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ORIGINAL ARTICLE

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Effects of fertilization and elevated CO_2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L.

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Abstract The effects of larval diet on the nutritional preferences of butterflies has rarely been examined. This study investigates whether alterations in the larval diet result in changes in adult preferences for nectar amino acids. Larvae of *Coenonympha pamphilus* were raised on fertilized or unfertilized *Festuca rubra*, grown under ambient (350 ppm) or elevated (750 ppm) atmospheric CO_2 environments. Fertilization led to marked increases in leaf nitrogen concentration. In plants grown under elevated CO_2 conditions, leaf water and nitrogen concentrations were significantly lower, and the C/N-ratio increased significantly. Fertilization of the host plant shortened the development time of *C. pamphilus* larvae, and pupal weight increased. In contrast, larvae of *C. pamphilus* developed significantly slower on *F. rubra* grown under elevated CO_2, but adult emergence weight was not affected by CO_2 treatment of the plant. *C. pamphilus* females showed a clear preference for nectar mimics containing amino acids, whereas males, regardless of treatment, either preferred the nectar mimic void of amino acids or showed no preference for the different solutions. Female butterflies raised on fertilized plants showed a significant decline in their preference for nectar mimics containing amino acids. A slight, but not significant, trend towards increased nectar amino acid preference was found in females raised on plants grown under elevated CO_2. We clearly demonstrate that alterations in larval host quality led to changes in butterfly nectar preferences. The ability of the butterfly to either rely less on nectar uptake or compensate for poor larval conditions represents a trade-off between larval and adult butterfly feeding.

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Introduction

Shifts in foliar chemistry due to fertilization and rising atmospheric CO_2 can affect feeding and growth rates, attained adult weight, and perhaps also realized fecundity in butterflies (Myers 1985; Lincoln et al. 1986; Baylis and Pierce 1991; Fischer and Fiedler 2000, Goverde et al. 2002).

Nitrogen concentration in larval food plants is a key factor for butterfly fitness (Myers and Post 1981; Slansky and Rodriguez 1987; Bink and Siepel 1996). The quality of the larval host plant is a major determinant of stored reserves, and the uptake and storage of nitrogenous compounds is an important factor determining fecundity (Boggs 1981, 1997b; Singer 1984; Karlsson 1994; Braby and Jones 1995). Herbivores have developed various ways to optimize their nitrogen uptake (Simpson and Neff 1983). Some larvae feed on the most nitrogen-rich plant part, moving to better positions or plants as the season progresses (McNiel and Southwood 1978; Pullin 1987). Furthermore, larvae may optimize their uptake by increasing their feeding rate and/or development time (Scriber and Slansky 1990; Simpson and Simpson 1990; Lincoln et al. 1993).

Fertilized plants clearly have a higher nitrogen concentration than unfertilized plants, so that larvae feeding on these plants are able to grow faster and/or accumulate more nitrogen for storage. These higher nitrogen levels have been found to increase butterfly survival (Myers and Post 1981; Myers 1985). Fertilized plants were more readily chosen by ovipositing *Pieris rapae* females and the larvae grew faster and attained heavier weights on fertilized plants (Myers 1985). *Lasiommata megera* pupal weight decreased and diapause induction was altered with decreasing leaf nitrogen concentration (Bink and Siepel 1996). *Jalmenus evagoras* females preferred to lay egg batches on fertilized plants;

larvae feeding on these plants attracted larger guard ants and consequently had a higher survival rate (Baylis and Pierce 1991). But in some cases increased leaf nitrogen may not benefit butterflies (Fischer and Fiedler 2000).

Elevated CO_2 clearly alters the quality of larval food plants by causing decreases in foliar nitrogen, increases in carbohydrates and alterations in various secondary metabolites (Fajer 1989; Cortufo et al. 1998; Koricheva et al. 1998; Penuelas and Estiarte 1998). These changes in foliar chemistry can result in higher consumption rates, reduced growth rates, increased mortality, reduced pupal mass and prolonged larval development times (e.g. Lincoln et al. 1993; Watt et al. 1995; Bezemer and Jones 1998; Coviella and Trumble 2000).

Despite variation in larval development time, butterfly weight, egg size and egg number between maternal lineages (Van-Dyck et al 1998, Goverde et al. 1999; Garcia 2000; Carpenter and Bloem 2002; Mevi-Schütz personal observation), the details of maternal origin of butterflies used in experiments are rarely given. Possible differences in maternal lineages with regard to nectar preferences could affect plant-pollinator interactions and lead to evolutionary changes.

Baker and Baker (1977, 1983, 1986) were able to show that nectar composition, nectar sugars and nectar amino acids correlated with pollinator type, and that pollinators with limited adult nitrogen resources selected flowers with high nectar amino acid concentrations. The selectivity and preference of female butterflies towards nectar mimics containing amino acids has been shown for several species (Alm et al. 1990; Erhardt and Rusterholz 1998; Rühle 2000; J. Mevi-Schütz per observation). But there are also butterfly species that react indifferently toward nectar mimics containing amino acids versus corresponding plain sugar solutions (Erhardt 1991, 1992; Romeis and Wackers 2000). Deterioration of larval food conditions may cause a shift toward more reliance on adult nitrogen uptake.

Resources used in reproduction may come from larval reserves, adult feeding, nuptial gifts or a combination of these sources (Boggs 1981, 1997b). Resource allocation of stored larval reserves versus adult uptake may result in differences in reproductive output and longevity (Boggs and Ross 1993; Boggs 1997a). Trade-offs in nutritional uptake between the larval and adult stage become apparent when larval host plant quality is altered. Grasses are the primary larval food source for Satyrid larvae, providing comparatively low nitrogen levels. Consequently, resource accumulation prolongs the developmental time of these larvae. Increasing leaf nitrogen concentrations by fertilization or decreasing nitrogen concentrations by elevating CO_2 levels in *Festuca rubra*, the primary host of *Coenonympha pamphilus*, may affect growth rate, adult weight and perhaps nectar amino acid preference in adults.

The objective of this study was to determine if and how alterations in the quality of the larval host plant, *F. rubra*, affect different developmental and adult traits of the butterfly, *C. pamphilus*. We predict that: (1) fertilization of the larval food plant will result in heavier individuals and cause a decrease in the butterflies preference for nectar amino acids, (2) increased atmospheric CO_2 will alter the larval food plant, resulting in smaller individuals and the butterflies preference for nectar amino acid will increase, (3) maternal lineage influences the response of the butterflies toward increases in atmospheric CO_2, and can affect nectar amino acid preference.

Methods

Host-plant treatments

F. rubra grass was grown from seeds (450 seeds/pot) in plastic pots (750 ml) containing soil from calcareous, unfertilized grassland in CO_2 chambers in a greenhouse at the University of Basel, Switzerland. Pots were randomly assigned to either ambient (350 ppm) or elevated (700 ppm) CO_2 growth chambers with day/night temperatures of 24/16°C. Both chambers received ambient sunlight as well as supplementary light during cloudy periods from 1,000 W broad spectrum mercury vapour lamps (Powerstar, Osram). The light period was set from 6 a.m. to 8 p.m. Plants were watered with 50 ml every other day. Because only one growth chamber per treatment was used, pot position was re-randomized within each chamber every other day and plants were transferred from one chamber to the other, reversing the CO_2 treatment between chambers every week throughout the experiment, to limit chamber effects. With this temporal replication, we tried to minimize pseudoreplication at the CO_2 level, however, the statistical analysis must be interpreted with caution. The grass was grown for 5 weeks before larvae were introduced.

As part of our ongoing research (Goverde et al. 2002), half of the pots in 1998 were fertilized once a week (50 ml Algoflash, Laboratoire Algochimie Z.I. Nord, Chateau-Renault, France: N:P:K=1:1:1) so that four treatments, each with eight pots, were obtained (ambient unfertilized, ambient fertilized, elevated unfertilized, elevated fertilized).

In order to concentrate on the CO_2 effect, fertilization was excluded in the 1999 trial, thereby increasing the number of tested individuals in the two remaining treatments (ambient, elevated).

Before placing freshly emerged larvae onto plants, leaf samples were taken (≈10 blades/pot), and the following parameters were analysed: leaf water concentration was calculated as the percentage between fresh and dry (80°C for 48 h) leaf mass. Dried foliar tissue was then ground to powder. Leaf nitrogen (N) and carbon (C) concentrations were determined using a CHN analyser (LECO Instruments, Model 932, St. Joseph, Mich.)

Study organism

C. pamphilus (Linnaeus 1756), Lepidoptera, Satyridinae, is a common butterfly found primarily on unfertilized pastures or meadows throughout Europe (). The species is polyvoltine and overwinters as half-grown larva (Ebert and Rennwald 1991). The primary larval host plant in our study area is *F. rubra* but larvae feed on a variety of grasses (Ebert and Rennwald 1991). Adults actively forage for nectar in a variety of plants, and are known to visit *Ranunculus bulbosus, Veronica teucrium, Leucanthemum vulgare* and *Centaurea jacea* (H.P. Rusterholz pers com). *C. pamphilus* females were collected at three different field sites in the Swiss Jura mountains south of Basel in 1998 and at three additional sites on the Dinkelberg, Germany in 1999. The butterflies were placed in cages (40 cm×20 cm×20 cm) containing pots of *F. rubra* for oviposition, and fed a balanced 20% sugar solution (sucrose:fructose:glucose=1:1:1). The eggs were placed on moist filter paper in petri dishes covered with nylon mesh.

Larval conditions in 1998

Offspring from 13 females were pooled and groups of five freshly emerged larvae were randomly assigned to either fertilized or unfertilized treatments and placed on the *F. rubra* plants in growth chambers with ambient and elevated levels of CO_2. Eight replicates per treatment were established giving a total of 32 replicates each with five larvae. The pupae were collected, weighed and placed in numbered compartments of a plastic box set over water to avoid desiccation. Upon emergence the butterflies were sexed. A total of 60 butterflies were obtained for amino acid preference testing.

Larval conditions in 1999

The offspring from six female butterflies (maternal lineage) collected from different field sites were at all times (from egg to butterfly) kept separately to determine whether maternal lineage influences emergence weight or nectar amino acid preference. Emerged larvae were weighed individually. Groups of four larvae (corresponding to the maternal lineage) were placed on six *F. rubra* plants, so that each maternal line was represented by six pots. These pots (n=36) were then randomly assigned to either an ambient or elevated CO_2 treatment (18 pots and 72 larvae/treatment). The *F. rubra* biomass production in the individual pots was adequate for larval development. The pupae were collected, placed into numbered compartments in a plastic box and set over water to avoid desiccation. The duration from pupation until butterfly emergence was recorded. Upon emergence, the butterflies were sexed, weighed and transferred to separate flight cages (50 cm×50 cm×50 cm) according to maternal lineage and sex. A total of 119 individuals were obtained for testing in 1999.

Nectar preference testing

Two test solutions mimicking *Lantana camara* nectar were used to determine amino acid preferences. This nectar composition was chosen because this flower is often visited by butterflies (though it does not occur naturally in *C. pamphilus* habitats), has a high amino acid concentration and has been used in several previous studies on amino acid preference (Alm et al. 1990; Erhardt and Rusterholz 1998). Both nectar mimics contained 0.547 M sucrose, 0.282 M glucose, and 0.316 M fructose per liter of distilled water. The nectar mimic with amino acids also contained the nonessential amino acids: 0.718 mM alanine, 0.421 mM asparagine, 0.326 mM glutamic acid, 0.931 mM glutamine, 2.371 mM glycine, 2.23 mM proline, 1.37 mM serine, 0.672 mM threonine and the essential amino acids: 0.201 mM arginine, 0.221 mM tyrosine and 0.137 mM valine. The test solutions were made with sodium-free substances.

Preferences were tested in a two-way test analogous to the method used by Erhardt and Rusterholz (1998). Prior to testing the butterflies for their nectar preferences, they were brought to the same nutritional level by feeding them to satiation with a balanced sugar solution (sucrose:glucose:fructose=1:1:1, 25% w/w) until they withdrew the proboscis. In order to test whether the preference tests were affected by this meal, the amounts of balanced sugar solution ingested were measured. Regression analysis showed that the amount prefed did not influence the number of tests completed in 1998 (r^2=0.052, P=0.085, n=58) or in 1999 (r^2=0.009, P=0.318, n=120), and did not influence the preference outcome either in 1998 (r^2=0.109, P=0.074, n=58) or in 1999 (r^2=0.001, P=0.829, n=120).

The test solution was offered to the butterflies in 0.5 µl droplets extruded from the tip of a Hamilton syringe (10 µl, gas tight, no. 701, 75 N). The butterflies were held and the proboscis was carefully unrolled into the droplet with a fine pin. The two test solutions (nectar mimics with or without amino acids) were each offered once in a trial and the order of presentation randomly chosen. The butterfly either consumed the droplet within 10 s or the trial was a reject. The preference test consisted of five trials. If a butterfly accepted both mimics in all five trials, it was assumed to be too hungry and was offered the balanced sugar solution again, after which the test was repeated. The degree of preference was based on the proportional number of drops accepted (number of nectar droplets with amino acids/total number of droplets). Values above 0.5 show a preference for nectar with amino acids, values below 0.5 show a preference for nectar without amino acids. Butterflies which rejected both solutions continuously or ingested less than two droplets were excluded from the analysis.

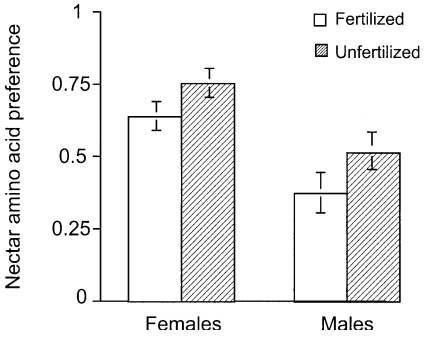

Fig. 1 1998: nectar amino acid preference response of adult *Coenonympha pamphilus* from larvae raised on fertilized (females: n=16, males: n=15) or unfertilized (females: n=15, males: n=14) *Festuca rubra* [pooled data of ambient (350 ppm) and elevated (700 ppm) CO_2 conditions]. *Y-axis* refers to the degree of preference, values above 0.5 show a clear preference for nectar with amino acids. Degree of preference described in methods (mean±SE)

Data analysis

Plant chemistry was analysed for differences between CO_2 and fertilization treatments in 1998 using a fully factorial ANOVA with CO_2 and fertilizer as fixed factors. In 1999, plant chemistry between the CO_2 treatments was analysed using the Student's t-test. Plant chemistry data which were not normally distributed were log transformed.

Differences in larval development time between the larval food treatments (ambient vs elevated CO_2) in 1999 were compared using ANOVA with CO_2 treatment, maternal lineage and sex as factors. Differences in pupal weight between treatments (fertilized vs unfertilized) in 1998 were log-transformed and tested by factorial ANOVA. Mann-Whitney rank sums were used to test differences in pupal duration in 1999 because the data were not normally distributed. Adult emergence weight differences between the larval food treatments (ambient vs elevated CO_2) in 1999 were compared using an ANOVA with CO_2 treatment, maternal lineage and sex as factors.

The effects of fertilization, CO_2, maternal lineage, emergence weight and sex on amino acid preference were tested with a fully factorial ANOVA. Stepwise reduction of the full models, removing the highest order interactions and always the least significant effect first were used when comparing nectar amino acid preference between the different larval food groups in both 1998 and 1999, employing type III sums of squares. The feeding responses, as shown in Fig. 1 and 2, for each treatment and sex were derived from the degree of nectar amino acid preference. The analyses were carried out using JMP Version 3.1 (SAS 1995).

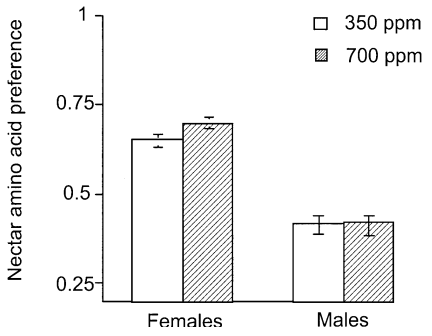

Fig. 2 1999: nectar amino acid preference response of adult *Coenonympha pamphilus* from larvae raised on *Festuca rubra* grown under ambient (350 ppm, females: n=24, males: n=34) and elevated (700 ppm, females: n=26, males: n=35) CO_2 conditions. *Y-axis* refers to the degree of preference, values above 0.5 show a clear preference for nectar with amino acids. Degree of preference described in methods (mean±SE)

Results

Plant chemistry

Leaf water and nitrogen concentrations were significantly lower in *F. rubra* grown under elevated CO_2 conditions in 1998 and 1999, and C/N ratios showed a significant increase under elevated CO_2 in both years (Table 1). Fertilization led to increased nitrogen concentrations in both ambient and elevated CO_2 conditions. Fertilized plants raised under ambient conditions had the highest nitrogen concentration, consequently the most marked differences in the C/N ratio occurred between ambient and elevated fertilized plants. Furthermore, a significant CO_2×fertilization interaction was apparent in 1998.

Development of *C. pamphilus*

In 1998

There was a significant difference in pupal weight ($F_{1,56}$=11.83, P=0.01, n=58), with the heaviest pupae obtained on the fertilized *F. rubra* plants (76.21±1.66 mg) compared to pupae obtained from unfertilized plants (71.48±2.72 mg). Pupal weight differed slightly ($F_{1,56}$= 5.63, P<0.05) between elevated CO_2 (64.1±1.58 mg) and ambient (71.5±2.8 mg) conditions.

In 1999

Larval development time (eclosion until pupation) was longer ($F_{1,100}$=32.04, P<0.0001, n=120) under elevated CO_2 (22.5±0.3 days) than under ambient conditions (20.8±0.3 days). Maternal lineage also affected the duration of larval development ($F_{5,100}$=3.22, P=0.0096). Furthermore, males developed 2.7 days faster than females ($F_{1,100}$=65.87, P<0.0001).

The pupal development time was unaffected by the CO_2 treatment (U=0.02, df=1, P=0.89).

Adult emergence weight was not affected by CO_2 treatment ($F_{1,95}$=1.16, P=0.28, n=120) or by maternal lineage ($F_{5,95}$=1.89, P=0.103). There was, however, a significant difference ($F_{1,95}$=92.51, P<0.0001) between females (51.18±1.27 mg) and males (31.91±1.08 mg).

Amino acid preference

In 1998

Female butterflies raised on *F. rubra* grown under all four combinations of CO_2 and fertilizer showed a significant preference (t=4.916, df=1, P=0.0001) for the nectar mimic containing amino acids (Fig. 1). In contrast, males raised under the above conditions showed no preference

Table 1 Effects of ambient (350 ppm) and elevated (750 ppm) carbon dioxide levels and fertilization on foliar chemistry of *Festuca rubra*, the larval host plant utilized by *Coenonympha pamphilus* larvae. Mmeans±SE, F-values 1998, t-values 1999

	Leaf water (%)		Leaf nitrogen (%)		C/N	
	350 ppm	700 ppm	350 ppm	700 ppm	350 ppm	700 ppm
1998 (n=32)						
Unfertilized	64.6±0.7	59.8±0.6	0.99±0.04	0.72±0.02	44.17±1.93	59.64±2.03
Fertilized	62.4±2.3	61.8±0.4	1.58±0.09	0.89±0.05	28.62±1.56	50.54±2.48
	df	*F*	*F*		*F*	
CO_2	1	4.79*	87.90***		85.01***	
Fertilizer	1	0.01	49.49***		36.93***	
CO_2×fertilizer	1	2.97	8.14**		2.53	
Error	28					
1999 (n=36)						
Unfertilized	71.9±1.36	60.8±1.40	1.07±0.05	0.71±0.05	41.50±1.70	66.50±1.80
	df	*t*	*t*		*t*	
CO_2	1	5.67***	5.14***		10.02***	

*$P\leq0.05$, **$P\leq0.01$, ***P<0.001

Table 2 1998: Effects of sex of the tested butterfly, fertilization of the larval host plant, and CO_2 treatment of the larval host plant on nectar amino acid preference of *Coenonympha pamphilus* for *Lantana camara* nectar mimics. (*n*=58)

	df	*SS*	*F*	*P*
Sex	1	0.9447	17.369	0.0001
Fertilization	1	0.2296	4.221	0.045
CO_2	1	0.0117	0.215	0.645
Error	54	2.9369		

Table 3 1999: Effects of sex of the tested butterfly, CO_2, treatment of the larval host plant and emergence weight of the tested butterfly on nectar amino acid preference of *Coenonympha pamphilus* for *Lantana camara* nectar mimcs. (*n*=120)

	df	*SS*	*F*	*P*
Sex	1	0.8976	47.89	0.0001
CO_2	1	0.0139	0.744	0.390
Sex*CO_2	1	0.1125	0.600	0.440
Weight	1	0.0028	0.149	0.700
Error	113	2.1181		

(t=0.46, df=1, P=0.6475) for the nectar mimic with amino acids (Fig. 1). A sex effect was apparent with males and females clearly accepting the nectar mimics differently (Table 2).

Fertilization of the larval plants caused a significant decline in the preference for nectar mimics with amino acids (Fig. 1, Table 2). The interaction sex by fertilization was not significant. Although there was a slight increase in the amino acid preference of females from the elevated CO_2 treatment, a statistically significant CO_2 effect was not found (Table 2).

In 1999

Female butterflies raised under both ambient and elevated CO_2 conditions showed a clear preference for the nectar mimics with amino acids over nectar mimic without amino acids (t=8.718, df=1, P=0.0001). Although amino acid preference by the elevated CO_2 group is numerically slightly higher (Fig. 2), as was the case in 1998, this effect was again not significant (Table 3).

Male butterflies raised under both ambient and elevated CO_2 larval conditions showed a significant preference toward the nectar mimic void of amino acids (t=4.494, df=1, P=0.0001, Fig. 2).

There was no maternal effect with regard to nectar amino acid preference. The offspring of the six females collected from different locations for oviposition in 1999 showed the same preference regardless of the collecting site (female offspring: $F_{5,31}$=0.21, P=0.956 and male offspring: $F_{5,51}$=0.806, P=0.551).

Discussion

Amino acid preference in butterflies

Female *C. pamphilus* showed a clear preference for the nectar mimic with amino acids over the nectar mimic without amino acids. Male butterflies accepted both mimics equally or preferred the mimic without amino acids. These results suggest a differential preference for adult resources between females and males. Different nutritional requirements for egg production and spermatophore synthesis could cause sex specific flower and nectar preferences (Wiklund and Ahrberg 1978; Rusterholz and Erhardt 2000). Female butterflies of *P. rapae*, *Inachis io*, *Aglais urticae*, *Lysandra bellargus*, *Polyommatus icarus*, *Maniola jurtina* and *Melanargia galathea* preferred nectar mimics with amino acids (Alm et al. 1990; Erhardt and Rusterholz 1998; Rühle 2000; J. Mevi-Schütz unpublished data), whereas males of these species showed no preference. But female preference is not universal (Erhardt 1991, 1992; Romeis and Wäckers 2000; Rühle 2000). Preference for nectar amino acids could be either sex or species-specific or may be influenced by larval conditions. Poor larval food quality increased *Araschnia levana* female butterfly preference for nectar amino acids (J. Mevi-Schütz and A. Erhardt unpublished data). Differences in male and female nectar source plants has been observed (Wiklund and Ahrberg 1978). The nitrogen stressed, overwintering, spring generation females of *Lysandra bellargus* preferred flowers with high concentrations of amino acids, whereas fall generation females showed no such preference (Rusterholz and Erhardt 2000). Furthermore, flowers visited by male or female *L. bellargus* during the spring differed in their amino acid composition.

Variations in larval conditions in earlier studies may have led to the discrepancy between the effect of nectar amino acids on egg weight. Murphy et al. (1983) used postdiapause larvae and reared them on two host plants in the greenhouse, whereas Moore and Singer (1987) collected mated females from the field. However, at present it remains unclear whether nectar amino acids influence the longevity and fecundity of butterflies (Hill 1989; Hill and Pierce 1989). Recent findings have shown that hawk moths use essential amino acids from larval reserves and synthesize non-essential amino acids from sugar for egg production (O'Brien et al. 2002).

Effects of host plant fertilization on amino acid preference

Fertilization of the larval food plant *F. rubra* increased foliar nitrogen, reduced larval development time, increased pupal weight and caused a significant decrease in the preference of female *C. pamphilus* butterflies for nectar mimics containing amino acids. The uptake of foliar nitrogen by larvae, due to fertilization, is known to increase larval growth, shorten development time and

improve food utilization (Mattson 1980; Taylor 1984; Myers 1985; Hunter and McNiel 1997; Grundel et al. 1998). Furthermore, pupal or adult weight is often, but not exclusively, a good indicator of realized fecundity (Danathanarayana 1975; Jones et al. 1982; Gilbert 1984; Nylin and Janz 1999; Fischer and Fiedler 2000). Larvae can store more reserves and emerge with high potential fecundity when host quality is high (Singer 1984).

Effects of elevated CO_2 on amino acid preference

The butterflies raised under elevated CO_2 conditions compensated for lower foliar nitrogen by increasing development time, emerging with the same weight as a butterfly feeding on ambiently grown foliage. Yet the slight increase in nectar amino acid preference by females raised under elevated CO_2 indicates a possible underlying deficiency. Higher consumption rates, increased development time and decreased growth rates have been shown for other lepidopteran larvae raised on elevated CO_2 foliage (Lincoln et al. 1986; Fajer 1989; Fajer et al. 1991; Lindroth et al. 1995). Higher carbohydrate levels in plants grown under elevated CO_2 produced larger individuals and/or increased lipid concentration, resulting in more fecund adults (Buse et al. 1998; Goverde et al. 1999). On the other hand, alterations in nectar quantity and quality due to elevated CO_2 (Osborne et al. 1997; Rusterholz and Erhardt 1998; Lake and Hughes 1999) could affect butterfly fitness in the future. Virtually nothing is known about how lifelong exposure to elevated CO_2 environments, including larval food and nectar composition, affects butterfly fitness.

Compensatory interaction between larval and adult uptake

Resources allocated by female butterflies to egg production are derived from larval reserves, adult diet and nuptial gifts (Boggs 1990). Reduction of nitrogen uptake may affect reproductive output.

A compensatory interaction between larval and adult uptake, especially under varying nutritional conditions, is likely. Butterflies coming from fertilized host plants may rely less on adult uptake, whereas those individuals subjected to inadequate larval nitrogen may rely more on adult resources. It is not clear whether increased or selective adult feeding can compensate for nutritional deficiencies carried over from inadequate larval uptake. Individuals with poor larval conditions have been shown to compensate by more extensive or more efficient adult foraging (May 1992). Larval food quality has been shown to affect adult weight and fecundity (Blais 1953; Hough and Pimentel 1978; Wiklund and Karlsson 1984; Pullin 1987; Braby and Jones 1995; Kelly and Debinski 1999). Yet the relationship between larval food quality, attained adult weight and realized fecundity has rarely been tested within one experiment. The moth *Epiphyas postvittana* raised on different larval food hosts, emerging with similar weights, showed significant differences in fecundity, suggesting that larval food quality overrides adult weight in determining fecundity (Danathanarayana 1975).

Conclusion

Shifts in nectar amino acid preference by butterflies due to fertilization or decreases in larval nitrogen uptake caused by elevated CO_2 demonstrate a compensatory shift between the larval and adult stage and a trade-off between larval and adult uptake of essential nutrients. Changes in nectar preference may lead to changes in flower preference and ultimately affect plant-pollinator dynamics. If the quality of larval food is permanently affected by increasing CO_2 levels, we may experience an increased reliance on alternative nitrogen sources by butterflies, such as nuptial gifts, pollen, rotting fruit, sap, and nectar. If, in addition to larval nutrient deficiencies, nectar amino acid production is reduced under elevated CO_2, as is the case in some plants of calcareous grasslands (Rusterholz and Erhardt 1998), negative effects of elevated CO_2 on butterfly fecundity may be exacerbated. Since larval food conditions influenced the outcome of amino acid preference in both the present study with *C. pamphilus* and in *A. levana* (J. Mevi-Schütz and A. Erhardt unpublished data), we assume that nutritional differences during the larval stage influence the effect of adult diet on fecundity. Further research under varying degrees of larval nitrogen uptake and adult amino acid availability may improve our understanding of the importance of nectar amino acids for butterfly fecundity.

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