Colorado Island. – Oxford Univ. Press.

Lill, J. T. and Marquis, R. J. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. – Oecologia 126: 418–428.

Loader, C. and Damman, H. 1991. Nitrogen content of food plants and vulnerability of Pieris rapae to natural enemies. – Ecology 72: 1586–1590.

Mackenzie, A. 1996. A tradeoff for host plant utilization in the black bean aphid. – Evolution 50: 155–162.

Marquis, R. J. 1991. Herbivore fauna of Piper (Piperaceae) in a Costa Rican wet forest: diversity, specificity and impact. – In: Price, P. W., Lewinsohn, T. M., Fernandes, G. W. et al. (eds), Plant–animal interactions: evolutionary ecology in tropical and temperate regions. Wiley & Sons, pp. 197–208.

Marquis, R. J. and Braker, H. E. 1994. Plant-herbivore interactions: diversity, specificity and impact. – In: McDade, L. A., Bawa, K. S., Hespenheide, H. A. et al. (eds), La Selva, ecology and natural history of Neotropical rain forest. Univ. of Chicago Press, pp. 261–281.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. – Annu. Rev. Ecol. Syst. 11: 119–161.

McKey, D. 1979. The distribution of secondary plant metabolites. – In: Rosenthal, J. P. and Janzen, D. (eds), Herbivores: their interactions with secondary plant metabolites. Academic Press, pp. 55–133.

Medina, R. F., Barbosa, P. and Waddell, K. 2005. Parasitism levels in Orgyia leucostigma feeding on two tree species: implications for the slow-growth- high-mortality hypothesis. – Entomol. Exp. Appl. 115: 193–197.

Novotny, V., Miller, S. E., Basset, Y. et al. 2002. Predictably simple: assemblages of caterpillars (Lepidoptera) feeding on rainforest trees in Papua, New Guinea. – Proc. R. Soc. Lond. B 269: 2337–2344.

Novotny, V., Basset, Y., Miller, S. E. et al. 2004. Local species richness of leaf-chewing insects feeding on woody plants from one hectare of a lowland rainforest. – Conserv. Biol. 18: 227–237.

Ohmart, C. P., Stewart, L. G. and Thomas, J. R. 1985. Effects of food quality, particularly nitrogen concentrations, of Eucalyptus blakelyi foliage on the growth of Paropsis atomaria larvae (Coleoptera: Chrysomelidae). – Oecologia 65: 543–549.

Orians, G. H. and Janzen, D. H. 1974. Why are embryos so tasty? – Am. Nat. 108: 581–592.

Osier, T. L. and Lindroth, R. L. 2001. Effects of genotype, nutrient availability and defoliation on aspen phytochemistry and insect performance. – J. Chem. Ecol. 27: 1289–1313.

Perkins, M. C., Woods, H. A., Harrison, J. F. et al. 2004. Dietary phosphorus affects the growth of larval Manduca sexta. – Arch. Insect Biochem. Physiol. 55: 153–168.

Price, P. W., Bouton, C., Gross, P. et al. 1980. Interactions among three trophic levels: influences of plants on interactions between insect herbivores and natural enemies. – Annu. Rev. Ecol. Syst. 11: 41–65.

Raupp, M. J. and Denno, R. F. 1983. Leaf age as a predictor of herbivore distribution and abundance. – In: Denno, R. F. and McClure, M. S. (eds), Variable plants and herbivores in natural and managed systems. Academic Press, pp. 91–124.

Rausher, M. D. 1981. Host plant selection by Battus philenor butterflies: the roles of predation, nutrition, and plant chemistry. – Ecol. Monogr. 51: 1–20.

Rosenthal, G. A. and Berenbaum, M. R. 1991. Herbivores: their interactions with secondary plant metabolites, 2nd ed. – Academic Press.

SAS. 2002. Version 8.1, SAS Institute Inc., Cary, NC, USA.

Scriber, J. M. and Slansky, F. J. 1981. The nutritional ecology of immature insects. – Annu. Rev. Entomol. 26: 183–211.

Singer, M. S. and Stireman, J. O., III. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. – Ecol. Lett. 8: 1247–1255.

Stamp, N. E. and Casey, T. M.. 1993. Caterpillars: ecological and evolutionary constraints on foraging. – Chapman and Hall.

Stireman, J. O., III., Dyer, L. A., Janzen, D. H. et al. 2005. Climatic unpredictability and parasitism of caterpillars: implications of global warming. – Proc. Natl Acad. Sci. USA 102: 17384–17387.

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