

Nutrient regulation in relation to diet breadth: a comparison of *Heliothis* sister species and a hybrid

Kwang Pum Lee^{1,*}, Spencer T. Behmer^{1,2} and Stephen J. Simpson^{1,3}

¹Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK, ²Department of Entomology, Texas A&M University, College Station, TX 77843-2475, USA and ³School of Biological Sciences, University of Sydney, Heydon-Laurence Building, A08, NSW 2006, Australia

*Author for correspondence (e-mail: kwangpumlee@hotmail.com)

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Summary

We examined the nutritional correlates of diet breadth in insect herbivores by comparing patterns of diet selection, nutrient balancing, post-ingestive utilization and development in two sister species of caterpillar and a hybrid between them. One species, *Heliothis virescens* (HV) has a broad host range, feeding on plants in at least 14 families. The other, *Heliothis subflexa* (HS), is a specialist on plants in the genus *Physalis* (Solanaceae). Experiments using synthetic foods showed that when the caterpillars were allowed to mix their diet, the generalist self-selected a higher-protein diet whereas the specialist ate almost equal amounts of protein and carbohydrate, which accords with differences between the two species in the nutrient content of their natural diets. When confined to nutritionally imbalanced diets, the generalist showed a propensity to over-eat high protein:carbohydrate (P:C) diets to a greater degree than did the specialist and maintained higher rates of development. The generalist

did not, however, over-eat low P:C diets to the same degree as the specialist. The hybrid selected a diet composition that was indistinguishable to that of its generalist father (HV), while its response to imbalanced diets was closely similar to that of the specialist mother (HS). The generalist converted ingested nutrient to growth with lower efficiency than did the specialist and the hybrid. Our findings imply that different behavioural and physiological traits linked to nutrient regulation are under genetic control and are explicable in terms of the different life-histories, feeding ecologies and presumed levels of nutritional heterogeneity in the environments of the two insects.

Key words: caterpillar, diet breadth, feeding, generalist, *Heliothis*, insect herbivore, macronutrient, nutrient regulation, protein:carbohydrate ratio, self-selection, specialist.

Introduction

The majority of insect herbivores are host-plant specialists, feeding on a single plant family, while many fewer species are broad generalists (Bernays and Chapman, 1994; Schoonhoven et al., 1998). Although numerous functional explanations have been offered to explain why host plant specialists are so common (Price et al., 1980; Bernays and Graham, 1988; Ehrlich and Murphy, 1988; Schultz, 1988), feeding is ultimately about securing nutrients (Slansky, 1993; Simpson et al., 2004) and relatively little is known about the behavioural and physiological correlates of diet breadth as they relate to nutrient acquisition, retention and allocation. Where differences in nutrient utilization have been sought between specialists and generalists, results have been ambiguous (Scriber and Feeny, 1979; Futuyma and Wasserman, 1981).

One aspect that might be predicted to differ between host-plant specialists and generalists is the variability of the nutritional environment (Lee et al., 2002; Lee et al., 2003;

Raubenheimer and Simpson, 2003). It is well established that the nutrient content of plants varies temporally and spatially within and between individuals, populations and species (McNeill and Southwood, 1978; Mattson, 1980; Scriber and Slansky, 1981; White, 1993), and since generalists feed on a wide range of plant families and are often highly mobile, they are likely to experience greater heterogeneity in the contents and mixtures of nutrients found in their natural diets (Raubenheimer and Simpson, 1999; Raubenheimer and Simpson, 2003). Being restricted to a narrow range of host-plant, however, specialists are more likely to encounter diets with lower nutritional variability compared to generalists, and may need to cope with qualitative nutritional impediments associated with dietary restriction (Behmer and Nes, 2003; De Moraes and Mescher, 2004). Another important aspect of the nutritional environment is the mean nutrient composition of the diet. For example, phytophagous species are known to differ widely in the ratio of protein to digestible carbohydrate that

supports optimal performance, with this ratio reflecting the composition of the natural diet (Simpson and Raubenheimer, 1993), but whether consistent differences in diet composition and nutrient requirements occur between specialists and generalists is not known.

Recently, detailed comparisons of the responses of host-plant specialist and generalist grasshoppers (Simpson et al., 2002; Raubenheimer and Simpson, 2003) and caterpillars (Lee et al., 2002; Lee et al., 2003) have indicated that the extent to which an insect over-consumes nutrients in excess in imbalanced foods is correlated with diet breadth. It has been hypothesized that these differences reflect an evolved trade-off between the short-term costs of over-eating excess nutrients and the probability of encountering nutritionally complementary foods in the future (Raubenheimer and Simpson, 1999; Raubenheimer and Simpson, 2003; Simpson et al., 2002).

In the present study, our aim was to explore the relationship between diet breadth and nutritional regulatory responses in two very closely related lepidopteran species and their hybrid crosses. *Heliothis virescens* and *H. subflexa* are hybridizable sister species (Laster, 1972; Sheck and Gould, 1996), which phylogenetic studies indicate recently diverged from a common polyphagous ancestral species (Mitter et al., 1993; Fang et al., 1997). Despite their close evolutionary relationship, the two species differ strikingly in their host-range. On the one hand, *H. virescens* larvae are extreme generalists, with a diet that includes 37 plant species from 14 different families, including the Malvaceae, Leguminosae, Gramineae and Solanaceae (Sheck and Gould, 1993). In contrast, *H. subflexa* larvae feed exclusively on plants in the genus *Physalis* in the Solanaceae. The present experiments were conceived and the data interpreted using the Geometric Framework (Simpson and Raubenheimer, 1993; Raubenheimer and Simpson, 1999; Raubenheimer and Simpson, 2004).

Materials and methods

Study organisms

Heliothis virescens (Fabricius) and *Heliothis subflexa* (Guenée) (abridged as HV and HS, respectively, hereafter) originated from a culture maintained at North Carolina State University, USA. Caterpillars of both species were individually reared in 25 ml polypots on a corn-milk-soy based artificial diet, modified from Burton (Burton, 1970), and maintained in an incubator (LMS Ltd, Kent, UK) set at 27°C with a 12 h:12 h L:D photoregime. Pupae were sexed and transferred to 30 cm×30 cm×30 cm aluminium insect cages (BioQuip, Gardena, CA, USA) containing small Eppendorf tubes with 10% sugar solution. Moths were mated in groups of 20–30 with roughly equal numbers of males and females. Eggs were collected from cloth mesh, which acted as an oviposition substrate, placed inside the cage at the top. These maintenance procedures were repeated for three generations for each species until the start of the experiment, to preclude any differences due to maternal effects.

Reciprocal crossing between the two species was undertaken in an attempt to yield both directions of F₁ hybrid. In each case, 15–20 newly eclosed males of one species were allowed to mate with an equivalent number of females from the other species. Eggs were obtained from the two reciprocal crosses, but only HS female × HV male eggs hatched.

Diets

Five synthetic foods differing in their content of protein and digestible carbohydrate (P:C, henceforth) were prepared (Lee et al., 2002; Lee et al., 2003; Lee et al., 2004): 7% protein with 35% carbohydrate (7:35), 14:28, 21:21, 28:14 and 35:7. The protein component contained a 3:1:1 mixture of casein, peptone and albumen, while digestible carbohydrate was sucrose. Other nutrients included essential micronutrients such as Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%) and a vitamin mix (0.2%). The remaining portion of the food was composed of the non-nutritive bulking agent, cellulose (54%). Dry ingredients were presented to the insects suspended at a 1:6 ratio in 1% agar solution.

Protocol

Caterpillars of HV (the generalist), HS (the specialist) and the SV hybrid cross (HS females × HV males) were allocated to one of six treatments: a choice treatment (35:7 paired with 14:28) and five no-choice treatments (7:35, 14:28, 21:21, 28:14 or 35:7). There were 12 replicates per treatment, and all experimental treatments were run concurrently.

Upon moulting to the final larval stadium (day 0), caterpillars were weighed to the nearest 0.1 mg (initial fresh mass), and placed individually into 9 cm diameter Petri dishes that had five 1 mm diameter perforations in the upper lid to allow ventilation. The choice caterpillars received two blocks of food, while the no-choice caterpillars received a single food block. The mass of each food block ranged between 1000 and 1500 mg, and was pre-weighed to the nearest 0.1 mg. This range of mass was chosen so that daily food consumption was never limited yet the surplus was minimal (Schmidt and Reese, 1986). Feeding dishes were sealed with a strip of Parafilm and kept in the incubator throughout the experimental period. After 24 h, any food remaining was collected and replaced with a fresh block of the same food. The collected food was dried to constant mass at 50°C and weighed to the nearest 0.1 mg.

To estimate daily food consumption (dry mass), 12 control blocks of each diet were dried to constant mass and used to construct a regression equation from which the initial dry mass of individual blocks of food was back-calculated.

Duration of the final stadium (until pupation) was recorded to the nearest day. Pupae were frozen and dried to constant mass in a desiccating oven at 50°C, weighed to the nearest 0.1 mg and lipid-extracted in three, 24-h changes of chloroform before being re-dried and re-weighed. Lipid-free carcasses were analyzed for nitrogen content using micro-Kjeldahl. The initial lipid and protein content of each experimental caterpillar were estimated from regression

equations from a sample of 12 freshly moulted final-instar larvae.

Statistical analysis

Nutrient consumption, nutrient growth and body mass data were analysed using general linear modeling procedures (PROC GLM) in SAS v. 8.2 (Cary, NC, USA). Pillai's trace statistic was used for multivariate tests (Scheiner, 2001). Efficiencies of conversion of ingested nutrient to body growth were investigated using ANCOVA in combination with graphical interpretation (Raubenheimer and Simpson, 1992). Where necessary, data were transformed to meet the underlying assumptions of these analyses. Feeding period and stadium duration were tested with accelerated failure-time analysis (PROC LIFEREG using Weibull distribution).

Results

Dietary self-selection: establishing the self-composed nutrient intake

Nutrient intake

The duration of feeding varied significantly between the genotypes ($\chi^2=56.18$, d.f.=2, $P<0.001$). In general, most HV caterpillars completed their feeding within 3 days, while the HS caterpillars continued to feed up to day 5. For the hybrids, feeding duration was intermediate between the parental species (day 4). The caterpillars also differed in their intake trajectory through nutrient space with development (Fig. 1; ANOVA on arctanC/P-transformed radian values pooled over the stadium for three genotypes: $F_{2,141}=46.80$, $P<0.001$). HV ingested a higher ratio of protein (P) to digestible carbohydrate (C) than

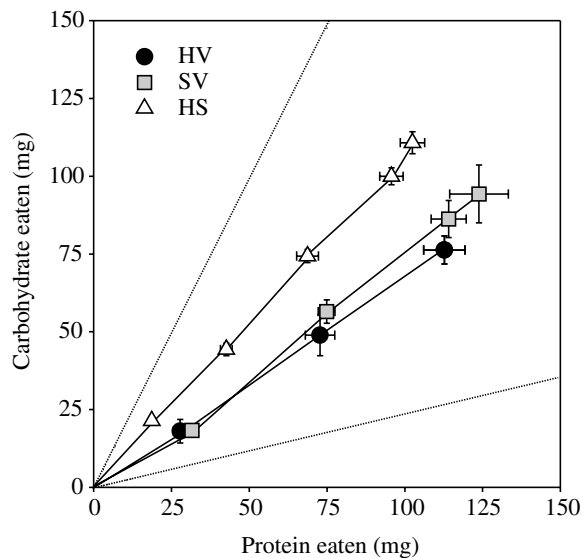


Fig. 1. Bivariate means (± 1 s.e.m.) of cumulative protein and carbohydrate consumed by HV, SV and HS caterpillars over the successive days during their entire final stadium in the choice test. The two dotted lines represent the protein:carbohydrate ratio of the two complementary foods (14:28 and 35:7 diets).

did HS (mean P:C of 1.6:1 versus 0.9:1, respectively; ANOVA contrast: $F_{1,142}=72.03$, $P<0.001$). The hybrid caterpillars (SV) followed an intake trajectory that was similar to that of HV (P:C=1.3:1; $F_{1,142}=1.82$, $P=0.180$). HS caterpillars ate considerably more carbohydrate than did the other caterpillars (ANOVA: $F_{2,33}=7.48$, $P=0.002$). Protein consumption was, however, similar across the three genotypes ($F_{2,33}=2.32$, $P=0.114$). The mean starting mass of HS caterpillars was significantly smaller than the other two groups ($F_{2,33}=15.83$, $P<0.001$). ANCOVA took this size difference into account, but the difference in starting size did not affect any of the results described.

Performance measures

All foods were suitable for the caterpillars to develop through the stadium, as evidenced by the fact all insects pupated. Stadium duration was significantly shorter for HV and was longest for HS, with SV development being intermediate (Fig. 2A; $\chi^2=57.96$, d.f.=2, $P<0.001$). SV reached a higher

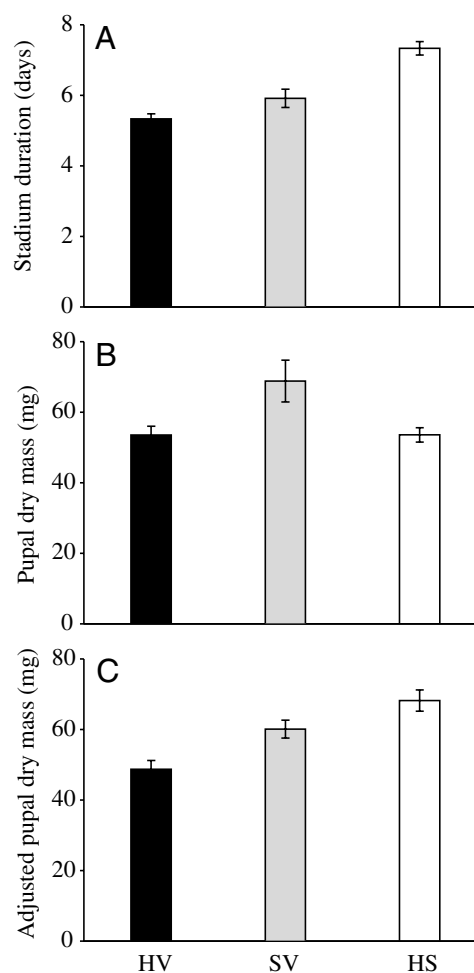


Fig. 2. Means (± 1 s.e.m.) of (A) stadium duration, (B) pupal dry mass of HV, SV and HS caterpillars in the choice test. Pupal mass was also adjusted for differences in initial fresh mass and demonstrated in (C) as the least square means (± 1 s.e.m.) calculated from ANCOVA.

pupal dry mass than did HV and HS insects (Fig. 2B; $F_{2,33}=5.10$, $P=0.012$). When ANCOVA was conducted to correct for differences in the initial fresh mass among insects, covariate-adjusted pupal dry mass was significantly reduced for HV *versus* both HS and SV (Fig. 2C; $F_{2,32}=12.42$, $P=0.001$). A strong covariate effect ($F_{1,32}=52.10$, $P<0.001$) indicated that heavier pupae developed from initially heavier caterpillars.

No-choice treatments: responses to dietary imbalance

Nutrient intake

As for self-selecting caterpillars, the period of feeding was the shortest for HV larvae, intermediate for the SV caterpillars and longest for the HS larvae ($\chi^2=96.84$, d.f.=2, $P<0.001$). For each genotype, we constructed intake arrays by connecting intake points across the five P:C diets. This allowed us to visualize the type of regulatory process the caterpillars employed when balancing the over-ingestion of nutrients in excess *versus* the under-ingestion of nutrients in deficit (Raubenheimer and Simpson, 1999; Raubenheimer and Simpson, 2003; Raubenheimer and Simpson, 2004). HV larvae displayed a conspicuous linear alignment of the intake points on four of the imbalanced diets (Fig. 3A; 35:7, 28:14, 21:21 and 14:28 diet). However, they ate substantially less on the extreme carbohydrate-biased 7:35, such that the line connecting the 14:28 and 7:35 intake points bent downwards (Fig. 3A) up until day 4, at which point the final intake array formed a straight line. All the HV caterpillars except for those on the 7:35 food ceased feeding prior to ecdysis by day 3. Extended development, and thus feeding, caused the intake point to move further along the 7:35 trajectory, and eventually to line up with the other points.

The manner in which HS larvae responded to a series of imbalanced foods (Fig. 3C) differed markedly compared to HV larvae, with the intake arrays forming convex curves over the first four days (days 0–4). However, these quadratic patterns later became linear as both extreme arms of the array (7:35 and 35:7) straightened progressively outwards with prolonged development. On the three near-optimal diets, most caterpillars completed feeding by day 5, while those on the extreme diets continued feeding until days 6–8.

The intake array constructed for the SV hybrid caterpillars (Fig. 3B) was similar to that for HS, with a few notable

exceptions. On the first feeding day (day 1), the intake array formed an arc-shaped pattern (Fig. 3B), but from day 2 onwards larvae increased consumption of the extreme carbohydrate-biased food (7:35) to such an extent that all

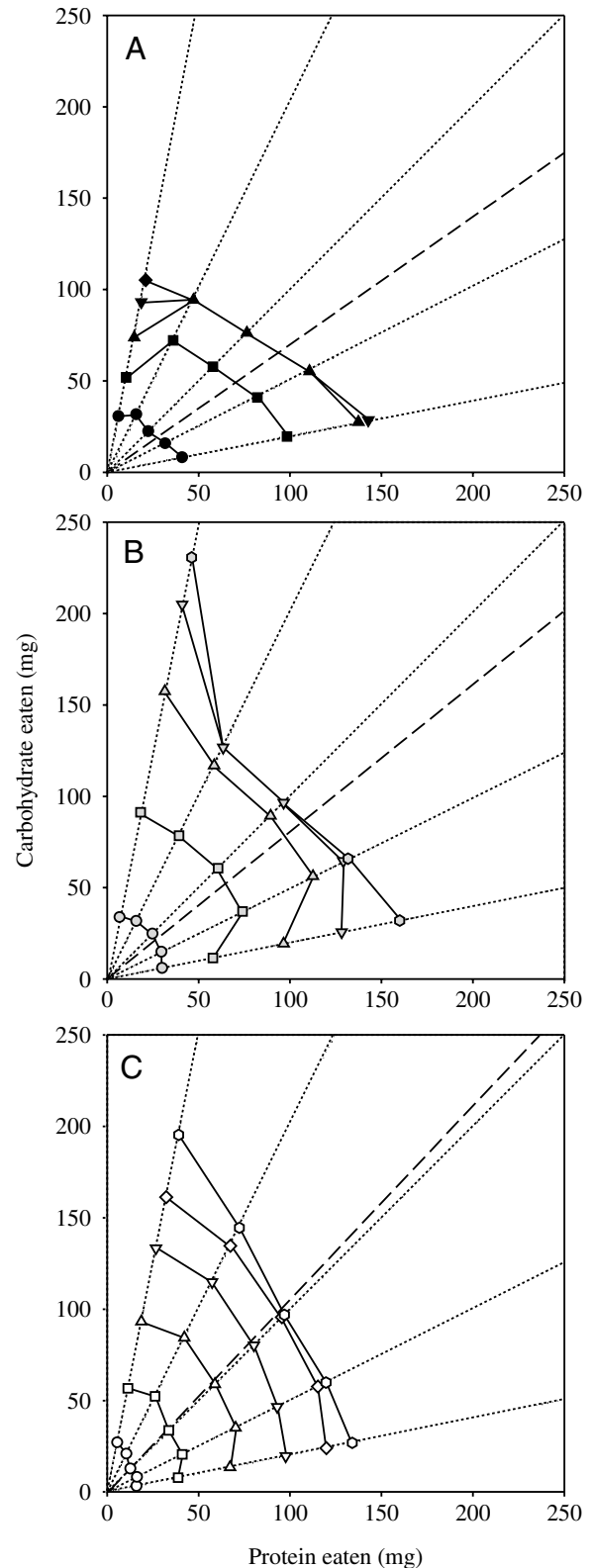


Fig. 3. Cumulative protein and carbohydrate consumed by (A) HV, (B) SV and (C) HS over the successive days and across the full final stadium in the no-choice test. In each plot, dotted lines from left to right indicate the nutrient ratios for 7:35, 14:28, 21:21, 28:14 and 35:7 P:C diets where caterpillars were restricted, respectively. The mean protein-carbohydrate intakes up to days 1, 2, 3, 4, 5 and beyond (days 6–8) are represented as following symbols: circle, square, triangle, inverted triangle, diamond and hexagon, respectively. Within each time interval, the intake points are connected with solid line (the intake array) to demonstrate the pattern of nutrient balancing strategy. Broken lines stand for the self-selected composition of protein and carbohydrate by three genotypes (data adapted from Fig. 1).

intake points except those of the extreme protein-biased food (35:7) were aligned in a linear configuration. Similar to HS, hybrid caterpillars strongly restrained their consumption on 35:7 diet compared to the other diets until day 4. Also similar was that hybrid caterpillars fed longer on the two extreme foods compared to those on the moderate diets. However the degree of such prolonged feeding was much greater than HS on the extreme carbohydrate-biased food, which resulted in a disproportionately extended intake on the 7:35 diet.

Performance measures

Survival was high for all caterpillars on all treatments, with only eight deaths (out of 180) occurring before pupation. Seven of these were HV caterpillars, but they were widely spread across the five food treatments. One HS caterpillar died (on the 14:28 diet), while all the SV caterpillars survived. Caterpillars that failed to survive were removed from all subsequent analyses. Stadium duration was shortest for HV and longest for HS caterpillars, with the hybrids (SV) being intermediate (Fig. 4A; $\chi^2=204.88$, d.f.=2, $P<0.001$). Dietary imbalance also affected stadium duration ($\chi^2=333.95$, d.f.=4, $P<0.001$), with all the caterpillars on the extreme carbohydrate-biased diet (7:35) developing slowly relative to those on the moderately

balanced foods (14:28, 21:21 and 28:14). In contrast, stadium duration on the extreme protein-biased diet (35:7) was notably delayed for SV larvae, but only marginally so for HV and HS caterpillars. This resulted in a strongly significant two-way interaction between genotype and P:C ratio ($\chi^2=213.61$, d.f.=8, $P<0.001$).

ANOVA of pupal dry mass exhibited significant effects due to genotype ($F_{2,156}=42.57$, $P<0.001$) and P:C ratio ($F_{4,156}=3.84$, $P=0.005$), with a significant interaction between them ($F_{8,156}=2.61$, $P=0.011$). Pupal dry mass of SV was higher than both of their parental species across the five imbalanced treatments (Fig. 4B). The effect of nutrient imbalance on pupal mass acted oppositely for HV and HS caterpillars, with the smallest pupae produced on the extreme carbohydrate- (7:35) and protein-biased (35:7) diets, respectively. ANCOVA with the initial fresh mass of caterpillars as the covariate yielded a strong covariate-by-genotype interaction ($F_{2,141}=21.27$, $P<0.001$), indicating a steeper increase in pupal mass with initial fresh mass for SV.

Body nutrient composition and utilization

Body composition of self-selecting caterpillars differed markedly depending on their genotype (MANOVA: $F_{4,66}=18.00$, $P<0.001$), with HV showing the highest proportion of nitrogen-to-lipid in body growth, SV being intermediate, and HS being the lowest (Fig. 5).

To investigate the effect of genotype and P:C ratio on the conversion efficiency of ingested nitrogen, nitrogen growth adjusted by initial fresh mass differences between the genotypes was plotted against nitrogen consumption (estimated as protein consumption divided by the conventional conversion factor of 6.25) in Fig. 6A. When ANCOVA was

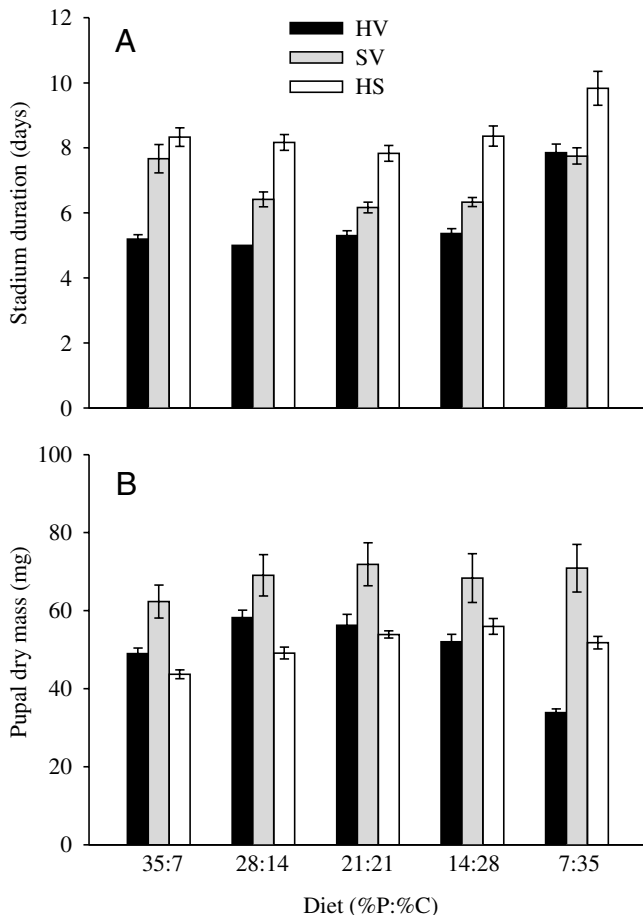


Fig. 4. Means (± 1 s.e.m.) of (A) stadium duration, (B) pupal dry mass of HV, SV and HS caterpillars in the no-choice test.

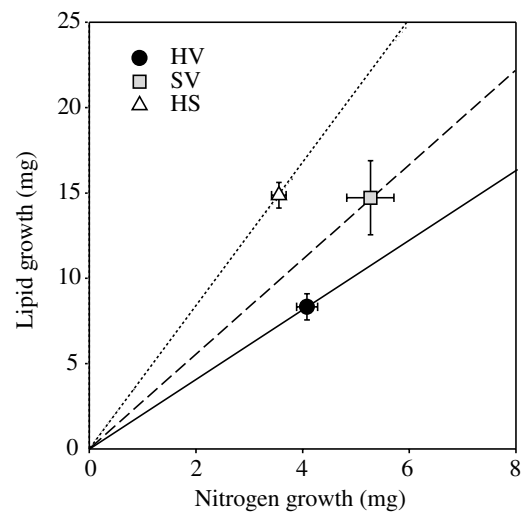


Fig. 5. Bivariate means (± 1 s.e.m.) of pupal nitrogen and lipid mass accumulated through the insect's final larval stadium, denoted as nitrogen and lipid growth, in the choice test. Solid, broken and dotted lines depict the proportions of nitrogen to lipid growth for HV, SV and HS caterpillars in that order.

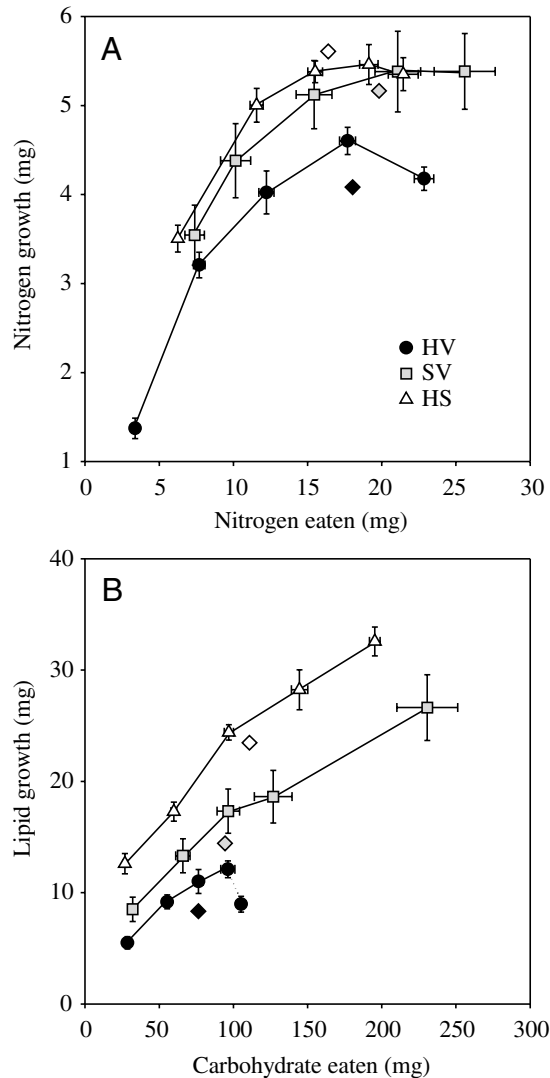


Fig. 6. Utilization plots describing the bivariate means (± 1 s.e.m.) of (A) nitrogen growth versus total nitrogen consumption and of (B) lipid growth versus total carbohydrate consumption in the no-choice test. Growth data presented for SV and HS caterpillars are corrected by multiplying the factor of 0.980 and 1.579 to each. This is done to account for their difference in initial fresh mass relative to HV caterpillars. In each genotype, the points from left to right are 7:35, 14:28, 21:21, 28:14 and 35:7 P:C diets for the nitrogen utilization plot, whereas such order is reversed for the lipid plot. Diamond symbols indicate the mean reference values for animals that are free to choose between 35:7 and 14:28 P:C diets (data adapted from Figs 1 and 5).

performed with nitrogen consumption as the covariate, there was a significant covariate-by-P:C ratio interaction ($F_{4,139}=5.77$, $P<0.001$), suggesting significant slope heterogeneity, which led us to a graphical interpretation of nitrogen utilization. Each genotype increased nitrogen growth with increasing protein intake as P:C ratio of the diet rose, but nitrogen growth slowed as nitrogen intake approached the self-selected nutrient intake. When protein intake exceeded the intake target level on high-protein diets, this excess did not

translate into increased nitrogen growth (Fig. 6A). There was a substantial effect of genotype, as seen by the fact that HV had substantially lower nitrogen gain per nitrogen intake relative to both SV and HS.

The conversion efficiency of ingested carbohydrate to lipid was also analyzed using ANCOVA, with initial mass-adjusted lipid growth and carbohydrate consumption as the response variable and covariate, respectively. There was a significant covariate-by-P:C ratio interaction ($F_{4,139}=2.78$, $P=0.029$). The lipid utilization plot (Fig. 6B) demonstrated that lipid growth tended to increase progressively with increased carbohydrate intake in all three genotypes, with one exception for HV caterpillars on extremely carbohydrate-biased diet (7:35). Lipid growth for the HV larvae on this diet was significantly lower compared to that on the 14:28 diet. It can also be seen that HS converted carbohydrate to body lipid with greatest efficiency, SV was intermediate, and HV the lowest (Fig. 6B).

Discussion

The generalist and specialist sister species, *H. virescens* (HV) and *H. subflexa* (HS), showed pronounced differences in the composition of their self-selected diet (i.e. intake target), their strategy for balancing intake of nutrient excesses against deficits when restricted to imbalanced diets (the rule of compromise), and their post-ingestive physiology and development. Data from the hybrid (SV) indicated that behavioural and physiological aspects of nutrient regulation are under genetic control, which suggests that these traits might have been selected in response to the nutritional environment. The noteworthy findings of the present study are next discussed in detail, with particular reference to each regulatory trait.

Compositions of the self-selected diet

The generalist HV caterpillars mixed a diet containing more protein than digestible carbohydrate, while the specialist-feeding HS selected almost equal amounts of these nutrients. This pattern was in accordance with results from studies showing that grass-feeding specialist locusts (*Locusta migratoria*) and caterpillars (*Spodoptera exempta*) selected lower P:C diets than did polyphagous relatives (*Schistocerca gregaria* and *Spodoptera littoralis*). This was interpreted as reflecting differences in host plant nitrogen content, grasses being on average less nitrogen-rich than forbs (Lee et al., 2002; Lee et al., 2003; Lee et al., 2004; Raubenheimer and Simpson, 2003). Consistent with such an explanation in the present case is the fact that HS larvae are behaviourally specialized to feed on *Physalis* fruit, a carbohydrate-rich food item (Sisterson and Gould, 1999; Oppenheim and Gould, 2002). Like most lepidopterous larvae (Simpson and Simpson, 1990; Waldbauer and Friedman, 1991), HV larvae selected higher levels of protein than carbohydrate, reflecting their renowned preference for nitrogen-rich plant reproductive tissues (e.g. floral buds, blossoms and anthers) and young foliar tissues (Telang et al., 2001). For the hybrid (SV), the self-selected diet composition was close to that of the generalist father (HV). A comparison

of male and female hybrids showed no statistical difference between the self-composed P:C ratios (1.3 and 1.4 for female and male SV, respectively; Student *t*-test on $\arctan C/P$ -transformed radian value of the final intake: $t=0.95$, d.f.=10, $P=0.367$). If the self-selected P:C ratio of the hybrid was inherited *via* sex chromosomes, we should have observed differences between the sexes. Instead, a possible dominant autosomal effect on the self-selected P:C ratio is suggested. Information from backcrossing is needed to determine the mode of inheritance (Futuyma and Peterson, 1985).

A second interesting finding from the choice experiment is that the generalist HV spent substantially less time feeding and developed much faster than did the specialist HS. Such rapid growth was positively correlated with the level of protein in their selected diet. This correlation makes sense because protein provides the building blocks for animal body tissue. Foraging and feeding can be hazardous, due to increased exposure to natural enemies (Bernays, 1997), and the risk of predation/parasitism accumulates with prolonged development and feeding (Moran and Hamilton, 1980; Loader and Damman, 1991; Benrey and Denno, 1997). It is widely thought that generalists are more susceptible to this kind of selective pressure than are specialists (Geitzner and Bernays, 1996; Oppenheim and Gould, 2002; Bernays et al., 2004). Rapid development and high protein consumption seen in HV are therefore explicable as evolved traits selected to reduce the higher predation risk experienced by this species under natural conditions. Hybrids' feeding period during the stadium, as well as their development rate, were intermediate between those of its parental species, suggesting the genetic control of these traits.

Dealing with imbalanced diets

When restricted to a one of a range of single foods, we found substantial differences between the two species in the way they balanced the intake of the surplus and deficient nutrients relative to the self-selected nutrient intake (as estimated from the choice treatment). Similar to other generalists studied to date (Lee et al., 2002; Simpson et al., 2002; Raubenheimer and Simpson, 2003), HV larvae demonstrated a linear intake array across the range of no-choice diets, except on the lowest-protein diet (7:35 P:C), where intake somewhat lagged behind the other diets. The slope of the linear P:C intake array was shallow (ca. -0.7), resulting in carbohydrate intake varying less than for protein across the array. Thus HV larvae over-ate protein to a substantial degree on high-protein diets, but did not over-consume carbohydrate to the same degree on low-protein diets.

Compared to HV, HS larvae were considerably less disposed to over-eat protein on protein-biased diets, and exhibited an arc-shaped intake array resembling the pattern described for other specialist feeders (Raubenheimer and Simpson, 2003; Lee et al., 2003; Lee et al., 2004). The array was tilted towards the vertical, indicating in tighter regulation of protein than carbohydrate intake (Lee et al., 2003; Lee et al., 2004). Results from these two closely related caterpillars corresponded well with our expectation based on the 'nutritional heterogeneity'

hypothesis. This predicts the opportunistic consumption of imbalanced diets to have evolved under circumstances in which animals are likely to encounter complementary foods, which will translate excess ingested nutrients into a balanced complement (Raubenheimer and Simpson, 1999; Simpson et al., 2002; Lee et al., 2002; Lee et al., 2003).

In the choice treatments we found that the hybrid SV larvae selected the same diet composition as their paternal generalist species, HV, but this was not the case for their intake array in the no-choice treatments. Here the overall configuration of their intake array was very much closer to the arc-shaped array of the maternal specialist species, HS; albeit that they spent an amount of time feeding during the stadium that was intermediate to their parental species. This suggests that the two traits, the self-selected intake target and the strategy for balancing intake of excesses and deficits on imbalanced diets, are controlled independently through separate genetic loci, with the possibility of genetic dominance operating in opposite directions. Despite their similarity to the specialist HS, SV larvae over-ate carbohydrate to a greater extent than did the maternal species on the extremely protein-deficient diet (7:35 P:C), which suggests a tighter regulatory response for protein in SV larvae (greater consumption of carbohydrate being coupled to greater intake of limiting protein).

The results from the no-choice experiments reinforced the conclusion that the generalist species has evolved rapid feeding and development as a strategy for reducing the ecological costs of predation and parasitism (see above). Thus, HV caterpillars over-consumed protein on high-protein diets to a greater degree than did the specialist species, and were therefore better able to maintain high development rates and to sustain pupal mass on such diets. As also seen for self-selecting larvae (see above), development time in hybrids was intermediate to their parent species, strengthening the indication of genetic influence.

Body chemical compositions and post-ingestive regulation

Differences between HV, HS and their hybrid in pupal chemical composition reflected differences in nutrient intake of the caterpillars. Hence, HV caterpillars accumulated a higher proportion of nitrogen to lipid mass over the final larval stadium than did HS larvae, while the hybrid SV larvae were intermediate. Such a parallel relationship between nutrient intake and body chemistry indicates genetic coupling of these traits and linkage of their regulatory mechanisms (Simpson and Raubenheimer, 1993). The body chemistry data also have implications for the effects of diet breadth on the ecological stoichiometry of insect herbivores (Sterner and Elser, 2002; Jaenike and Markow, 2003).

Consistent with earlier reports on caterpillars (Lee et al., 2002; Lee et al., 2003; Lee et al., 2004), in all three experimental groups (HV, HS, SV) nitrogen growth was regulated more tightly than lipid growth in the face of dietary manipulation. In each of the three genotypes, nitrogen ingested in excess of their different self-selected levels of protein was not converted into extra nitrogen growth,

indicating post-ingestive mechanisms of nitrogen regulation (Zanotto et al., 1993; Thompson and Redak, 2000). Tight coupling between patterns of nitrogen utilization and the position of the self-composed protein intake is to be expected (Simpson and Raubenheimer, 1993; Raubenheimer and Simpson, 2004), and the present data show that evolved changes in one accompany the other. Compared to HS and SV caterpillars on the 7:35 P:C diet, HV pupae had lower nitrogen content but this was explicable in terms of the different patterns of intake. Thus reduced nitrogen growth for HV stemmed from the fact that these caterpillars had eaten relatively smaller amounts of the extreme carbohydrate-biased diet (7:35 P:C), with a concomitant depression of protein intake (Fig. 3A).

In marked contrast to nitrogen, all caterpillars tended to accumulate more lipid as the P:C ratio decreased, with an exception demonstrated for HV caterpillars on extreme carbohydrate-rich diet (7:35 P:C). HV larvae over-consumed carbohydrate on low-protein diets to a lesser degree than did HS and SV larvae (Fig. 3A), and as a result will have suffered depletion of energy reserves for maintenance during the prolonged stadium duration on low-protein diets, seen most notably on the 7:35 P:C diet (Fig. 4A).

The generalist HV larvae converted ingested nutrients (both protein and carbohydrate) to body growth with lower efficiency compared to the specialist HS and hybrid SV caterpillars. It is possible that the fast growing HV used more of their ingested nutrients to fuel rapid development rate rather than allocating them to body tissue.

Conclusions and prospectus

In the present study, we have provided evidence for evolved differences in various aspects of nutrient regulation related to diet breadth by comparing two closely related caterpillar species. Such differences are explicable in terms of the different life-histories, feeding ecologies and presumed levels of nutritional heterogeneity in the environment of the two insects, leading us to highlight the importance of ecological factors shaping the mechanisms associated with nutritional homeostasis in insect herbivores. By describing the nutritional responses of a hybrid between the two species, we have demonstrated unprecedented evidence for the genetic foundations of such responses. Our results also indicate that certain regulatory traits may be controlled independently from others, while some traits seem tightly coupled. The nature of genetic coupling between nutritional regulatory mechanisms within individuals and the extent to which there is genetic variation in such responses within and between populations of a species remain to be elucidated and offer a rich vein for future research.

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