Butterfly fitness under changing food qualities

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General introduction

Animals depend indispensably, directly or indirectly, on green plants, the most voluminous compartment of living matter and primary source of energy-rich compounds for animal life (Schoonhoven et al. 2006). Most animal species are insects (May 1988), and the biomass of insects is enormous (Pimentel and Andow 1984), despite their small body size. Plants and insects are united by intricate relationships (Schoonhoven et al. 2006). First fossilized records of insect-plant interactions date from approximately 400 million years ago (Labandeira 1998), but it is not clear whether plants enhanced the evolution of the insects (Strong et al. 1984), or the opposite interaction was also important (Labandeira and Sepkoski 1993), resulting in a co-evolutionary process (Ehrlich and Raven 1964). However, specialization of herbivores on plants occurred over millions of years (Price 1997), and specialization on host plants is the rule rather than the exception (Bernays and Graham 1988), since nutrients gained from feeding are required in optimal levels to maximize animal fitness (Simpson and Raubenheimer 2007).

Quality and/or quantity of larval host plants in nature can change due to seasonal variation (Rodrigues and Moreira 2004), fertilization (Goverde and Erhardt 2003), air pollution (Huges and Voland 1988), elevated atmospheric CO_2 (Bazin et al. 2002) or herbivory (Awmack and Leather 2002), and plants have developed numerous defence mechanisms to deter herbivores from feeding (Schoonhoven et al. 2006). However, in butterflies larvae of almost all species are herbivorous, and the amount of nitrogen gained from variable host plants influences their development (Myers 1985; Mevi-Schütz et al. 2003) and accordingly their reproduction (Oberhauser 1988; Bissoondath and Wiklund 1996; Boggs 1997a; O'Brien et al. 2002).

Parental effects, such as provisioning to eggs, oviposition behaviour of mothers and epigenetic inheritance (Mousseau and Fox 1998a; Poulin and Thomas 2008), prepare larvae to adverse larval host plant conditions. For example, if host plant quality is indicative of future conditions, then it would improve progeny's fitness if the offspring phenotype is adjusted to these host plant conditions (Fox et al. 1995; Spitzer 2004). This phenomenon, based on acquired parental effects, is called 'transgenerational acclimatization' and could have profound implications for ecological and evolutional processes (Fox et al. 1995; Spitzer 2004).

In butterflies, maternal but also paternal effects could potentially improve progeny's fitness on variable and adverse host plant qualities, since butterflies have a mating system in which males transfer nutrients to females at mating, often referred to as 'nuptial gifts' (Thornhill 1976). For instance, female butterflies can use male-derived nutrients for egg production (Boggs and Gilbert 1979), and nuptial gifts can significantly increase female fecundity (Rutowski et al. 1987; Karlsson 1998).

Butterflies are holometabolous insects and have the ability to compensate for a nitrogen-poor diet both as herbivorous larvae as well as nectar-feeding adults. For instance, adult butterflies feed on pollen, rotting fruits, mud puddles, carrion or dung. But floral nectar is by far the most common and widespread adult butterfly food source (Gilbert and Singer 1975). Floral nectar provides sugars, water and amino acids for pollinators (Ziegler 1956; Lüttge 1961; Baker and Baker 1973, 1986a), and flowers adapted to pollination by butterflies contain higher levels of amino acids than flowers that are pollinated, for instance, by bees (Baker and Baker 1986b). Furthermore, females of some butterfly species select for amino acid-rich nectar (Alm et al. 1990; Mevi-Schütz and Erhardt 2003; Mevi-Schütz et al. 2003; but see Erhardt 1991, 1992), and

nectar amino acids can be used to increase fecundity and to compensate for nitrogen deficiencies acquired during the larval phase (Mevi-Schütz and Erhardt 2005). Thus, a compensatory interaction between larval and adult nitrogen uptake is likely when larval resources are limited.

There are various potential strategies that butterflies could use to deal with the varying quality and quantity of food sources in order to maximize their fitness and reproduction (Fig. 1).

The present thesis investigates how different food qualities over the whole life cycle and across generations affect butterfly development and reproduction, and addresses the following main questions: (1) What effects have varying nitrogen concentration and different levels of host plant defence during larval phase on larval and adult performance in butterflies? (2) Can adult feeding in male and female butterflies compensate for deficiencies acquired during the larval stage, or even increase butterfly fitness and reproductive success? (3) Does parental food quality affect the next generation by parental effects (increased provisioning to offspring, maternal oviposition choice or transgenerational acclimatization)?

The **first chapter** describes effects of different host plant qualities on larval performance and adult feeding behavior in butterflies. Nitrogen is a key factor for development and fitness in insects (Bink and Siepel 1996; Mevi-Schütz and Erhardt 2005; Schoonhoven et al. 2006), and herbivores consume on average 10–20% of the annual net primary production in terrestrial ecosystems to acquire the needed amount of nitrogen (Cyr and Pace 1993). As a consequence, plants have developed different strategies to deter herbivores from feeding, and insects in turn have developed corresponding strategies to deal with suboptimal food qualities (Schoonhoven et al. 2006).

Effects of high- and low nitrogen concentration in combination with different levels of silica in host plants on larval development of the grass-feeding butterfly *Coenonympha pamphilus* were investigated. Silica is the main anti-herbivore deterrent in grasses (Vicari and Bazely 1993; Massey et al. 2007). Furthermore, effects of different larval host qualities on relative consumption of amino acid nectar in male and female butterflies were tested to investigate the link between larval and adult nitrogen acquisition.

The **second chapter** gives an example about the relationship between nitrogen acquired during larval and adult feeding and its effect on female butterfly reproduction and provisioning to offspring. Although nitrogen used in egg production is mainly derived from stored larval reserves (Boggs 1981, 1997a; O'Brien et al. 2002), adult female diet is also a potential nitrogen source (Boggs 1997a; O'Brien et al. 2002). For example, *Araschnia levana* and *Bicyclus anynana* females can use amino acids from the adult diet to increase their reproduction (Mevi-Schütz and Erhardt 2005; Bauerfeind and Fischer 2009).

Effects of high- and low amino acid concentrations during larval and adult feeding on female butterfly reproduction and provisioning to offspring were tested using *C. pamphilus*, a butterfly species belonging to another subfamily than the previously investigated *A. levana* and *B. anynana*.

In the **third chapter**, corresponding to the second chapter, effects of high- and low nitrogen levels over the whole butterfly life cycle on realized male butterfly reproduction were investigated. In insects, males can transfer nutrients to females at mating, often referred to as 'nuptial gifts' (Thornhill 1976). Furthermore, radiotracer studies on several butterfly species demonstrated that amino acids acquired during male larval and adult feeding built into spermatophores can be used by females for egg production (Boggs and Gilbert 1979; Wiklund et al. 1993).

Figure 1

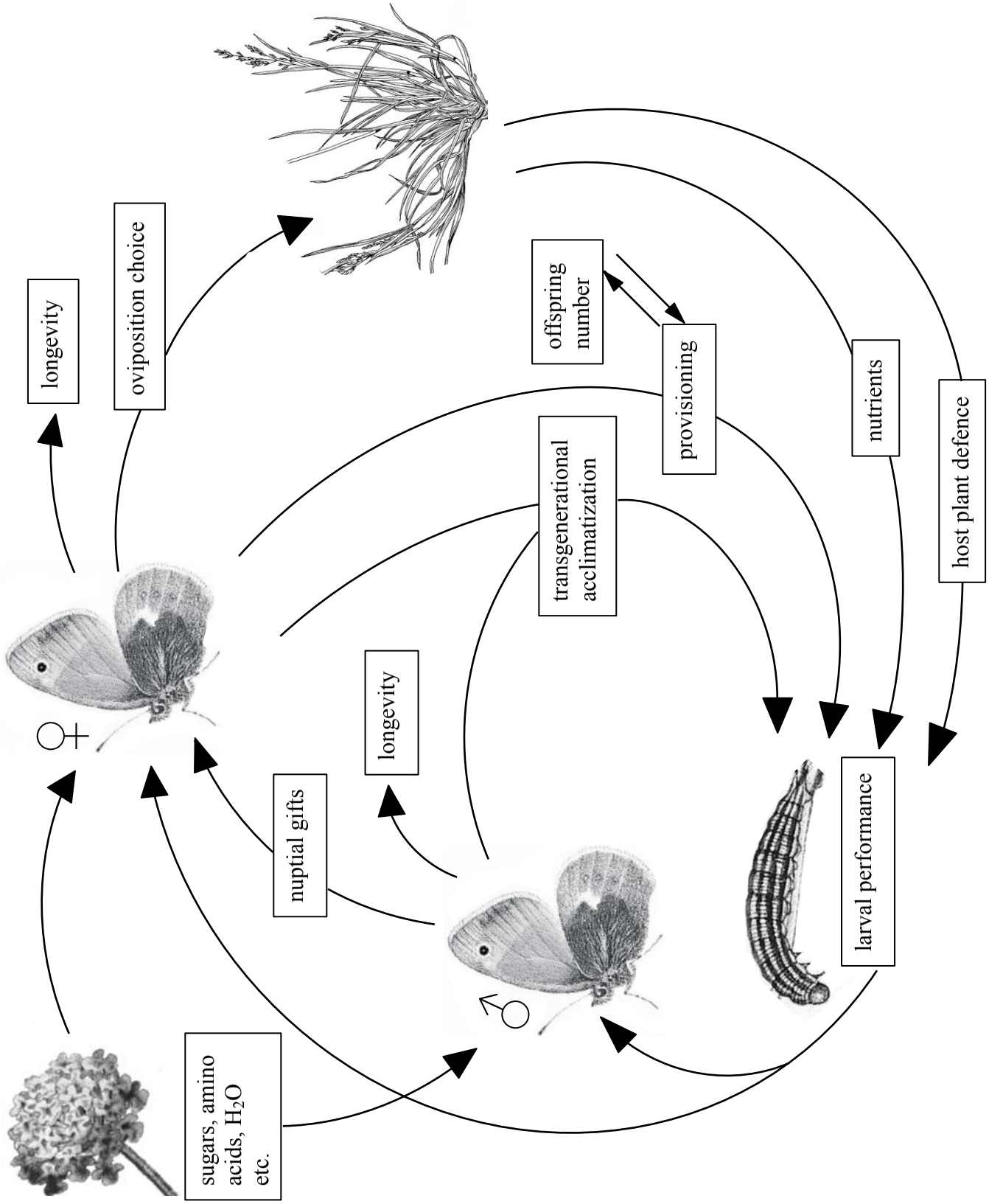


Fig. 1 Graphical illustration of effects of larval and adult food sources on resource allocation, performance and reproduction in butterflies

However, to date, effects of nectar amino acids on male reproduction are unknown.

Effects of high- and low amino acid concentrations during larval and adult feeding on male butterfly reproduction and provisioning to offspring were tested using *C. pamphilus* males. Effects of nitrogen on realized male reproduction were measured indirectly via nuptial gifts, by female performance. This method took into account the important role of female butterflies in passing male nutrients to offspring.

The **fourth chapter** pays attention to effects of nectar sugars on male butterfly fitness and reproduction and provisioning to offspring. Floral nectar contains mainly water and sugars (Baker and Baker 1986a) that are primarily used to cover energy requirements for general maintenance (Murphy et al. 1983) and flight expenditure (Willers et al. 1987). The remaining nutrients could enhance reproduction, since female butterflies can use sugars from the male spermatophore for egg production (Boggs 1997b). Although male butterflies contribute substantial amounts of nutrients to reproduction (Svärd 1985, Svärd and Wiklund 1989), effects of nectar sugars on male realized reproduction are unknown.

In an analogous way as in chapter three, effects of nectar sugars in the adult diet of *C. pamphilus* males on reproductive success and provisioning to offspring were measured indirectly via nuptial gifts, by female performance. Furthermore, relationships between investment into reproduction and male body maintenance were characterized.

The **fifth chapter** tests for a phenomenon called 'transgenerational acclimatization'. If the host plant quality encountered by the parental generation is indicative for future conditions, then it would be advantageous to produce offspring that is adjusted to these anticipated host plant conditions (Fox et al. 1995; Spitzer 2004). This phenomenon could prepare larvae of the F_1 generation to optimally utilize the resources by parental experience. Thus, transgenerational acclimatization could have profound implications for our understanding of evolutional and ecological processes (Fox et al. 1995; Spitzer 2004).
C. pamphilus butterflies were used to test if maternal and paternal larval host experience adjusted progeny performance on their respective diet.

The **sixth chapter** investigates the relationship between oviposition choice and transgenerational acclimatization. Chapter five has shown that parents can utilize their experience of the environment to adapt their offspring's phenotype to the same environmental conditions. Thus, offspring would then perform best under environmental conditions experienced by their parents due to transgenerational phenotypic plasticity. However, evidence that parents can subsequently ensure the appropriate environmental conditions in order that offspring benefit from transgenerational acclimatization has never been demonstrated.

Here, we combine transgenerational acclimatization with oviposition behaviour, for what we believe is the first time, by asking whether mothers can indeed align both the adaptation and allocation of their progeny to the environmental conditions that their own experience predicts those progeny to encounter.

Chapter 1

Host plant defence in the larval stage affects feeding behaviour in adult butterflies

Abstract

Nitrogen is a key nutrient for fitness in insects, but host plant defence can deter herbivores from acquiring it. Therefore, coping with host plant defence is a predominant issue for herbivores. Butterflies have the ability to compensate for a nitrogen-poor diet both as herbivorous larvae as well as nectar-feeding adults. We examined if silica (S), the main anti-herbivore defence in grasses (in both fertilized nitrogen-rich (F+) and nitrogen-poor (F-) *Festuca rubra* host plants), affects larval development and accordingly adult feeding behaviour in the small heath butterfly *Coenonympha pamphilus*. High silica levels in nitrogen-poor host plants (F-/S+) negatively affected larval performance, and as a consequence, female and even male butterflies preferred to consume amino acid-rich nectar. Our findings show for the first time that plant defence in larval host plants affects feeding behaviour in adult butterflies, and that even male butterflies, which have so far been thought to be indifferent to nectar amino acids, preferred to consume amino acid-rich nectar. Hence larval food quality can influence plant-pollinator dynamics.

Key words: amino acids, *Coenonympha pamphilus*, *Festuca rubra*, larval feeding, Lepidoptera, nectar, preference, Satyrinae, silica

Introduction

To acquire organic nitrogen is indispensable for normal insect growth and reproductive success (Schoonhoven et al. 2006). For example, in butterflies, larvae of almost all species are herbivorous, and the amount of nitrogen gained from host plants influences their development (Myers 1985; Mevi-Schütz et al. 2003) and accordingly their reproduction (Bissoondath and Wiklund 1996; Mevi-Schütz and Erhardt 2005; Cahenzli and Erhardt 2012a). Optimizing nitrogen acquisition is therefore crucial during the entire life cycle of butterflies and herbivorous insects in general. Thus, host plant quantity and quality are critical factors for the development of herbivore insects. However, plants have developed many strategies to limit biomass loss to herbivores, and food utilization of herbivores depends not only on the nutrient content in host plants, but also on structural and chemical plant defence (Schoonhoven et al. 2006). Nevertheless, food specialists are often adapted to chemical plant defences and may even benefit from secondary plant metabolites (Schoonhoven et al. 2006). Thus, physical defences may be more effective in deterring herbivores than chemical deterrents. For instance, physical defences such as silica are considered to be more important than chemical defences in deterring herbivory on grasses (Vicari and Bazely 1993; Massey et al. 2007), although grasses can also use chemical defences (Barton and Koricheva 2010). For example, grasses contain high silica levels in their leaves, about 10–20 times higher than typically found in dicotyledonous plants (Russel 1961), and grazing of herbivores induces even higher silica levels (Massey et al. 2007). In insects, high silica levels in grasses can cause increased mandible wear, can reduce the efficiency of converting ingested food to body mass and can decrease the amount of nitrogen absorbed from larval food, thus resulting in reduced growth rates and fitness (Massey et al. 2006; Massey and Hartley 2009). A high silica content in host plants could also

inhibit compensatory feeding in insects, especially when larval food quality or quantity is insufficient. Furthermore, silica and nitrogen may interact and influence herbivore performance and nitrogen acquisition on host plants (Reynolds et al. 2009). Thus, larvae of satyrid butterflies, which primarily feed on grasses, have to cope with trade-offs between nitrogen acquisition and silica avoidance.

Butterflies are holometabolous insects and as adults primarily feed on floral nectar, which can contain significant amounts of amino acids (Baker and Baker 1986). Thus, butterflies can compensate for nutritional deficiencies acquired during the larval phase by adult feeding (Mevi-Schütz and Erhardt 2005). In fact, female butterflies of several species prefer to consume amino acid-rich nectar (Alm et al. 1990; Erhardt and Rusterholz 1998; but see Erhardt 1991, 1992), and female butterfly fecundity can be increased by the availability of amino acids in the adult diet (Mevi-Schütz and Erhardt 2005; Bauerfeind and Fischer 2009; Cahenzli and Erhardt 2012a; but see Moore & Singer 1987; Hill 1989; Mevi-Schütz and Erhardt 2003a). In contrast, male butterflies showed no preference toward amino acid-rich nectar (Alm et al. 1990; Erhardt 1991, 1992; Erhardt and Rusterholz 1998; Mevi-Schütz et al. 2003; Mevi-Schütz and Erhardt 2003b). Different nutritional requirements for egg production and spermatophore synthesis could underlie sex specific flower and nectar preferences (Rusterholz and Erhardt 2000). However, a compensatory interaction between larval and adult nitrogen uptake is likely when larval resources are inadequate. So far, only one study has investigated influences of larval host plant defence on adult butterfly feeding behaviour, but has not been able to show an effect (Goverde et al. 2008). Nonetheless, larval host plant defence is an important plant trait for phytophagous insects, potentially also influencing nectar feeding in adult butterflies, thus ultimately affecting plant-pollinator interactions.

This study addresses the relationship between larval and adult feeding behaviour under the influence of different levels of nitrogen in larval host plants and host plant defence. We examined effects of increased silica levels (S+), different nitrogen concentrations (F+ versus F-) and the interaction of these two factors on larval development and relative consumption of amino acid nectar in *C. pamphilus* L. (Lepidoptera: Satyrinae) males and females. We predicted (1) that larvae reared on host plants with a low nitrogen level (F-/S- and F-/S+) would perform worse than larvae reared with a high nitrogen supply (F+/S- and F+/S+), (2) that a high silica level in host plants would reduce nitrogen uptake in larvae, and (3) that adverse larval food conditions would affect relative consumption of amino acid nectar in adult butterflies.

Materials and methods

Study species

C. pamphilus, the small heath, is found on various meadow types (Lepidopterologen-Arbeitsgruppe 1987), and *Festuca rubra* is the favoured larval host plant (Goverde and Erhardt 2003). Butterflies in this experiment originated from five *C. pamphilus* females caught on unfertilized meadows in the northern Jura mountains (Liesberg 47° 24′ N, 7° 25′ E and Nenzlingen 47° 26′ N, 7° 33′ E, Switzerland). Females were placed in cages (40 cm x 20 cm x 20 cm), and fed a balanced 20% (w/w) sugar solution (sucrose:fructose:glucose = 1:1:1). Eggs were collected and placed separately in Petri dishes in order to later trace back each butterfly to the ovipositing female (lineage).

Plant material

Festuca rubra L. (Poaceae) larval host plants were grown in 750 ml plastic pots filled with nutrient-poor soil (seeding compost, Compo Sana, Switzerland) in a

greenhouse with supplement sunlight (1000 W broad spectrum, light period from 06.00 to 20.00) and were randomly assigned to one of the four treatments: Fertilized grass with supplement silica (F+/S+), fertilized grass without supplement silica (F+/S-), unfertilized grass with supplement silica (F-/S+) and unfertilized grass without supplement silica (F-/S-). (F+/S+) and (F+/S-) pots were fertilized once a week with 2dl fertilizer (50 ml Algoflash/1.5 l water) (Algoflash, Laboratoire Algochemie Z. I. Nord, Chateau- Renault, France: N:P:K = 1:1:1). (F-/S+) and (F+/S+) host plants received supplement soluble silica in the form $NaSiO_3 9H_2O$ (150mg/L) every third day. (F-/S-) larval host plants received only deionized water. All pots were watered when necessary with deionized water. Prior to introducing the butterfly larvae, host plant quality was analysed from seven-week-old grass samples. Dry leafs (drying by 80° C for 48 hours) were ground for leaf nitrogen (N) analysis using a CHN analyser (LECO instruments, model 1932, St. Joseph, MI). Leaf water content was calculated as the difference between dry and fresh leaf mass. Foliar silica content was determined by fusing oven-dried leaf samples (100 mg) in sodium hydroxide using the autoclave-induced digestion (AID) (Elliot and Snyder 1991), and was analyzed with the colorimetric silicomolybdate technique (Allen 1989).

Larval treatment

Forty larvae from each of the five ovipositing females (N = 200) were randomly assigned to the four larval treatments and fed ad libitum, resulting in 50 larvae per treatment. The larvae were kept separately by maternal lineage and were reared individually in Petri dishes after two weeks. Pupae were collected and placed in individual plastic boxes until emergence. We recorded the developmental traits: larval hatching mass within 24 hours (mg), larval duration (number of days from when the larva hatched to pupation), pupal mass on the 5th day after pupation (mg) and forewing length (mm; lateral wingspan of the left forewing). Furthermore, mortality was calculated as the percentage of larvae of every maternal lineage in the four treatment groups that did not achieve adult stage.

Testing nectar amino acid consumption

Two test solutions mimicking *Lantana camara* nectar were used to determine relative consumption of amino acid nectar. This nectar composition has been used in several previous studies on amino acid preference testing in *C. pamphilus* (Mevi-Schütz et al. 2003) and other butterfly species (Alm et al. 1990; Erhardt and Rusterholz 1998; Mevi-Schütz and Erhardt 2003b), as the amino acid concentration is high, and *L. camara* is often visited by butterflies (although this plant species does not naturally occur in the habitat of *C. pamphilus*). The artificial nectar of the groups fed without amino acids contained only sucrose, glucose and fructose, whereas the diet of the amino acid fed groups corresponded to the complete nutrient spectrum of *L. camara* nectar, additionally containing nonessential and essential amino acids (for exact composition see Alm et al. 1990).

Relative consumption of amino acid nectar was tested in a two-way test analogous to several other nectar amino acid preference tests (Rusterholz & Erhardt 1997; Mevi-Schütz and Erhardt 2003b; Mevi-Schütz et al. 2003). All butterflies were tested the second day after emergence to prevent aging effects and different egg loads in females. Prior to testing the butterflies for their relative consumption of amino acid nectar, they were brought to the same nutritional level by feeding them with a balanced sugar solution (sucrose:glucose:fructose = 1:1:1, 25% w/w). In order to test whether the relative consumption of amino acid nectar was affected by this meal, the amounts of

balanced sugar solution ingested were measured.

The test solution was offered to the butterflies in 0.5 µl droplets. We held the butterflies and dipped the unrolled proboscis with the help of a needle into the nectar mimic. The two test solutions (nectar mimics with or without amino acids) were each offered once in a trial and the order of presentation was chosen randomly. The butterfly either consumed the droplet within 15 s or the trial was evaluated as a reject. Testing the relative consumption of amino acid nectar consisted of five trials. The degree of relative consumption of amino acid nectar was based on the proportion of drops accepted (number of nectar droplets with amino acids/total number of droplets). Values above 0.5 show an increased relative consumption for nectar with amino acids, values below 0.5 show an increased relative consumption for nectar without amino acids (Mevi-Schütz et al. 2003). Butterflies that rejected either solutions continuously or ingested less than two droplets were excluded from the analysis.

Statistical analysis

Plant chemistry was analysed for differences between silica (S+ versus S-) and fertilization (F+ versus F-) treatments using a fully factorial ANOVA.

Pupal mass and forewing length were analysed with mixed-effects models, and larval duration, relative consumption of amino acid nectar and mortality with generalized linear mixed-effects models due to non-normal data structure (Crawley 2007). Developmental traits and relative consumption of amino acid nectar were tested against the categorical variables sex, fertilization (F+ versus F.) and silica (S+ versus S-), and the random factor lineage. Developmental traits were additionally tested against the continuous covariate larval hatching mass, and adult relative consumption of amino acid nectar against the continuous covariate amount of consumed artificial nectar. A stepwise model reduction was employed, with the least significant interaction always removed first (Crawley 2007).

Tukey multiple comparisons ($P < 0.05$) were performed between the levels of significant factors. All statistical analyses were calculated with R Statistical Software (Version 2.9.1; R Development Core Team 2009).

Table 1: Mixed effects ANCOVA of the effects of sex, silica (S), fertilization (Fert), lineage (L) and larval hatching mass (LHM) on Coenonympha pamphilus butterflies.

	Larval duration *(df = 1, 141)*			Pupal mass *(df = 1, 140)*			Forewing length *(df = 1, 139)*		
	F	*P*	R^2	*F*	*P*	R^2	*F*	*P*	R^2
Sex	67.99	**<0.001**	0.33	583.36	**<0.001**	0.81	265.82	**<0.001**	0.66
S	52.23	**<0.001**	0.27	0.94	0.34	<0.01	6.55	**0.012**	0.05
Fert	10.07	**0.002**	0.07	0.52	0.47	<0.01	2.11	0.15	0.01
L	0.04	0.86	0.01	2.24	0.23	0.43	8.10	0.07	0.73
LHM	0.62	0.57	<0.01	0.40	0.53	<0.01	1.60	0.21	0.01
S × Fert	27.44	**<0.001**	0.16	6.15	**0.014**	0.04	7.83	**0.006**	0.05

Silica treatment is shown as larval host plants grown with supplement silica vs. no supplement silica, and fertilization treatment is shown as fertilized vs. unfertilized larval host plants. $R^2 = F(F+df)^{-1}$. *df* in lineage = 1, 3. A stepwise model reduction of these models was employed, with the least significant (n.s. = not significant) interactions always removed first (Crawley 2007).

Results

Plant material

Foliar nitrogen content was significantly affected by fertilization (ANOVA: $F_{1,36}$ = 315.52, $P < 0.001$) and supplement silica ($F_{1,36}$ = 17.32, $P < 0.001$). Furthermore, there was a significant interaction between these two factors ($F_{1,36}$ = 9.94, P = 0.003). Fertilized host plants (Tukey multiple comparison: F+/S- 5.18^{a} ± 0.06 % N, F+/S+ 5.04^{a} ± 0.05 % N) had a significantly higher foliar nitrogen content than unfertilized plants (F-/S- 3.23^{b} ± 0.24 % N, F-/S+ 2.26^{c} ± 0.09 % N).

Foliar silica content was significantly affected by fertilization (ANOVA: $F_{1,36}$ = 248.38, $P < 0.001$) and supplement silica ($F_{1,36}$ = 406.10, $P < 0.001$). There was a significant interaction between these two factors ($F_{1,36}$ = 94.37, $P < 0.001$). Supplemented host plants (Tukey multiple comparison: F-/S+ 3.17^{a} ± 0.12 % S, F+/S+ 1.24^{b} ± 0.07 % S) had a significantly higher silica content than plants raised without supplement silica (F-/S- 0.91^{c} ± 0.06 % S, F+/S- 0.45^{d} ± 0.03 % S).

Butterfly development

Larval duration was significantly affected by sex, silica level and fertilization of larval host plants, whereas lineage and larval hatching mass had no effects (Table 1). Furthermore, there was a significant interaction between silica level and fertilization ($F_{1,141}$ = 27.44, $P < 0.001$). (F-/S+)-females and males had a significantly longer larval duration than larvae of the other treatment groups (Table 2).

Pupal mass was significantly affected by sex, whereas all other measured parameters had no effects (Table 1). There were significant interactions between silica level and fertilization (Table 1) and between sex and fertilization (Mixed effects ANCOVA: $F_{1,140}$ = 5.79, P = 0.017). (F+/S+)-females had a higher pupal mass than (F-/S+)-females, whereas pupal mass differed not among the other treatment groups and among males (Table 2).

Forewing length was significantly affected by sex and silica level, whereas lineage had only a marginal effect (Table 1). Fertilization of larval host plants and larval hatching mass had no significant effects on forewing length, but there were significant interactions between silica level and fertilization (Table 1), sex and lineage (Mixed effects ANCOVA: $F_{1, 139}$ = 4.99, P = 0.027) and between sex and fertilization ($F_{1, 139}$ = 12.47, P = 0.008). (F-/S+)-females had significantly shorter forewings than females of the other treatments, whereas forewing length differed not among male treatment groups (Table 2).

Mortality did not differ significantly between treatment groups (F-/S- 17%, F-/S+ 10%, F+/S- 28%, F+/S+ 25%; $F_{3,16}$ = 0.94, P = 0.45) and was not significantly affected by host plant silica level ($F_{1,13}$ = 0.56, P = 0.47) and maternal lineage ($F_{1,3}$ = 4.01, P = 0.14), whereas fertilization of host plants only had a marginal effect ($F_{1,13}$ = 3.47, P = 0.09).

Table 2: Treatment means for *Coenonympha pamphilus* butterflies.

	Sex	F+/S-	F+/S+	F-/S-	F-/S+
Larval duration (days)	♀	27.79^{a} ± 0.60	27.12^{a} ± 0.62	29.25^{a} ± 0.56	34.55^{b} ± 1.15
	♂	24.50^{ab} ± 0.50	23.87^{a} ± 0.35	26.17^{b} ± 0.60	29.45^{c} ± 0.68
Pupal mass (mg)	♀	79.72^{ab} ± 1.75	81.00^{a} ± 1.54	77.65^{ab} ± 1.59	74.82^{b} ± 1.26
	♂	57.07 ± 1.17	58.04 ± 1.25	60.05 ± 0.92	57.82 ± 1.09
Forewing length (mm)	♀	14.00^{a} ± 0.10	13.95^{a} ± 0.13	13.82^{a} ± 0.15	13.22^{b} ± 0.12
	♂	12.17 ± 0.12	12.33 ± 0.14	12.55 ± 0.11	12.21 ± 0.13

Larvae were reared on fertilized (F+) or unfertilized (F-) larval host plants treated with (S+) or without (S-) supplement silica. Means ± SE. Different letters indicate significant differences among treatment groups (Tukey multiple comparisons, $P < 0.05$).

Table 3: Mixed effects ANCOVA of the effects of the factors sex, silica (S), fertilization (Fert), lineage (L) and amount of consumed artificial nectar (ACAN) on relative consumption of amino acid nectar of *Coenonympha pamphilus* butterflies.

	F	*P*	R^2	*df*
Sex	1.94	0.17	0.02	1, 121
S	13.95	**<0.001**	0.10	1, 121
Fert	3.29	0.07	0.03	1, 121
L	0.06	0.82	<0.01	1, 3
ACAN	0.01	0.94	<0.01	1, 121

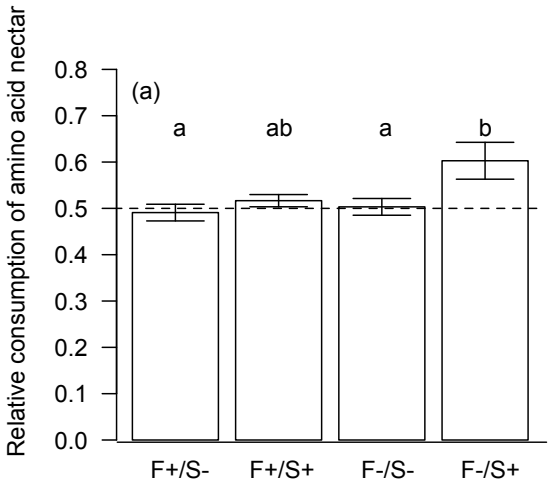
Silica treatment is shown as larval host plants grown with supplement silica vs. no supplement silica, and fertilization treatment is shown as fertilized vs. unfertilized larval host plants. $R^2 = F(F+df)^{-1}$.

Relative consumption of amino acid nectar

Nectar amino acid consumption was significantly affected by silica level, whereas fertilization had a marginal effect, and sex, lineage and the amount of consumed artificial nectar had no effects (Table 3). Tukey multiple comparisons showed that (F-/S+)-males (N = 65, P = 0.025) and (F-/S+)-females (N = 65, P = 0.036) increased their relative consumption of amino acid nectar compared to (F+/S-)-butterflies (Figure 1). Furthermore, (F-/S+)-females showed an increased relative consumption of amino acid nectar compared to (F-/S-)-females (N = 65, P = 0.034; Figure 1a).

Figure 1

A



B

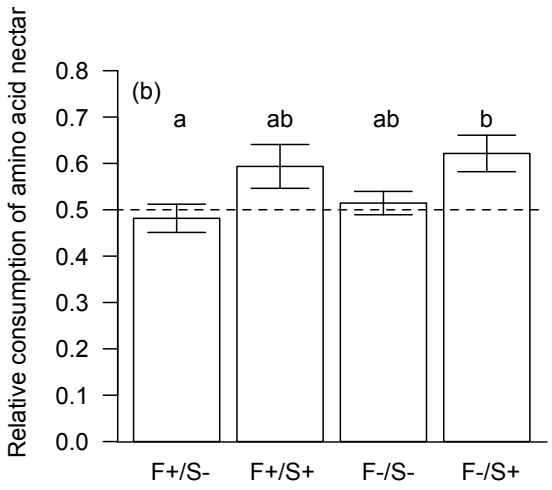


Figure 1: Relative consumption of amino acid nectar of female (a) and male (b) *Coenonympha pamphilus* butterflies raised on fertilized (F+) and unfertilized (F-) *Festuca rubra* host plants treated with (S+) or without (S-) supplement silica (means ± SE). The degree of relative consumption of amino acid nectar was based on the proportional number of droplets accepted (nectar mimic droplets with amino acids/total number of droplets). Random foraging (acceptance of both nectar mimics equally) is indicated by a value of 0.5 (dashed line). Different letters indicate significant differences among treatment groups (Tukey multiple comparisons (P < 0.05)).

Discussion

Direct effects of host plant nitrogen concentration

The nitrogen content in larval host plants (high/low) had a significant effect on larval duration, whereas there was no direct effect on pupal mass and forewing length (Table 1). Larvae can compensate for low nitrogen concentrations in their host plants by higher relative consumption rates (Lavoie and Oberhauser 2004) or by increased efficiency of larval nitrogen utilization (Slansky and Feeny 1977). Furthermore, (F-/S+)-larvae prolonged their feeding periods (Table 2). Prevention of larval compensatory feeding by feeding last larval instar only with quantitatively limited amounts of food significantly decreased pupal mass and adult emergence mass in *C. pamphilus* (Cahenzli and Erhardt 2012a). Without increased host plant defence, compensatory feeding behaviour compensated for low nitrogen concentration in host plants, and (F-/S-)-larvae achieved similar pupal mass and forewing length as larvae raised on host plants with high nitrogen content (Table 2).

Nitrogen content in larval host plants marginally affected relative consumption of amino acid nectar in *C. pamphilus* butterflies, revealing slight nutritional deficiencies from a low nitrogen level during larval feeding in this study (Table 3). This is in accordance with the nectar amino acid preference found in *C. pamphilus* and *A. levana* females raised on nitrogen-poor larval host plants (Mevi-Schütz et al. 2003; Mevi-Schütz and Erhardt 2003b).

Direct effects of high- and low silica level

High silica levels in host plants affected adult forewing length and larval duration, whereas there was no direct effect on pupal mass (Table 1). Several previous studies found negative effects of silica on insect growth, whereas other studies found no effects (Massey et al. 2006; Massey and Hartley 2009; Reynolds et al. 2009). However, an increased silica level in host plants significantly affected relative consumption of amino acid nectar in adult butterflies (Table 3, Figure 1), indicating nutritional deficiencies from larval feeding triggered by host plant defence. Moreover, the silica treatment allowed us to document for the first time that also male butterflies show an increased relative consumption of amino acid nectar, Our findings are in contrast to previous studies, in which *C. pamphilus* males did not prefer amino acid-rich nectar, and it was assumed that males compensated deficiencies from larval feeding by prolonged larval feeding periods (Mevi-Schütz and Erhardt 2003b). In our study, high silica levels in host plants negatively affected feeding during larval stage, and (F-/S+)-male butterflies showed a significantly increased relative consumption of amino acid nectar. In contrast, the only other study investigating effects of host plant defence on adult butterfly feeding behaviour found that male and female *Polyommatus icarus* butterflies reared on host plants with varying cyanogenic glycosides levels showed no preference for amino acid-rich nectar and it was assumed that larvae potentially metabolised the surplus of nitrogen in cyanogenic plants for their growth (Goverde et al. 2008). Our research shows that both low nitrogen content as well as the silica level in larval host plants can increase relative consumption of amino acid nectar in adult butterflies.

Interaction of nitrogen content and silica level

Interactions between silica level and nitrogen content affected butterfly development in this study (Table 1). The larval compensatory feeding, influenced by low nitrogen content, was disrupted by supplementary silica level in (F-/S+)-host plants. As a consequence, (F-/S+)-females

achieved the lowest pupal mass and forewing length (Table 2). In holometabolous insects larval resources are allocated to different imaginal discs during metamorphosis (Shingleton et al. 2007). Thus, silica level in host plants affected forewing length more than pupal mass (Table 1, 2), suggesting that under limited nitrogen availability, allocation to wing formation was decreased. The level of silica in nitrogen low host plants could negatively affect female fitness, as butterfly mass and reproduction are positively correlated (Mevi-Schütz and Erhardt 2005; Cahenzli and Erhardt 2012a, b), and thorax mass and forewing geometry affect flight performance (Berwaerts et al. 2002), which can be important for dispersal, foraging, reproduction and predator avoidance. Furthermore, larval duration in the (F-/S+)-treatment group was significantly longer than in the other treatment groups (Table 2), and prolonged larval duration may increase the exposure time to predators, parasites and other adverse factors (Clancy and Price 1987; Williams 1999). In contrast, supplementary silica levels had no negative effects on butterflies raised on fertilized host plants (Table 2). Correspondingly, only locusts fed with suboptimal protein:carbohydrate ratios in their artificial diet were negatively affected by the secondary plant metabolite tannic acid (Simpson and Raubenheimer 2001). Thus, host plant defence is affected by fertilization, and host plant quality is determined by several factors that are not independent of each other. These interactions can influence herbivores differently.

Compensatory feeding in the adult stage

Allocation patterns of nutrients at each developmental stage are not independent of each other and also interact with the nutritional environment (Simpson and Raubenheimer 1993; Boggs 2009). Our study shows that compensatory feeding from the larval phase continued in the adult stage. Both females as well as males (F-/S+), showed an increased relative consumption of amino acid nectar compared to (F+/S-)-butterflies that were raised under optimal larval food conditions. These results confirm that larval and adult feeding are interconnected in *C. pamphilus*, and correspond with previous findings (O'Brien et al. 2002, 2005; Mevi-Schütz and Erhardt 2003b; Cahenzli and Erhardt 2012 a). Nectar amino acids acquired in the adult phase compensated for nutritional deficiencies from the larval phase in *A. levana* females raised on nitrogen-poor host plants, resulting in almost the same fecundity as females reared on nitrogen-rich host plants (Mevi-Schütz and Erhardt 2005). Furthermore, male butterflies can use nectar amino acids to enhance their reproduction (Cahenzli and Erhardt 2013). Thus, nectar covers not only current energy requirements in the adult stage, but can also compensate for deficiencies acquired during larval feeding and benefit reproduction.

Conclusions

This study shows that nitrogen concentration in larval host plants and the silica level in host plant defence interact and affect butterfly performance, as well as the relative consumption of amino acid nectar by *C. pamphilus* butterflies, thus documenting the complexity of plant-herbivore interactions. Furthermore, we verified that larval and adult feeding are interconnected in *C. pamphilus*, and we show that both female and male butterflies reared under adverse larval food conditions preferred to consume amino acid-rich nectar. Thus, varying larval host quality, be it chemically or structurally, affects adult consumption of amino acid nectar. This in turn may lead to changes in flower preferences and could ultimately affect plant-pollinator dynamics (Mevi-Schütz et al. 2003).

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References

Allen SE (1989) *Chemical Analysis of Ecological Materials*. 2nd edn. Blackwell, Oxford

Alm J, Ohnmeiss TE, Lanza J, Vriesenga L (1990) Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia 84:53–57

Baker HG, Baker I (1986) The occurrence and significance of amino acids in floral nectar. Plant Syst Evol 151:175–186

Barton KE, Koricheva J (2010) The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am Nat 175:481–493

Bauerfeind SS, Fischer K (2009) Effects of larval starvation and adult diet derived amino acids on reproduction in a fruit-feeding butterfly. Entomol Exp Appl 130:229–237

Berwaerts K, Van Dyck, H, Aerts P (2002) Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Funct Ecol 16:484–491

Bissoondath CJ, Wiklund C (1996) Male butterfly investment in successive ejaculates in relation to mating system. Behav Ecol Sociobiol 39:285–292

Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. Funct Ecol 23:27–37

Cahenzli F, Erhardt A (2012a) Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. Oecologia 169:1005–1014

Cahenzli F, Erhardt A (2012b). Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos 121: 1417-1423

Cahenzli F, Erhardt A (2013). Nectar amino acids enhance reproduction in male butterflies. Oecologia 171: 197-205

Clancy KM, Price PW (1987) Rapid herbivore growth enhances enemy attack − sublethal plant defenses remain a paradox. Ecology 68:733–737

Crawley MJ (2007) The R book. John Wiley & Sons Ltd, West Sussex, England

Elliott CL, Snyder GH (1991) Autoclave-Induced Digestion for the Colorimetric Determination of Silicon in Rice Straw? J Agricultural Food Chemistry 39:1118–1119

Erhardt A (1991) Nectar sugar and amino acid preferences of *Battus philenor* (Lepidoptera, Papilionidae). Ecol Entomol 16:425–434

Erhardt A (1992) Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). Oecologia 90:581–585

Erhardt A, Rusterholz HP (1998) Do peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds? Oecologia 117:536–542

Goverde M, Bazin A, Kéry M, Shykoff J A, Erhardt A (2008) Positive effects of cyanogenic glycosides in food plants on larval development of the common blue butterfly. Oecologia 157:409–418

Goverde M, Erhardt A (2003) Effects of elevated CO2 on development and larval food-plant preference in the butterfly

Coenonympha pamphilus (Lepidoptera, Satyridae). Glob Change Biol 9:74–83

Hill CJ (1989) The effect of adult diet on the biology of butterflies. II. The common crow butterfly, Euploea core corinna. Oecologia 81:258–266

Lavoie B, Oberhauser KS (2004) Compensatory Feeding in *Danaus plexippus* (Lepidoptera:Nymphalidae) in Response to Variation in Host Plant Quality. Environmental Entomol 33:1062–1069

Lepidopterologen-Arbeitsgruppe (1987) Tagfalter und ihre lebensräume, Band 1. Schweizerischer Bund für Naturschutz. Switzerland

Massey FP, Ennos AR, Hartley SE (2006) Silica in grasses as defence against insect herbivores: contrasting effects on folivores and phloem feeder. J Anim Ecol 75:595–603

Massey FP, Ennos AR, Hartley SE (2007) Grasses and the resource availability hypothesis: the importance of silica-based defences. J Ecol 95:414–424

Massey FP, Hartley SE (2009) Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. J Anim Ecol 78:281–291

Mevi-Schütz J, Erhardt A (2003a) Effects of nectar amino acids on fecundity of the wall brown butterfly (*Lasiomata megera* L.). Basic Appl Ecol 4:413–421

Mevi-Schütz J, Erhardt A (2003b) Larval nutrition affects female nectar amino acid preference in the map butterfly (*Araschnia levana*). Ecology 84:2788–2794

Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long awaited link. Am Nat 165:411–419

Mevi-Schütz J, Goverde M, Erhardt A (2003) Effects of fertilization and elevated CO2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L. Behav Ecol Sociobiol 54:36–43

Moore RA, Singer MC (1987) Effects of maternal age and adult diet on egg weight in the butterfly *Euphydryas editha*. Ecol Entomol 12:401–408

Myers JH (1985) Effect of physiological condition of the host plant on the oviposition choice of the cabbage white butterfly, *Pieris rapae.* J Anim Ecol 54:193-204

O'Brien DM, Boggs CL, Fogel ML (2002) Renewable and non-renewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. Proc Natl Acad Sci USA 99:4413–4418

O'Brien DM, Boggs CL, Fogel ML (2005) The amino acids used in reproduction by butterflies: a comperative study of dietary sources using compound-specific stable isotpe analysis. Physiol Biochem Zool 78:819-827

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reynolds OL, Keeping MG, Meyer JH (2009) Silicon-augmented resistance of plants to herbivorous insects: a review. Annals Appl Biol 155:171–186

Russel EW (1961) *Soil conditions and plant growth*. New York: Longmans Green

Rusterholz H-P, Erhardt A (1997) Preferences for nectar sugars in the peacock butterfly, *Inachis io*. Ecol Entomol 22:220–224

Rusterholz H-P, Erhardt A (2000) Can nectar properties explain sexspecific flower preferences in the adonis blue butterfly *Lysandra bellargus*? Ecol Entomol 25:81–90

Schoonhoven LM, Van Loon JJA, Dicke M (2006) Insect – plant biology. 2nd edition, Oxford University Press, Oxford, UK

Shingleton AW, Frankino WA, Flatt T, Nijhout HF, Emlen DJ (2007) Size and shape: The developmental regulation of static allometry in insects. Bio Essays 29:536–548

Simpson SJ, Raubenheimer ED (1993) A multi-level analysis of feeding

behaviour: the geometry of nutritional decisions. Phil Trans R Soc Lond B 342: 381–402

Simpson SJ, Raubenheimer ED (2001) The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. Ecology 82:422-439

Slansky F, Feeny P (1977) Stabilization of rate of nitrogen accumulation by larvae of cabbage butterfly on wild and cultivated food plants. Ecol Monogr 47:209–228

Vicari M, Bazely DR (1993) Do grasses fight back – the case for antiherbivore defenses. Trends Ecol Evol 8:137–141

Williams IS (1999) Slow-growth, high-mortality - a general hypothesis, or is it? Ecol Entomol 24:490–495

Chapter 2

Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies

Abstract

Butterfly-pollinated flowers offer nectar with higher amino acid concentrations than most flowers pollinated by other animals, and female butterflies of some species prefer to consume amino acid-rich nectar. However, for over 30 years, there has been an ongoing discussion about whether nectar amino acids benefit butterfly fitness. A clear positive effect was only shown for the nectar-feeding *Araschnia levana,* and females of the fruit-feeding *Bicyclus anynana* also increased offspring quality when they were fed amino acids as adults. Thus, severe doubts remain about the general signifcance of these single positive results. We therefore tested a further species from a phylogenetically different butterfly subfamily, the small heath (Coenonympha *pamphilus* L., Satyrinae), taking into account feeding conditions over the whole life cycle of this species. *C. pamphilus* females receiving nectar amino acids as adults, irrespective of larval food quality, produced heavier larvae and also increased the hatching success of their eggs over the oviposition period. Furthermore, females raised under nitrogen-poor larval conditions tended to use nectar amino acids to increase the number of eggs laid. Thus, *C. pamphilus* females used nectar amino acids primarily to increase their offspring quality, and secondly tended to increase offspring quantity, if larval resources were scarce, showing a resource allocation pattern differing from both *B. anynana* and *A. levana.* Our study supports the old postulate that nectar amino acids generally enhance butterfly fitness.

Keywords: Butterfly reproduction, *Coenonympha pamphilus*, Larval feeding, Lepidoptera, pollination

Introduction

Approximately two-thirds of all flowering plants are pollinated by insects (Schoonhoven et al. 2006). Apart from olfactory and visible attractants, flowers lure potential pollinators with rewards such as nectar, pollen, or oil. Nectar is composed mainly of water and sugars, but can also contain significant amounts of amino acids (Ziegler 1956; Lüttge 1961; Baker and Baker 1973, 1986; Baker 1975). Amino acid concentration in floral nectar is relatively constant within species and can typify certain plant families and genera (Baker and Baker 1986). Moreover, the amino acid concentration in floral nectar correlates with specific pollinator types, and nectar of flowers adapted to pollination by butterflies contains higher levels of amino acids than most flowers pollinated by other animal types (Baker and Baker 1975, 1986). As a consequence of this finding, there has been an ongoing discussion for over 30 years as to whether amino acids obtained from nectar in the adult diet enhance butterfly fitness.

Insect eggs consist primarily of protein (Engleman 1984), and the amount of acquired nitrogen is a key factor for fitness and reproduction in insects, but larval host plants often do not provide optimal amounts of nitrogen (Schoonhoven et al. 2006). Hence, the limiting resource for reproduction seems to be nitrogenous compounds (Mattson 1980; Boggs 1981), and insects are dependent on dietary sources for 10 out of 20 amino acids (Dadd 1973). Thereby,

resource allocation differs under unconstrained, benign conditions and stressful, resource-poor environments (Boggs 2009). However, butterflies can also synthesise non-essential amino acids from carbohydrates in adult nectar diet (O'Brien et al. 2002).

Depending on an animal's stage of development and current environmental circumstances, nutrients are required at optimal levels to maximize fitness (Simpson and Raubenheimer 1993). Furthermore, allocation patterns of nutrients at each developmental stage are not independent of each other and also interact with the nutritional environment (Boggs 2009). Thus, in holometabolous insects, larvae and adults can feed on different food sources and both stages can contribute nutrients to reproduction. In butterflies, nitrogen used in egg production is mainly derived from stored larval reserves (Boggs 1981, 1997a; O'Brien et al. 2002), but the importance of larval reserves declines with increasing quality of adult nutrition (Boggs 2009). Nitrogen sources in the adult stage are adult diet (Boggs 1997a; O'Brien et al. 2002) and 'nuptial gifts' that females receive in the form of spermatophores from males at mating (Boggs and Gilbert 1979; Boggs 1981). Hence, female butterfly fecundity can be increased by the availability of amino acid-rich nectar in the adult diet (Mevi-Schütz and Erhardt 2005), by a plain sugar solution enriched with amino acids and salts (Bauerfeind and Fischer 2009), by the utilization of nitrogen-rich pollen (O'Brien et al. 2003), and by nitrogen-rich nuptial gifts received during mating (Wiklund et al. 1993; Karlsson 1998; Arnqvist and Nilsson 2000). Furthermore, female butterflies of some species prefer to consume amino acid-rich nectar (Alm et al. 1990; Erhardt and Rusterholz 1998; Mevi-Schütz and Erhardt 2002), suggesting that amino acids derived from adult diet are generally important for female butterfly reproduction. For instance, female butterflies of *Araschnia levana* and *Coenonympha pamphilus* L. (Lepidoptera: Satyrinae) raised on nitrogen-poor larval host plants showed an increased preference for amino acid-rich nectar (Mevi-Schütz and Erhardt 2003a; Mevi-Schütz et al. 2003).

Another important factor affecting resource allocation patterns is aging. Food intake for any given life stage does not necessarily match life-history trait requirements for that particular life stage (Boggs 2009). For example, nectar intake varies with age (Boggs and Ross 1993; Boggs 1997b), and the source (larval or adult income) of egg nutrients varies over the female oviposition period (Boggs 1997a, b), thereby also affecting fecundity (Boggs and Freeman 2005).

Nevertheless, the role of amino acids in nectar for butterfly reproduction remains controversial. For instance, females of the tropical papilionids *Battus philenor* and *Ornithoptera priamus* (Erhardt 1991, 1992) and male butterflies in general did not show a preference for amino acid-rich nectar (e.g., Mevi-Schütz and Erhardt 2003a; Mevi-Schütz et al. 2003). Furthermore, female fecundity of several butterfly species was not influenced by nectar amino acids (Moore and Singer 1987; Hill 1989; Mevi-Schütz and Erhardt 2003b), leaving some ambiguity about the general significance of nectar amino acids for butterfly reproduction. To date, only two studies have shown conclusive evidence of a beneficial effect of amino acids from adult diet on butterfly fecundity (Mevi-Schütz and Erhardt 2005; Bauerfeind and Fischer 2009). However, in the study of Mevi-Schütz and Erhardt (2005), positive fitness effects of nectar amino acids in the adult diet of *A. levana* females only became apparent when larvae were raised under nitrogen-poor dietary conditions. In contrast, increased egg size resulting from an amino acid-rich adult diet in the fruit- feeding *Bicyclus anynana* was unaffected by larval food conditions (Bauerfeind and Fischer 2009). Previous studies which found no effect of amino acids

in the adult diet on butterfly reproduction neglected nutrients acquired during the larval phase. As, in these studies, larvae were raised under benign conditions, positive effects of nectar amino acids from adult feeding might have been masked (Moore and Singer 1987; Hill 1989; Mevi-Schütz and Erhardt 2003b). Hence, further manipulative studies of resource acquisition and utilization in phylogenetically diverse butterflies are needed to test the hypothesized universality of the relationship between nectar amino acids, reproduction, and larval food stress among butterflies, as stated by Jervis and Boggs (2005).

The objective of the present study was therefore to clarify this issue by investigating the small heath (*Coenonympha pamphilus* L., Satyrinae), an additional butterfly species from a different subfamily than the previously tested nectar-feeding *A. levana* and fruit-feeding *B. anynana*, taking into account the food quality of the juveniles as well as of the adults. We measured not only quantitative reproductive parameters (i.e. egg number) but also qualitative, fitness-relevant traits such as hatching success of eggs and progeny's larval hatching mass, and refer to these traits as offspring quality in this paper. As nutrients from adult feeding can be stored and can be used only later during the oviposition period after resources from the larval phase are depleted (Boggs 1997a), we also analysed time patterns of reproductive parameters over the whole female oviposition period.

Materials and methods

Study species

Coenonympha pamphilus, the small heath, is a common butterfly in Eurasia and is found primarily on unfertilized meadows. It generally has three generations per year and overwinters as half-grown larva (Ebert and Rennwald 1991). Larvae of this species feed on a variety of grasses (*Cynosurus*, *Poa*, and *Anthoxanthum* spp.; Koch 1991), but *Festuca rubra* is favored (Goverde and Erhardt 2003). Eighteen C. pamphilus females were collected from an unfertilized meadow in the northern Jura mountains (Liesberg BL, Switzerland) and placed in cages containing pots of *F. rubra* for oviposition. The eggs were carefully collected (the butterflies laid their eggs directly on the cage netting so it was necessary to cut the netting to collect the eggs) and placed in Petri dishes.

Plant material

Larval food plants of *F. rubra* were grown in 750-ml plastic pots filled with untreated calcareous soil from a nutrient-poor meadow near Liesberg BL in Switzerland. Each pot was planted with 450 seeds (UFA Samen, Basel, Switzer- land). Plants were grown in a greenhouse at the University of Basel, with ambient sun light in summer and supplement light (1,000 W broad spectrum, light period from 0600 to 2000 hours) during cloudy weather conditions and a day/ night cycle of 25/19°C. All pots were watered when necessary. High-quality larval food plants were obtained by fertilizing half the pots once a week with 50 ml Algoflash (Laboratoire Algochemie Z. I. Nord, Chateau-Renault, France; N:P:K = 1:1:1). The low-quality larval food plants received only water. Prior to introducing the larvae, the following plant quality parameters were analyzed from 8-week-old grass samples: Leaf water content was calculated as the difference between dry and fresh leaf mass (drying by 80°C for 48 h), dry leaves were ground for leaf nitrogen (N) and carbon (C) analysis using a CHN analyzer (LECO Instruments, model 1932; St. Joseph, MI, USA).

Larval diet

L1 larvae from the 18 ovipositing females were randomly assigned to either the high-

or low-quality larval food plants and kept in groups of 10. All lineages were included in the analysis.

Larvae were raised in the same greenhouse under the same climatic conditions as larval food plants. Larvae from each female were reared separately in order to later trace back each butterfly to the ovipositing female. After 10 days, larvae were separated and kept individually in Petri dishes enclosed with nylon mesh. About a third of all larvae entered diapause and had therefore to be excluded from the experiment. There was no effect of larval food quality on the proportion of larvae entering diapause (χ^2 = 2.46, *df* = 1, *P* = 0.12). The larvae continued to receive their assigned larval food quality diet. To achieve a difference in nitrogen reserves from the larval phase, larvae raised on high-quality larval food plants received an abundant supply of fertilized *F. rubra* ad libitum, whereas larvae from the low-quality treatment received a quantitatively limited amount of unfertilized larval host plants during the last larval instar (ca. 50% of the amount of the high-quality larvae). Considering not only food quality but also the amount of available food is important in order to evaluate effects of food quality on performance in feeding experiments due to potential compensatory feeding (Carvalho et al. 2005; Simpson and Raubenheimer 2007). Pupae were collected and placed in individual compartments until emergence.

Butterfly diet

Females were weighed within 24 h after emergence (Mettler Toledo Ab 204-S; Mettler Instruments, Switzerland), placed in individual nylon mesh cages (20 cm x 20 cm x 40 cm), and allowed to mate once with an unrelated male. All butterflies that did not mate within the first 2 days after emergence were excluded from the analysis. Female butterflies from the high- and low-quality larval food treatments were randomly assigned to a nectar diet treatment consisting either nectar mimic with amino acids (AA) or without amino acids (NAA). Four treatment groups resulted: high- quality larval food and adult diet with amino acids (high/ AA, *n* = 13), high-quality larval food and adult diet without amino acids (high/NAA, *n* = 16), low-quality larval food and adult diet with amino acids (low/AA, *n* = 11) and low- quality larval food and adult diet without amino acids (low/ NAA, *n* = 13).

A nectar mimic of the plant *Lantana camara* was used in this experiment. Although this plant does not occur naturally in the habitat of *C. pamphilus*, nectar mimics of *L. camara* have been used in former experiments with *C. pamphilus* (Mevi-Schütz and Erhardt 2003a) as well as with a number of other butterfly species (Alm et al. 1990; Erhardt and Rusterholz 1998; Mevi-Schütz et al. 2003; Mevi-Schütz and Erhardt 2005) and have become established as a reference for comparing studies of different butterfly species. The nectar mimic of the group fed without amino acids contained only sucrose, glucose, and fructose, whereas the diet of the amino acid-fed group corresponded to the complete nutrient spectrum of *L. camara* nectar, additionally containing nonessential and essential amino acids (for exact composition, see Alm et al. 1990). The test solutions were made with sodium-free substances.

Preliminary experiments showed that *C. pamphilus* butterflies rejected a daily feeding (Cahenzli and Erhardt, unpublished data). In contrast to the varying availability of floral nectar quality and quantity in nature (Rusterholz and Erhardt 1998a, b), nectar supply at the artificial feeding station was not limited. In the present experiment, the butterflies consumed obviously enough nutrients by feeding only every second day. Furthermore, the butterflies were kept in cages, reducing their mobility and diminishing their energy consumption. We therefore fed the butterflies their respective nectar diet every second day and allowed

them to consume nectar until they voluntarily left the feeding station. *C. pamphilus* butterflies did not recognize the artificial feeding station as a natural nectar source, so we placed the butterflies beside the nectar-filled tube and dipped the rolled-out proboscis with the help of a needle into the nectar mimic to initiate feeding. To measure the amount of nectar consumed, we used a 100-µl Hamilton syringe.

Reproductive parameters

Larval host plants for oviposition were offered, but in our experiment *C. pamphilus* females laid their eggs mainly on the cage netting. We recorded the longevity of each butterfly and collected all eggs laid by cutting them out of the cage netting.

Egg duration (number of days from when the egg was laid to when larva hatched), the egg hatching success (number of eggs hatched per female), and larval hatching mass (mg) of progeny were recorded for all eggs collected from each butterfly. Previous studies with other butterfly species showed that egg mass and larval hatching mass are tightly correlated (Karlsson and Wiklund 1984; Nakasuji and Kimura 1984) and larger eggs produce larger hatchlings (Fischer et al. 2002). Thus, the measured larval hatching mass in this study is a good substitute for egg mass. To measure larval hatching mass instead of egg mass was necessary because *C. pamphilus* females frequently lay their eggs on the cage netting, which made weighing eggs without destroying them impossible. Eggs laid on the same day by the same butterfly were placed together in Petri dishes. Freshly hatched larvae were weighed within 24 h (Mettler M3; Mettler Instruments) before they were fed. Furthermore, we characterized relationships between butterfly mass and reproduction, between butterfly age and number of eggs, and between larval hatching success and larval hatching mass.

Statistical analysis

Plant quality parameters (leaf water concentration, leaf nitrogen, and C/N ratio) of fertilized and unfertilized larval food plants were compared using a one-way MANOVA.

The larval quality traits $\log_n$ transformed larval duration, pupal duration and adult emergence mass, the reproductive traits $\log_n$-transformed longevity of female butterflies, the $\log_n$-transformed average amount of nectar mimic consumed by females, total number of eggs laid, hatching success of eggs, and progeny's egg duration and larval hatching mass were analyzed with mixed-effects models. Because related individuals (individuals from the same maternal lineage) are not independent of each other, effects and interactions were tested against the random factor lineage. Larval quality traits were tested against the factors larval food quality (low, high) and sex. The reproductive traits were tested against the factors nectar amino acid diet (AA vs. NAA) and larval food quality (high vs. low), and the covariates female emergence mass and the average amount of nectar consumed per feeding. The average amount of nectar mimic consumed was tested against the factor nectar diet and the covariate female emergence mass. A stepwise model reduction of these models was employed, with the least significant interactions always removed first (Crawley 2007). Tukey–Kramer's HSD comparisons ($P < 0.05$) were performed between the levels of significant factors.

Mixed-effects models with temporal pseudoreplication (repeated measures on the same females) were used to test whether day of oviposition, adult diet, individual, and the interaction between day of oviposition and adult diet affected time patterns of reproductive traits. Number of eggs and larval hatching success of eggs over time were analyzed with generalized linear mixed models with temporal pseudoreplication due

to non-normal data structure (Crawley 2007). Furthermore, quadratic regression was used to characterize trends in the time pattern of hatching success of eggs over the oviposition period from females fed with nectar without amino acids, whereas the time pattern of hatching success of eggs from females fed with amino acid-rich nectar was analyzed with linear regression (Crawley 2007). The time pattern of number of eggs laid over the oviposition period of females fed with nectar lacking amino acids was characterized with exponential regressions, whereas the corresponding time pattern of females fed with amino acid-rich nectar was analyzed with quadratic regression (Crawley 2007). Time patterns of progeny's larval hatching mass were analyzed using quadratic regressions (Crawley 2007).

Correlation analysis was used to examine if female emergence mass affected total number of eggs laid positively or negatively. We also used correlation analysis to detect a possible trade-off between total number of eggs laid and progeny's larval hatching mass, as it was not clear whether number of eggs or larval hatching mass is the dependent variable.

To maintain consistency in the investigation of the effect of nectar amino acids on female butterffy reproduction, we used analogous statistical analyses as in the experiment with *A. levana* (Mevi-Schütz and Erhardt 2005). All statistical analyses were calculated with R Statistical Software (v.2.9.1; R Development Core Team 2009). All treatment means are indicated with standard errors.

Results

Laval host plant quality

Fertilized *F. rubra* had a significantly higher plant quality (leaf nitrogen, water content and C/N ratio) than unfertilized larval food plants ($F_{1,26} = 79.88$, $P < 0.001$). As expected, fertilized *F. rubra* had significantly higher leaf nitrogen (low-quality: 2.85 ± 0.08 g N / 100 g dry weight; high-quality: 4.16 ± 0.07 g N / g dry weight; $P < 0.001$), a higher water content (low-quality: 0.08 ± 0.01 g H_2O / 100g dry weight; high-quality: 0.12 ± 0.01 g H_2O / 100g dry weight; $P = 0.006$) and a lower C/N ratio (low-quality: 16.23 ± 0.53; high-quality: 10.37 ± 0.25; $P < 0.001$) than unfertilized larval food plants.

Effects of larval diet

Larval duration (high-quality: females 24.69 ± 0.54 days; males 22.25 ± 0.31 days; low-quality females 26.48 ± 0.62 days; males 24.47 ± 0.53 days) was significantly affected by larval diet quality ($F_{1,49} = 27.31$, $P < 0.001$), sex ($F_{1,49} = 23.63$, $P < 0.001$) and marginally by lineage ($F_{1,49} = 3.51$, $P = 0.076$). Additionally, there was a marginal interaction between sex and larval diet quality ($F_{1,49} = 3.54$, $P = 0.06$), since in males the difference of larval duration between low- and high-quality larval food treatment groups was more pronounced than in females.

Pupal duration (high-quality: females 7.52 ± 0.12 days; males 8.36 ± 0.74 days; low-quality: females 7.52 ± 0.10 days; males 7.67 ± 0.10 days) was not affect by larval diet quality ($F_{1,50} = 0.28$, $P = 0.60$), by sex ($F_{1,50} = 2.42$, $P = 0.12$), or by lineage ($F_{1,50} = 0.10$, $P = 0.75$).

Emergence mass (high-quality: females 31.55 ± 1.04 mg; males 21.45 ± 0.52 mg; low-quality: females 26.18 ± 0.77 mg; males 18.73 ± 0.91 mg) was significantly affected by larval diet quality ($F_{1,49} = 56.84$, $P < 0.001$) and sex ($F_{1,49} = 157.80$, $P < 0.001$), whereas lineage had no significant effect ($F_{1,49} = 1.60$, $P = 0.22$). Additionally, there was a significant interaction between sex and larval diet quality ($F_{1,49} = 4.25$, $P = 0.042$), since in males the difference of emergence mass between low- and high-quality larval food treatment groups was more pronounced than in females.

Table 1: Treatment means for *Coenonympha pamphilus* butterflies.

	Low/NAA	Low/AA	High/NAA	High/AA
Total no. eggs laid	$84.15^{a} \pm 4.71$	$98.64^{ab} \pm 8.06$	$132.75^{c} \pm 5.73$	$121.15^{bc} \pm 7.37$
Egg duration (day)	7.74 ± 0.06	7.82 ± 0.12	7.77 ± 0.06	7.62 ± 0.08
Hatching success	0.87 ± 0.02	0.85 ± 0.04	0.82 ± 0.05	0.88 ± 0.03
Hatching mass (mg)	$0.163^{bc} \pm 0.005$	$0.185^{a} \pm 0.006$	$0.159^{c} \pm 0.004$	$0.178^{ab} \pm 0.005$
Nectar/feeding (µl)	9.35 ± 0.56	9.58 ± 0.72	9.46 ± 0.35	10.01 ± 0.47
Longevity (day)	22.77 ± 11.04	24.18 ± 10.34	24.07 ± 11.27	26.46 ± 10.56

Note: Butterflies were raised on a low- or high-quality larval diet and fed a nectar mimic with (AA) or without (NAA) amino acids (means ± SE). Different letters indicate significant differences among treatment groups (Tukey-Kramer HSD, $P < 0.05$).

Effects of nectar amino acids on female reproduction

The reproductive traits total number of eggs laid, progeny's larval hatching mass, egg duration, hatching success of eggs, female longevity and the amount of consumed nectar were affected differently by the measured parameters:

The total number of eggs laid by females differed significantly among the four treatment groups ($F_{3,49} = 12.1$, $P < 0.001$; Table 1). Total number of eggs laid was significantly affected by larval food quality, whereas nectar diet, average amount of nectar consumed, emergence mass and lineage had no significant effects (Table 2). Additionally, there was a significant interaction between larval and adult diet quality (Table 2).

Progeny's larval hatching mass differed significantly among the four treatment groups ($F_{3,49} = 5.71$, $P = 0.003$; Figu 1, Table 1). Larval food quality, emergence mass and lineage did not affect progeny's mean larval hatching mass, whereas nectar diet had a significant, and the average amount of consumed nectar only a marginal effect (Table 2). There was no trade-off between number of eggs and progeny's larval hatching mass in females fed nectar without amino acids ($r = -0.10$, df = 27, $P = 0.61$) as well as in females fed nectar containing amino acids ($r = 0.28$, df = 22, $P = 0.18$).

Egg duration ($F_{3,49} = 0.73$, $P = 0.6$; Table 1) and hatching success of eggs ($F_{3,49} = 0.45$, $P = 0.8$; Table 1) did not differ significantly among the four treatment groups and were not influenced by larval food quality, female emergence mass, average amount of nectar mimic consumed, adult nectar diet or lineage (Table 2).

Figure 1

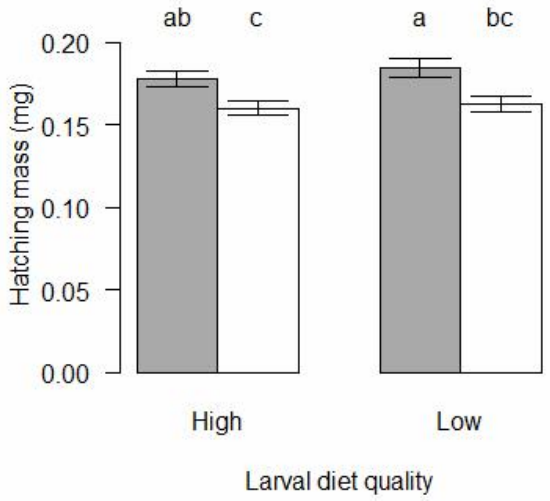

Fig. 1 Hatching mass of larvae laid by *Coenonympha pamphilus* butterflies raised on low-quality (= low) or high-quality (= high) *Festuca rubra* and fed nectar mimics with amino acids (= AA; dark bars) or without amino acids (= NAA; bright bars). Mean ± SE. Different letters indicate significant differences for larval hatching mass (high/NAA-high/AA: $P = 0.047$; low/AA-high/AA: $P = 0.8$; low/NAA-high/AA: $P = 0.2$; low/AA-high/NAA: $P = 0.005$; low/NAA-high/NAA: $P = 0.1$; low/NAA-low/AA: P = 0.03).

Table 2: Effects of larval food quality, emergence mass, adult nectar diet, daily amount of nectar consumed and lineage on reproduction of *Coenonympha pamphilus* butterflies. *F* values, *P* values and the effect size (*R*) of the ANCOVA are presented.

	Number of eggs (*df* = 1, 30)			Egg duration (*df* = 1, 31)			Egg hatching success (*df* = 1, 31)			Larval hatching mass (*df* = 1, 31)			Longevity (*df* = 1, 31)		
	F	*P*	*R*	*F*	*P*	*R*	*F*	*P*	*R*	*F*	*P*	*R*	*F*	*P*	*R*
Nectar quality	<0.01	0.98	0.00	0.19	0.66	0.08	0.42	0.52	0.11	17.11	**<0.001**	0.60	0.28	0.60	0.09
Larval food quality	33.75	**<0.001**	0.73	0.69	0.41	0.15	1.75	0.20	0.23	0.90	0.35	0.17	0.84	0.37	0.16
Emergence mass	1.14	0.29	0.19	2.09	0.16	0.25	3.07	0.09	0.30	0.08	0.78	0.05	1.78	0.19	0.23
Amount of nectar	2.71	0.11	0.29	0.64	0.43	0.14	0.62	0.44	0.14	4.07	0.053	0.34	1.38	0.17	0.21
Lineage	<0.001	1.00	0.00	1.78	0.19	0.23	0.002	0.97	0.01	1.06	0.32	0.25	2.02	0.17	0.33
Larval food quality x nectar quality	4.50	**0.042**	0.36	-	n.s	-	-	n.s.	-	-	n.s.	-	-	n.s.	-

Note: Emergence mass is shown as an expression of larval food quality. Adult nectar diet is shown as AA versus NAA.
$R = \sqrt{(F)}\ (\sqrt{(F+df)})^{-1}$, df in lineage = 16.

Table 3: Effects of day of oviposition, adult nectar diet and individual on time patterns of reproductive parameters of *Coenonympha pamphilus* butterflies. *Z* or *t* values, *P* values, degrees of freedom (*df*) and the effect size (*R*) of the ANCOVA are presented.

	Egg number				Larval hatching mass				Larval hatching success			
	z	*P*	*R*	*df*	*t*	*P*	*R*	*df*	*z*	*P*	*R*	*df*
Day of oviposition	-15.05	**<0.001**	0.43	1,994	21.58	**<0.001**	0.95	1,754	5.78	**<0.001**	0.21	1,754
Individual	3.92	**<0.001**	0.49	1,50	0.41	0.65	0.03	1,50	1.41	0.16	0.20	1,50
Adult diet	-1.97	**0.049**	0.27	1,994	4.32	**<0.001**	0.52	1,754	3.79	**<0.001**	0.47	1,754
Adult diet x day of ovipostion	-	n.s.	-	-	-	n.s.	-	-	-4.32	**<0.001**	0.16	1,754

Note: Adult nectar diet is shown as AA versus NAA. $R = t(\sqrt{(t^2+df)})^{-1}$ or $z(\sqrt{(z^2+df)})^{-1}$

Female longevity did not differ among the four treatment groups ($F_{3,49} = 0.34$, $P = 0.8$; Table 1) and was not influenced by larval food quality, average amount of nectar mimic consumed, adult nectar diet or lineage (Table 2). Emergence mass had a marginal effect on longevity (Table 2).

Adult treatment groups fed with the nectar mimic containing amino acids (high/AA & low/AA) did not consume more nectar than those fed with the nectar mimic without amino acids (high/NAA versus low/NAA; $F_{3,49} = 0.4$, $P = 0.8$; Table 1). Larval food quality ($F_{1,32} = 0.47$, $P = 0.50$), female emergence mass ($F_{1,49} < 0.01$, $P = 0.95$), nectar diet quality ($F_{1,32} = 0.55$, $P = 0.46$) and lineage ($F_{1,49} = 0.78$, $P = 0.39$) had no significant effects on nectar consumption.

Female emergence mass was positively correlated with the number of eggs laid ($r = 0.40$, $N = 53$, $P = 0.003$), since heavier females laid more eggs.

Figure 2

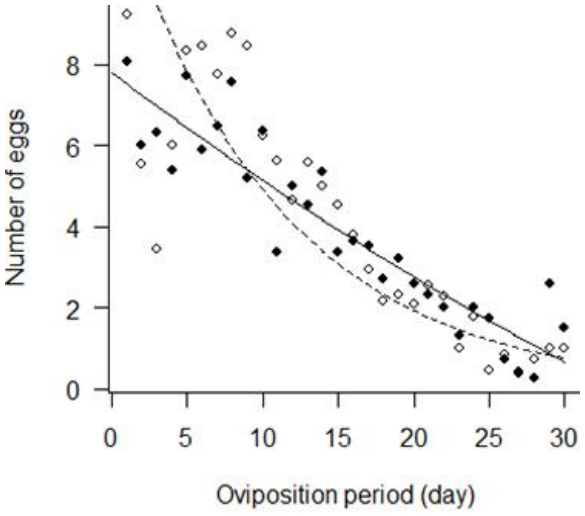

Fig. 2 Regression analyses show that the number of eggs laid from *Coenonympha pamphilus* females fed with amino acid-rich nectar (*filled dots, solid lines*; $R^2 = 0.85$, $N = 30$, $P < 0.001$) versus females receiving no amino acids (*empty dots*, *dashed lines*; $R^2 = 0.79$, $N = 29$, $P < 0.001$) both decrease over oviposition period. There was no difference between high- and low-quality larval food groups.

Time effects over oviposition period

The reproductive traits number of eggs laid, progeny's larval hatching mass and hatching success of eggs were affected differently by the measured parameters over the female oviposition period. Furthermore, regression analyses revealed how time patterns changed over the female oviposition period:

Generalized linear mixed-model analysis revealed that day of oviposition, individual and adult diet had a significant effect on the time pattern of number of eggs laid (Table 3). Furthermore, exponential regression analysis showed that the number of eggs laid of females that received no amino acids as adults decreased over female oviposition period (high/NAA & low/NAA; $R^2 = 0.79$, $N = 29$, $P < 0.001$; Figure 2). Quadratic regression analysis showed that also the number of eggs laid of the females fed amino acid-rich nectar decreased over the female oviposition period (high/AA & low/AA; $R^2 = 0.85$, $N = 30$, $P < 0.001$).

Generalized linear mixed-model analysis showed significant effects of adult diet and day of oviposition on time patterns of progeny' s larval hatching mass (Table 3). Quadratic regression analyses revealed that progeny's larval hatching mass decreased over the oviposition period in both adult feeding groups (high/AA & low/AA: $R^2 = 0.82$, $N = 28$, $P < 0.001$; high/NAA & low/NAA: $R^2 = 0.85$, $N = 29$, $P < 0.001$).

Day of oviposition, adult nectar diet and the interaction between day and adult diet affected significantly the time pattern of hatching success of eggs, whereas individual had no significant effect (Table 3). Quadratic regression analysis showed that hatching success of eggs increased over the oviposition period for AA females ($R^2 = 0.74$, $N = 24$, $P < 0.001$, Figure 3). For NAA females, linear regression analysis revealed that hatching success of eggs increased up to day twelve and decreased afterwards ($R^2 = 0.57$, $N = 27$, $P < 0.001$; Figure 3).

Figure 3

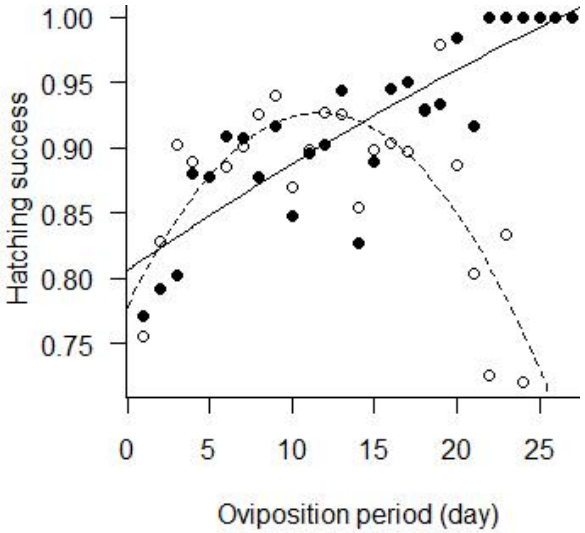

Fig. 3 Regression analyses show that hatching success of eggs from *Coenonympha pamphilus* females fed with amino acid-rich nectar (*filled dots, solid lines*; $R^2 = 0.74$, $N = 24$, $P < 0.001$) increased significantly, whereas females receiving no amino acids (*empty dots*, *dashed lines*; $R^2 = 0.57$, $N = 27$, $P < 0.001$) showed first an increase and afterwards a decrease. There was no difference between high- and low-quality larval food groups.

Discussion

Effects of nectar amino acids on butterfly reproduction

This study clearly shows that floral nectar amino acids can influence reproduction in female *Coenonympha pamphilus* butterflies. Similar to the fruit-feeding *B. anynana* females (Bauerfeind and Fischer 2009), *C. pamphilus* females receiving amino acids in their adult diet produced significantly heavier offspring (Figure 1; Tables 1, 2). Several previous studies with other butterfly species showed benefits of increased egg and larval size (Murphy et al. 1983; Braby 1994; Fischer et al. 2003; Seko and Nakasuji 2004; Fischer et al. 2006; but see Wiklund and Persson 1983; Karlsson and Wiklund 1984; Wiklund and Karlsson 1984). Furthermore, *C. pamphilus* lay their eggs not only on benign larval food plants but also on dead plant material, from which freshly hatched larvae must find new host plants (Wiklund 1984). In general, larger hatchling larvae can travel longer distances, thereby increasing the likelihood to find fresh larval food plants, as stated by Murphy et al. (1983). However, further work is required to determine the advantage of heavier larval masses in *C. pamphilus*. Furthermore, a disadvantage regarding sibling competition was found for species of Hesperioidea with bigger eggs and longer egg duration (Garcia-Barros 2000). However, this was not apparent in our study, since egg duration was the same for all treatment groups (Table 1).

In addition, we did not observe a trade-off between progeny's larval hatching mass (as a substitute for egg mass) and number of eggs in our study, an analogous result as in *A. levana* (Mevi-Schütz and Erhardt 2005). Furthermore, females raised on high-quality larval food plants laid similar numbers of eggs in the present study as did *C. pamphilus* females in another experiment (Karlsson and Wiklund 2005).

Nitrogen-rich larval food significantly affected emergence mass and the number of eggs laid (Tables 1, 2). Furthermore, emergence mass and the number of eggs laid were positively correlated. The achieved adult mass of females raised under variable larval food conditions also correlated strongly with their reproductive success in other butterfly species (Mevi-Schütz and Erhardt 2005; Bauerfeind and Fischer 2009). Low/AA females used nectar amino acids primarily to increase larval hatching mass and egg hatching success over time (Tables 1, 3; Figures 1, 3), but obviously also invested additional nitrogen from adult feeding to slightly increase the number of eggs, as indicated by the significant interaction between larval and adult diet quality on egg number.

The findings of the present study support the idea that reproduction can be increased by amino acids obtained from adult diet, and

this may even be a general feature in nectar feeding butterflies. It also demonstrates that allocation patterns of amino acids acquired from adult diet differ between different butterfly subfamilies, possibly reflecting different life history traits and strategies.

Time effects over oviposition period

In our study, the number of eggs laid over the female oviposition period decreased in all four treatment groups. Females fed with nectar lacking amino acids tended to lay more eggs early in their life, whereas females fed amino acid-rich nectar tended to extended the number of eggs over the whole oviposition period (Figure 2), possibly relying on the better adult food quality (Boggs 1997b). A corresponding result has been found in the fruit-feeding *B*. *anynana* (Bauerfeind and Fischer 2009).

The progeny's hatching mass of larvae decreased steadily with increasing female age in the present study. This result is in accordance with the decreasing egg mass produced by *C*. *pamphilus* females towards the end of their life (Wickman and Karlsson 1987) and the decreasing egg mass with increasing age of several other butterfly species (Wiklund and Persson 1983; Karlsson and Wiklund 1984; Mevi-Schütz and Erhardt 2003a, 2005; Bauerfeind and Fischer 2009; but see Bauerfeind and Fischer 2007).

However, patterns of hatching success of eggs differed considerably between females fed with or without amino acid-rich nectar (Figure 3). Hatching success of eggs steadily increased with female age for butterflies fed with amino acid-rich nectar, whereas females that received no amino acids as adults reached a peak hatching success of eggs before it decreased again. Similarly, differences in hatching success of eggs were more pronounced in later phases of the oviposition period in differently fed females of the fruit-feeding butterfly *B*. *anynana* (Geister et al. 2008). Nutrient types that are scarce or non-existent in adult food indeed seem to limit reproduction, causing it to decline to zero as juvenile stores are depleted (Boggs 1997b). For instance, nitrogen-rich pollen in the adult diet significantly prolonged lifetime fecundity and oviposition rate in female *Heliconius charitonia* butterflies (Dunlap-Pianka et al. 1977). How- ever, the differing time patterns of the hatching success over a female's oviposition period were not reflected in the quantitative analysis between treatment groups in *C*. *pamphilus* (Tables 1, 2). This seemingly contradictory finding could be caused by the fact that the potential to realize positive effects of nectar amino acids on female fecundity is truncated by the relatively short life span of *C*. *pamphilus*. This in turn may represent a life history strategy adapted to temperate, nutrient-poor habitats. It is, however, remarkable that *C*. *pamphilus* females show a resource allocation pattern which is more pronounced and more successful in longer lived butterflies living under more benign environmental conditions, such as *Charaxes fulvescens* (Molleman et al. 2008) or the tropical *Heliconius* butterflies (Dunlap-Pianka et al. 1977).

Regarding allocation patterns of larval versus adult nutrients for reproduction, *C*. *pamphilus* is a nectar-feeding species with some mature eggs at emergence and therefore an intermediate type between 'capital' and 'income' breeder (Boggs 1997a, b; Casas et al. 2005). Our study reveals a remarkably differentiated resource use strategy of *C*. *pamphilus*. Egg number depended mainly on larval resources (Table 2), but the significant effect of nectar diet quality on the number of eggs laid over female oviposition period (Table 3), and the significant interaction between adult and larval diet on the total number of eggs laid, also reveal slight effects of nectar amino acids. In contrast, only adequate adult resources (i. e. amino acid-rich nectar) increased progeny's larval hatching mass

(Tables 1, 2; Figure 1) and could compensate for a decline in hatching success of eggs in *C. pamphilus* (Fig. 3). These results support the findings of Casas et al. (2005) in the ectoparasitoid *Eupelmus vuilletti*, indicating that a strict classification of breeding types, based on resource allocation of larval reserves versus adult incoming nutrient use, may be difficult, as different reproduction parameters and nutrient use are highly variable within a single species.

Conclusions

This study shows that the uptake of amino acids in floral nectar can enhance reproduction in female *C. pamphilus* butterflies. However, resource allocation of amino acids from adult diet in combination with nitrogen gained from the larval phase may affect female reproduction in different butterfly species in different ways. Thus, further work is required to clarify if and how amino acids gained from floral nectar and adult diet in general affect butterfly reproduction. Nevertheless, the results of the present study support previous findings suggesting a coevolutionary process between butterflies and flowers dependent on butterfly pollination.

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References

Alm J, Ohnmeiss TE, Lanza J, Vriesenga L (1990) Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia 84:53–57

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164

Baker HG, Baker I (1973) Amino-acids in nectar and their evolutionary significance. Nature 241:543–545

Baker HG, Baker I (1975) Studies of nectar-constitution and pollinator-plant coevolution. In: L. E. Gilbert and P. H. Raven (eds) Coevolution of animals and plants. University of Texas Press, Austin, pp 100–140

Baker HG, Baker I (1986) The occurrence and significance of amino acids in floral nectar. Plant Syst Evol 151:175–186

Baker HG (1975) Sugar concentrations in nectars from hummingbird flowers. Biotropica 7:37–41

Bauerfeind SS, Fischer K (2007) Effects of adult nutrition on female reproduction in a fruit-feeding butterfly: The role of fruit decay and dietary lipids. J Insect Physiol 53:964–973

Bauerfeind SS, Fischer K (2009) Effects of larval starvation and adult diet derived amino acids on reproduction in a fruit-feeding butterfly. Entomol Exp Appl 130:229–237

Boggs CL (1981) Nutritional and life-history determinants of resource allocation in holometabolous insects. Am Nat 117:692–709

Boggs CL (1997a) Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. Ecology 78:192–202

Boggs CL (1997b) Reproductive allocation from reserves and income in butterfly

species with differing adult diets. Ecology 78:181–191
Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. Funct Ecol 23:27–37
Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206:83–84
Boggs CL, Ross CL (1993) The effect of adult food limitation on the life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). Ecology 74:433–441
Boggs CL, Freeman KD (2005) Larval food limitation in butterflies: effects on adult resource allocation and fitness. Oecologia 144:353–361
Braby MF (1994) The significance of egg size variation in butterflies in relation to hostplant quality. Oikos 71:119–129
Casas J, Pincebourde S, Mandon N, Vannier F, Poujol R, Giron D (2005) Lifetime nutrient dynamics reveal simultaneous capital and income breeding in a parasitoid. Ecology 86:545–554
Carvalho GB, Kapahi P, Benzer S (2005). Compensatory ingestion upon dietary restriction in *Drosophila melanogaster*. Nat Methods 2: 813–815
Crawley MJ (2007) The R book. John Wiley & Sons Ltd, West Sussex, England
Dadd RH (1973) Insect nutrition: Current developments and metabolic implications. Annu Rev Entomol 18:381–420
Dunlap-Pianka H, Boggs CL, Gilbert LE (1977) Ovarian dynamics in heliconiine butterflies: programmed senescence versus eternal youth. Science 197:487–490
Ebert G, Rennwald E (1991) Die Schmetterlinge Baden-Württembergs. Eugen Ulmer, Stuttgrad, Germany
Engleman F (1984) Ecological Entomology. Wiley, New York
Erhardt A (1991) Nectar sugar and amino acid preferences of *Battus philenor* (Lepidoptera, Papilionidae). Ecol Entomol 16:425–434
Erhardt A (1992) Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). Oecologia 90:581–585
Erhardt A, Rusterholz HP (1998) Do peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds? Oecologia 117:536–542
Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2003) Fitness consequences of temperature-mediated egg size plasticity in a butterfly. Funct Ecol 17:803–810
Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2006) Do mothers producing large offspring have to sacrifice fecundity? J Evolution Biol 19:380–391
Fischer K, Zwaan BJ, Brakefield PM (2002) How does egg size relate to body size in butterflies? Oecologia 3:375–379
Garcia-Barros E (2000) Body size, egg size and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperoidea). Biol J Linn Soc 70:251–284
Geister TL, Lorenz MW, Hoffmann KH, Fischer K (2008) Adult nutrition and butterfly fitness: effects of diet quality on reproductive output, egg composition, and egg hatching success. Front Zool 5:10
Goverde M, Erhardt A (2003) Effects of elevated CO2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyridae). Global Change Biol 9:74–83
Hill CJ (1989) The effect of adult diet on the biology of butterflies. II. The common crow butterfly, *Euploea core corinna*. Oecologia 81:258–266
Jervis MA, Boggs CL (2005) Linking nectar amino acids to fitness in female butterflies. Trends Ecol Evol 20:585–587
Karlsson B (1998) Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. Ecology 79:2931–2940

Karlsson B, Wiklund C (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly, *Lasiommata megera*. Oikos 43:376–385

Karlsson B, Wiklund C (2005) Butterfly life history and temperature adaptations; dry open habitats select for increased fecundity and longevity. J Anim Ecol 74:99–104

Koch M (1991) Schmetterlinge. Neumann Verlag, Radebeul, Germany. pp 138–139

Lüttge U (1961) Über die zusammensetzung des nektars und den mechanismus seiner sekretion. I. Planta 56:189–212

Mattson, WJ (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol Evol S 11:119–161

Mevi-Schütz J, Erhardt A (2002) Can *Inachis io* detect nectar amino acids at low concentrations? Physiol Entomol 27:256–260

Mevi-Schütz J, Erhardt A (2003a) Larval nutrition affects female nectar amino acid preference in the map butterfly (*Araschnia levana*). Ecology 84:2788–2794

Mevi-Schütz J, Erhardt A (2003b) Effects of nectar amino acids on fecundity of the wall brown butterfly (*Lasiomata megera* L.). Basic Appl Ecol 4:413–421

Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long awaited link. Am Nat 165:411–419

Mevi-Schütz J, Goverde M, Erhardt A (2003) Effects of fertilization and elevated CO2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L. Behav Ecol Sociobiol 54:36–43

Molleman F, Ding J, Wang J, Zwaan BJ, Carey JR, Brakefield PM (2008) Adult diet affects lifespan and reproduction of the fruit-feeding butterfly *Charaxes fulvescens*. Entomol Exp Appl 129:54–65 doi:

Moore RA, Singer MC (1987) Effects of maternal age and adult diet on egg weight in the butterfly *Euphydryas editha*. Ecol Entomol 12:401–408

Murphy DD, Launer AE, Ehrlich PR (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. Oecologia 56:257–263

Nakasuji F, Kimura M (1984) Seasonal polymorphism of egg size in a migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperiidae). Kontyu 52:253–259

O'Brien DM, Boggs CL, Fogel ML (2002) Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. PNAS 99:4413–4418

O'Brien DM, Boggs CL, Fogel ML (2003) Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. Proc R Soc Lond B 270:2631–2636

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

Rusterholz HP, Erhardt A (1998a). Interactions of butterflies with their nectar plants under present conditions and elevated levels of atmospheric CO2. PhD, dissertation, Department of Integrative Biology, Section Conservation Biology (NLU), University of Basel, Basel, Switzerland

Rusterholz HP, Erhardt A (1998b). Effects of elevated CO2 on flowering phenology and nectar production in important nectar plants for butterflies of calcareous grasslands. Oecologia 113:341–349

Schoonhoven LM, Van Loon JJA, Dicke M (2006) Insect – plant biology. 2nd edition, Oxford University Press, Oxford, UK

Seko T, Nakasuji F (2004) Effect of egg size variation on survival rate, development and fecundity of offspring in a migrant skipper, *Parnara guttata guttata* (Lepidoptera : Hesperiidae). Appl Entomol Zool 39:171–176

Simpson SJ, Raubenheimer ED (1993) A multi-level analysis of feeding behaviour:

the geometry of nutritional decisions. Phil Trans R Soc Lond B 342: 381–402

Simpson SJ, Raubenheimer ED (2007). Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. J Gerontol 62A: 707–713

Wickman P-O, Karlsson B (1987) Changes in egg colour, egg weight and oviposition rate with the number of eggs laid by wild females of the small heath butterfly, *Coenonympha pamphilus*. Ecol Entomol 12:109–114

Wiklund C (1984) Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. Oecologia 63:23–29

Wiklund C, Kaitala A, Lindfors V, Abenius J (1993) Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). Behav Ecol Sociobiol 33:25–33

Wiklund C, Kalrsson B (1984) Egg Size Variation in Satyrid Butterflies: Adaptive vs Historical, "Bauplan", and Mechanistic Explanations. Oikos 43:391–400

Wiklund C, Persson A (1983) Fecundity, and the relation of egg weight to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? Oikos 40:53–63

Ziegler H (1956) Untersuchungen über die leitung und sekretion der assimilate. Planta 47:447–500

Chapter 3

Nectar amino acids enhance male butterfly reproduction

Abstract

After over 30 years of research, it was recently shown that nectar amino acids increase female butterfly fecundity. However, little attention has been paid to the effect of nectar amino acids on male butterfly reproduction. Here, we show that larval food conditions (nitrogen-rich vs. nitrogen-poor host plants) and adult diet quality (nectar with or without amino acids) affected the amount of consumed nectar in *Coenonympha pamphilus* males. Furthermore, amino acids in the nectar diet of males increased progeny's larval hatching mass, irrespective of paternal larval reserves. Our study takes the whole reproductive cycle of male butterflies into account, and also considers the role of females in passing male nutrients to offspring, as males' realized reproduction was examined indirectly via nuptial gifts, by female performance. With this comprehensive approach, we demonstrate for the first time that nectar amino acids can improve male butterfly reproduction, supporting the old postulate that nectar amino acids generally enhance butterfly fitness.

Keywords: Butterfly reproduction, Larval feeding, Lepidoptera, Pollination

Introduction

Floral nectar is the most common and widespread adult butterfly food source (Gilbert and Singer 1975), and provides sugars, water, minerals, and amino acids for pollinators (Ziegler 1956; Lüttge 1961; Baker and Baker 1986). Furthermore, female *Heliconius* butterflies can utilize amino acid-rich pollen (Gilbert 1972; Dunlap- Pianka et al. 1977; O'Brien et al. 2003), and fruit-feeding *Bicyclus anynana* females can use amino acids from their adult diet to increase their reproduction (Bauerfeind and Fischer 2009; but see Bauerfeind and Fischer 2005). However, female butterflies of nectar-feeding species have for long been thought to be unaffected by nectar amino acids (Murphy 1983; Moore and Singer 1987; Hill 1989; Hill and Pierce 1989; O'Brien et al. 2000; Romeis and Wäckers 2002; Mevi-Schütz and Erhardt 2003), although butterfly-pollinated flowers contain higher levels of amino acids than flowers that are pollinated, for instance, by bees (Baker and Baker 1986). Only recently, it has also been shown that nectar amino acids can increase female butterfly reproduction (Mevi-Schütz and Erhardt 2005; Cahenzli and Erhardt 2012a). In contrast, effects of nectar amino acids on male butterfly reproduction are unknown.

The amount of acquired nitrogen is a key factor for fitness and reproduction in insects (Schoonhoven et al. 2006), and nitrogen is primarily acquired during the larval phase (Boggs 1997; O'Brien et al. 2002). However, larval host plants often do not provide optimal amounts of nitrogen (Schoonhoven et al. 2006). To maximize fitness, nutrients are required in optimal levels according to an animal's stage of development and current environmental circumstances (Simpson and Raubenheimer 1993). Furthermore, allocation patterns of nutrients at different developmental stages are not independent of each other, and the importance of larval reserves declines with increasing quality of adult nutrition (Boggs 2009). In Lepidoptera, spermatophores of males are an additional nutritional resource for females (Boggs and Gilbert 1979). However, spermatophores of Lepidoptera are a costly physiological product built from limited resources (Oberhauser 1988), and the common assumption that males have an almost unlimited reproductive capacity seems to be obsolete in

Lepidoptera (Lewis and Wedell 2007). This suggests that male butterflies have a demand for amino acid-rich food sources in their adult diet (e.g., floral nectar) in order to supplement larval resources for the production of spermatophores. Furthermore, radiotracer studies of several butterfly species demonstrated that amino acids acquired during male larval and adult feeding built into spermatophores can enhance female fitness and reproduction (Boggs and Gilbert 1979; Boggs and Watt 1981; Boggs 1981; Wedell 1996; Wiklund et al. 1993; Karlsson 1998). Thus, male and female resource budgets can be linked via nuptial gifts transferred to females at mating (Boggs 2009). Hence, the role of female butterflies in passing male nutrients to offspring should be included in analyzing male butterfly reproduction. We therefore examined males' realized reproduction indirectly via nuptial gifts, by female performance, in an analogous way as in a previous study (Cahenzli and Erhardt 2012b). This approach allowed for a more realistic assessment of male contributions to offspring than purely measuring male reproductive traits such as spermatophore mass or nitrogen content, as no relationship was found between ejaculate mass and protein content of ejaculates produced by *Pieris rapae* and *P. brassicae* males mating for the first time (Bissoondath and Wiklund 1996), and as spermatophore mass did not influence female reproductive output in several other butterfly species (Jones et al. 1986; Oberhauser 1989; Svärd and Wiklund 1991; Ward and Landolt 1995) and was therefore not a clear indicator for realized fecundity. In addition, amino acids from spermatophores are not necessarily invested in egg production, but can also be incorporated into female somatic tissue (Boggs and Gilbert 1979; Boggs and Watt 1981; Boggs 1981; Wedell 1996; Wiklund et al. 1993). Nevertheless, spermatophore quality can affect the amount of females' nitrogen put into reproduction (Wedell 1996).

Given the many indications that male butterflies may benefit from additional amino acids in their adult diet due to significant investments in reproduction, the objective of the present study was to investigate effects of nectar amino acids on the reproduction of male butterflies, taking into account male larval reserves and influences of females on passing male nutrients to offspring. We expected that adding amino acids in the adult diet of males enhances male and female fitness, and that this effect is more pronounced in males emerging with little larval reserves.

Materials and methods

Study species

The small heath butterfly, *Coenonympha pamphilus* L. (Lepidoptera: Satyrinae), occurs in unfertilized to lightly fertilized grasslands (Lepidopterologen-Arbeitsgruppe 1987). Larvae feed on a variety of grasses differing in nutritional quality (Goverde and Erhardt 2003), and varying resources from the larval phase may therefore be common in this species. We considered *C. pamphilus* as an appropriate study species to measure effects of nitrogen on reproduction for several reasons. First, nitrogen acquired during the whole life cycle can affect reproduction. Second, *C. pamphilus* females emerge with only 5–13 % mature eggs of their total potential egg number (Goverde et al. 2002; Goverde and Erhardt 2003). Thus, females of this species seem to have a particularly high potential to utilize male-derived nutrients from nuptial gifts for egg production. Third, *C. pamphilus* females can use nectar amino acids to increase their reproductive success (Cahenzli and Erhardt 2012a). However, *C. pamphilus* is monandrous (Wickman 1985) and males of polyandrous species invest more in spermatophores (Svärd and Wiklund 1989) and may therefore seem more appropriate to study effects of male-derived nutrients for female fitness. On the other hand, *C. pamphilus* males with their

own territories can copulate as many as four times more than non-resident males (Wickman 1985), therefore incurring non-trivial costs in producing ejaculates (Svärd 1985), and females of most butterfly species mate only once or just one to two times during their lifetime (Ehrlich and Ehrlich 1978; Svärd and Wiklund 1989).

The butterflies used in this experiment originated from 16 *C. pamphilus* females caught on an unfertilized meadow in the northern Jura mountains (Liesberg BL, Switzerland).

Plant material

Larval food plants *Festuca rubra* were grown in 750-ml plastic pots filled with untreated calcareous soil from the butterfly's origin place. Plants were grown in a greenhouse at the University of Basel with supplement sunlight (1,000 W broad spectrum, light period from 0600 to 2000 hours) during cloudy weather conditions and a day/ night cycle of 25 °C/19 °C. All pots were watered when necessary. High-quality larval food plants were obtained by fertilizing half the pots once a week with 50 ml Algoflash (N:P:K = 1:1:1; Laboratoire Algochemie Z.I. Nord, Chateau-Renault, France). The low-quality larval food plants received only water. Prior to introducing the larvae, dry leaves (drying by 80 °C for 48 h) were ground for leaf nitrogen (N) analysis using a CHN analyser (LECO instruments, model 1932; St. Joseph, MI, USA).

Larval rearing

To account for possible nitrogen allocation patterns from larval to the adult stage, and to test whether larval reserves influence potential effects of nectar amino acids on male reproduction, the nitrogen level in larval food was controlled by rearing larvae on high-quality or low-quality host plants. Male larvae from the 16 ovipositing females were randomly assigned to either the high- or low-quality larval host plants, and were reared separately in order to later trace back each butterfly to the ovipositing female (lineage). After 2 weeks, larvae were separated and kept individually in Petri dishes. We reared unsexed larvae on high- and low-quality host plants and used only females raised on low-quality larval food to minimize effects of female nutritional conditions. Females raised on high-quality plants were released. The larvae continued to receive their assigned larval food quality diet. The high-quality larval food group received an abundant supply of fertilized *F. rubra* ad libitum, whereas the low-quality larval food group was reared on unfertilized host plants. To prevent compensatory feeding of the low-quality larval group, last instar larvae received a limited quantity of unfertilized host plants (ca. 50 % of the amount of the high-quality larvae). Measuring the amount and quality of available food in the low-quality feeding treatment is necessary to avoid overestimating effects of a low nitrogen level in host plants (Carvalho et al. 2005).

Butterfly diet

Males were kept in plastic boxes (0.6 l), whereas females were placed in individual nylon mesh cages (20 cm 9 20 cm 9 40 cm). Male butterflies from the high- and low-quality larval food groups were randomly assigned to a nectar diet treatment consisting either of a nectar mimic with amino acids (AA), or without amino acids (NAA). Females were fed with nectar without amino acids to minimize effects of female nutritional conditions. Male butterflies were fed three times before they were allowed to mate once with an unrelated female. All butterflies were fed by hand (Cahenzli and Erhardt 2012a, b). Four treatment groups resulted: high-quality larval food and adult diet with amino acids (high/AA, n = 14), high-quality larval food and adult diet without amino acids (high/NAA, n = 19), low-quality larval food and adult diet with amino acids (low/AA, n = 17) and low-

quality larval food and adult diet without amino acids (low/NAA, n = 22).

A nectar mimic of the plant *Lantana camara* was used in this experiment. Although this plant does not naturally occur in the habitat of *C. pamphilus*, it was used in several former studies (Alm et al. 1990; Mevi-Schütz et al. 2003; Mevi-Schütz and Erhardt 2003, 2005; Cahenzli and Erhardt 2012a). The nectar mimic of the group fed without amino acids contained only sucrose, glucose, and fructose, whereas the diet of the amino acid-fed group corresponded to the complete nutrient spectrum of *L. camara* nectar, additionally containing nonessential and essential amino acids (for exact composition, see Alm et al. 1990).

Preliminary experiments showed that *C. pamphilus* butterflies rejected a daily feeding (Cahenzli and Erhardt 2012a, b). We therefore fed the butterflies their respective nectar diet every second day and allowed them to consume nectar until they voluntarily left the feeding station. The butterflies did not recognize the artificial feeding station as a natural nectar source. We therefore placed the butterflies beside the nectar-filled tube and dipped the rolled out proboscis with the help of a needle into the nectar mimic to initiate feeding. To measure the amount of nectar consumed we used a 100-ll Hamilton syringe.

Reproductive parameters

Butterflies were weighed within 24 h after emerging, and the longevity of each butterfly was recorded (number of days from hatching out of the pupa to death). We counted and collected all eggs of every single female every day. Eggs were placed in Petri dishes covered with nylon mesh until the larvae hatched.

Progeny's egg duration (number of days from when the egg was laid to eclosion), hatching success of eggs (number of eggs hatched per butterfly), and larval hatching mass of offspring (mg) were recorded for all eggs collected from each butterfly. Freshly hatched larvae were weighed within 24 h.

Statistical analysis

Males' larval development and reproductive traits were analyzed with mixed-effects models (Table 1) (Crawley 2007). Males' larval development was tested against the categorical variable larval food quality (low, high) and the factor lineage. Larval duration was $\log_n$ transformed.

The reproductive traits were tested against the categorical variables larval food quality (low, high) and nectar amino acid diet (AA/NAA), the continuous covariates male and female emergence mass and the average amount of nectar consumed per feeding by male butterflies, and the factor lineage (Table 1). Male longevity, egg hatching success and egg duration were analyzed with generalized linear mixed-effects models due to non-normal data structure (Crawley 2007). The average amount of nectar mimic consumed was tested against the categorical variables larval food quality and nectar diet and the continuous covariate male emergence mass. To test if males fed with amino acid-rich nectar consumed more nectar than males fed nectar lacking amino acids, irrespective of male emergence mass, the continuous variable 'amount of consumed nectar' was divided by male emergence mass and analyzed with a two-sided t test. Furthermore, a mixed-effects model with temporal pseudoreplication (repeated measures on the same females) was used to test if larval hatching mass changed over female oviposition period (Crawley 2007). The model used day of oviposition and individual and males' larval and adult diet as factors.

There was no significant difference in average amount of nectar mimics consumed among females paired with males from different treatment groups ($F_{1,52}$ = 1.03, P = 0.4). Therefore, the continuous variable females' amount of consumed nectar was not incorporated in

Table 1: Effects of larval diet quality (larval), male emergence mass (♂ EM), nectar quality (nectar), amount of consumed nectar (amount), lineage (L) and female emergence mass (♀ EM) on reproduction of male *Coenonympha pamphilus* butterflies. *F* values, *P* values and the effect size (*R*) are presented

		larval	♂ EM	nectar	amount	L	♀ EM	Effect direction
Egg number	*F*	0.80	0.06	1.89	0.003	2.32	5.02	Heavy ♀ > Light ♀
(*df* = 52)	*P*	0.37	0.80	0.18	0.96	0.15	**0.03**	
	R	0.12	0.03	0.19	0.01	0.39	0.30	
Egg duration	*F*	4.29	0.45	0.23	0.07	0.38	0.33	High > Low
(*df* = 52)	*P*	**0.04**	0.63	0.96	0.80	0.55	0.57	
	R	0.19	0.09	0.01	0.07	0.21	0.11	
Egg hatching	*F*	1.03	0.65	3.54	1.30	0.35	0.31	
success	*P*	0.31	0.42	0.066	0.26	0.56	0.58	
(*df* = 52)	*R*	0.14	0.12	0.14	0.18	0.21	0.10	
Larval hatching	*F*	1.15	0.19	4.60	0.72	0.09	1.35	AA > NAA (until
mass	*P*	0.29	0.66	**0.037**	0.40	0.76	0.25	day 6 of oviposition
(*df* = 52)	*R*	0.14	0.06	0.28	0.12	0.08	0.16	period)
Amount	*F*	61.22	0.22	5.80	-	0.15	-	High > Low,
(*df* = 54)	*P*	**<0.001**	0.64	**0.019**	-	0.70	-	AA > NAA
	R	0.73	0.06	0.31	-	0.11	-	
♂ Longevity	*F*	1.87	0.33	0.18	3.42	0.76	-	
(*df* = 53)	*P*	0.18	0.57	0.67	0.074	0.40	-	
	R	0.18	0.08	0.06	0.25	0.23	-	

Note: Larval diet is high-quality (High) versus low-quality (Low) and nectar is containing amino acids (AA) or lacking amino acids (NAA). $R = \sqrt{(F)}\ (\sqrt{(F+df)})^{-1}$. Lineage (*df* = 13).

the analyses of reproductive parameters. A stepwise model reduction was employed, with the least significant inter- action always removed first (Crawley 2007). Two-sided t tests were performed between treatment groups.

Correlation analysis was used to examine if female emergence mass affected total number of eggs laid and longevity positively or negatively, and to characterize the relationship between longevity and the average amount of consumed nectar. We also used correlation analysis to detect a possible trade-off between the total number of eggs laid and progeny's larval hatching mass. All statistical analyses were calculated with R Statistical Software (v.2.9.1; R Development Core Team 2009).

Results

Male larval development

Fertilized larval host plants (6.80 ± 0.07 g N / 100 g dry weight) had a significantly higher nitrogen content than unfertilized plants (5.35 ± 0.18 g N / 100 g dry weight, $t_{19.94}$= 7.59, $P < 0.001$). Larval host plant quality significantly affected larval duration of males ($F_{1,55}$ = 96.66, $P < 0.001$), whereas lineage had no significant effect ($F_{1,13}$ = 0.03, P = 0.9). Males reared on high-quality host plants had a significantly shorter larval duration (27.12 ± 3.31 days) than males reared on low-quality host plants (37.51 ± 5.74 days, t = 9.62, N = 72, $P < 0.001$).

Male emergence mass was significantly affected by larval diet quality ($F_{1,55}$ = 27.72, $P < 0.001$), but lineage had no significant effect ($F_{1,13}$ = 0.001, $P > 0.99$). Males reared on high-quality host plants had a significantly higher emergence mass (25.66 ± 5.16 mg) than males reared on

Table 2: Treatment means of reproductive parameters for *Coenonympha pamphilus* butterflies.

	Low/NAA			Low/AA			High/NAA			High/AA		
Egg number	74.05	±	34.82	57.53	±	36.72	73.42	±	24.33	74.36	±	23.44
Egg duration (days)	5.99	±	0.32	6.06	±	0.29	6.27	±	0.41	6.26	±	0.33
Egg hatching success	0.92	±	0.06	0.90	±	0.18	0.90	±	0.13	0.86	±	0.17
Larval hatching mass (mg)	0.182	±	0.016	0.193	±	0.019	0.180	±	0.023	0.187	±	0.023
♂ Nectar consumption (µl)	4.61	±	0.74	4.89	±	0.63	6.21	±	1.17	7.00	±	1.40
♂ longevity (days)	18.73	±	10.46	18.79	±	13.53	23.23	±	15.05	18.33	±	9.77
♀ longevity (days)	17.63	±	9.01	15.40	±	9.50	18.80	±	7.19	18.14	±	5.72

Note: Male butterflies were raised on a low- or high-quality larval diet ('Low' and 'High', respectively) and fed a nectar mimic with (AA) or without (NAA) amino acids and were mated once with an unrelated female reared on low-quality host plants fed with nectar lacking amino acids (means ± SE).

low-quality host plants (20.10 ± 4.28 mg, $t = 5.51$, $N = 72$, $P < 0.001$).

Male reproduction

Total number of eggs laid was significantly affected by female emergence mass, whereas male's nectar quality, male's larval food quality, male emergence mass, the average amount of male's consumed nectar and lineage had no significant effect (Table 1, 2). Heavier females laid more eggs than lighter females ($r = 0.30$, $N = 72$, $P = 0.02$).

Progeny's egg duration was not significantly affected by male's nectar diet quality, male emergence mass, male's average amount of nectar mimic consumed, female emergence mass or lineage, whereas male's larval food quality had a significant effect (Table 1, 2). Egg duration of progeny descending from males that had been reared on high-quality host plants (6.25 ± 0.07 days) was significantly longer than from males that had been reared on low-quality host plants (6.02 ± 0.05 days, $t = 2.89$, $N = 72$, $P = 0.005$).

Hatching success of eggs was not significantly influenced by male's larval food quality, male emergence mass, male's average amount of nectar mimic consumed, female emergence mass or lineage, whereas male's nectar diet quality had a marginal effect (Table 1, 2). However, there was no significant difference between hatching success of eggs of females mated to males fed with nectar containing (0.88 ± 0.03) or lacking amino acids (0.91 ± 0.02, $t = 0.94$, $N = 72$, $P = 0.35$).

Nectar diet quality of males had a significant effect on progeny's larval hatching mass, whereas male's larval food quality, male emergence mass, the males' average amount of consumed nectar, female emergence mass and lineage had no significant effect (Table 1, 2). Females mated to males fed with amino acid-rich nectar produced marginally heavier larvae (0.190 ± 0.004 mg) than females mated to males fed with nectar lacking amino acids (0.181 ± 0.003 mg, $t = 1.84$, $N = 72$, $P = 0.07$). Mixed-model analysis with temporal pseudoreplication showed a significant effect of day of oviposition on the time pattern of progeny' s larval hatching mass ($t = 15.73$, $N = 72$, $P < 0.001$), whereas adult diet had a marginal effect ($t = 1.93$, $N = 72$, $P = 0.058$). Individual ($t = 0.40$, $N = 72$, $P = 0.69$) and larval diet ($t = 0.68$, $N = 72$, $P = 0.50$) had no significant effect. During the first six days of female oviposition period, larvae descending from males fed with amino acid-rich nectar (0.195 ± 0.004 mg) were significantly heavier than larvae descending from males fed with nectar lacking amino acids (NAA: 0.186 ± 0.003 mg, $t = 2.01$, $N = 72$, $P = 0.049$), whereas there was no difference after day six (AA: 0.175 ± 0.004 mg, NAA: 0.174 ± 0.004 mg, $t = 0.12$, $N = 72$, $P = 0.91$, Figure 1). There was no trade-off between number of eggs and progeny's larval hatching mass in the offspring of males fed high-quality larval food ($r = 0.11$, $N = 33$, $P = 0.55$), whereas there was a marginal trade-off in the offspring of

Figure 1

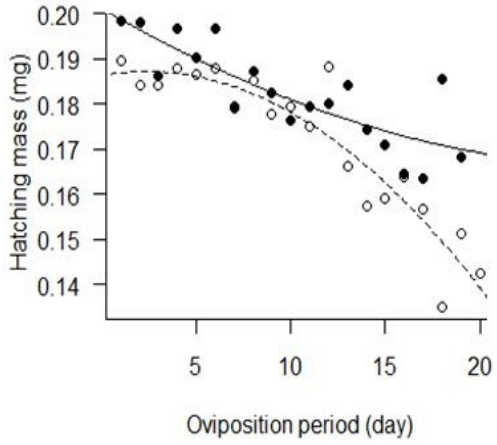

Fig. 1 Mixed-model analysis with temporal pseudoreplication showed a significant effect of day of oviposition on time patterns of progeny' s larval hatching mass in *Coenonympha pamphilus* (t = 15.73, N = 72, P < 0.001). During the first six days of female oviposition period, larvae descending from males fed with amino acid-rich nectar were significantly heavier than larvae descending from males fed with nectar lacking amino acids (t = 2.01, N = 72, P = 0.049), whereas there was no difference after day six (t = 0.12, N = 72, P = 0.91).

males that had been reared on low-quality host plants, since females that produced lighter larvae tended to lay more eggs (r = 0.57, N = 39, P = 0.06).

Male's nectar consumption was not significantly affected by male emergence mass and lineage. Male's larval food quality and nectar diet quality had a significant effect on nectar consumption (Table 1, 2). Males that had been reared on high-quality larval host plants (6.55 ± 0.23 µl) consumed significantly more nectar per feeding than males that had been reared on low-quality host plants (4.73 ± 0.11 µl, t = 7.74, N = 72, P < 0.001), and males consumed significantly more amino acid-rich nectar (0.28 ± 0.01 µl nectar /mg body mass) than nectar lacking amino acids (0.24 ± 0.01 µl nectar /mg body mass, t = 2.54, N = 72, P = 0.014).

Longevity of male butterflies was not significantly affected by nectar diet quality, male's larval food quality, emergence mass or lineage, whereas the average amount of consumed nectar mimic had a marginal effect (Table 1, 2). However, there was no correlation between average amount of consumed nectar mimic and longevity (r = -0.16, N = 72, P = 0.30).

Longevity of female butterflies was influenced by female emergence mass ($F_{1,52}$ = 6.22, P = 0.017); emergence mass and longevity were positively correlated (r = 0.37, N = 72, P = 0.003), since heavier females lived longer than lighter females. Male's adult nectar diet quality ($F_{1,52}$ = 2.07, P = 0.16), male emergence mass ($F_{1,52}$ = 2.40, P = 0.13), the male's average amount of nectar mimic consumed ($F_{1,52}$ < 0.01, P = 0.95) and lineage ($F_{1,52}$ = 1.32, P = 0.27) had no significant effect. Male's larval food quality had a marginal effect on female longevity ($F_{1,52}$ = 2.99, P = 0.09); females mated with males that had been reared on high-quality larval host plants tended to live longer than females mated with males that had been reared on low-quality larval host plants.

Discussion

Effects of larval food conditions on male butterfly reproduction

In this experiment, larval food quality significantly affected male emergence mass, but had no significant effects on the total number of eggs laid, progeny's larval hatching mass, and hatching success of eggs (Table 1), suggesting that resources acquired during the larval phase play only a limited role for male reproduction in *C. pamphilus*. In contrast, initial spermatophore mass was related to male emergence mass in other butterfly species (Oberhauser 1988; Bissoondath and Wiklund 1996; but see Svärd 1985). However, larval food conditions significantly affected males' larval duration and emergence mass, and by hatching earlier and with a larger body size, the probability of occupying a territory increases, resulting in higher mating success, since larger males in

territories mate significantly more often than males without a territory (Wickman 1985). Thus, larval food conditions may still have fitness-relevant effects.

Males' larval food quality significantly affected nectar consumption (Table 1), and heavier males consumed more nectar than lighter males. Bigger males can ingest more food per feeding, and a bigger body size requires more energy for somatic maintenance. But larval food quality of males also affected their nectar consumption independently from emergence mass, indicating that larval and adult feeding in holometabolous insects are interconnected (Boggs 1997).

We did not find a trade-off between the number of eggs and progeny's larval hatching mass in females mated with males fed high-quality larval food, whereas a marginal trade-off appeared in females mated with males that had been reared on low-quality larval host plants, since females that laid more eggs tended to produce lighter larvae, indicating a lower spermatophore quality or a reduced nutrient quantity in spermatophores secreted by males that had been reared on low-quality larval host plants. Resource allocation differs under unconstrained, benign conditions and stressful, resource-poor environments (Boggs 2009).

Effects of nectar amino acids on male butterfly reproduction

Male reproductive success is influenced not only by larval resources but also by adult food conditions (Cahenzli and Erhardt 2012b). In our study, hatching mass of larvae descending from males fed with amino acid-rich nectar was increased during the first 6 days of female oviposition period. It is likely that males used nectar amino acids to enhance spermatophore quality, and that females reacted to better spermatophore quality by increasing larval hatching mass. However, larval hatching mass did not differ anymore between treatment groups (AA vs. NAA) after day 6 of the female oviposition period, suggesting that females had depleted potential male resources (Figure 1). Previous studies showed that female butterflies can incorporate male-derived nutrients almost immediately into eggs (Boggs and Gilbert 1979; Boggs and Watt 1981; Boggs 1997). However, *C. pamphilus* males transfer relatively small ejaculates, corresponding to a mere 1.5 % of male body mass (Svärd and Wiklund 1989). The present findings are all the more relevant because they show that nectar-derived nutrients can improve male reproductive success even in a species where males deliver small nuptial gifts, documenting a nutritional pathway likely also present in other butterfly species producing bigger spermatophores than *C. pamphilus* and potentially relying more on nuptial gifts.

Several previous studies with other butterfly species showed benefits of increased egg and larval size (Murphy et al. 1983; Braby 1994; Fischer et al. 2003, 2006; Seko and Nakasuji 2004; but see Wiklund and Persson 1983; Wiklund and Karlsson 1984). *C. pamphilus* females like other Satyrid butterflies often lay their eggs on unfavorable plant material, and freshly hatched larvae must find better host plants (Wiklund 1984). In general, larger hatchling larvae can travel longer distances, thereby increasing the likelihood to find appropriate larval food plants (Murphy et al. 1983). Thus, floral nectar amino acids in butterfly diet may increase the reproductive success of males by inducing heavier larval hatching mass of offspring.

In a previous study, Lederhouse et al. (1990) found that a combination of electrolytes and amino acids enabled males of *Papilio glaucus* to produce more offspring than control males. However, actual effects of amino acids on offspring were not tested in that study. Furthermore, Beck (2007) found that males of some tropical butterfly species fed an amino acid-rich sucrose solution lived significantly longer. However, the amino acid concentration used in that study was

at an unnatural high level, and the amount of consumed nectar was not measured.

In nectar preference tests carried out so far, *C. pamphilus* males did not discriminate between nectar containing or lacking amino acids (Mevi-Schu¨tz et al. 2003). In the present study, nectar quality significantly affected nectar consumption (Table 1), and males fed amino acid-rich nectar consumed significantly more nectar than males fed with nectar without amino acids, showing a latent preference in males for amino acids in their adult diet. It is likely that the increased nectar consumption was caused by a tendency to achieve the optimal quantity of nitrogen for maximizing fitness (Simpson and Raubenheimer 1993). However, males increasing their amount of consumed amino acid-rich nectar also ingested more carbohydrates, and female butterflies can also use nitrogen from other sources to synthesize non-essential amino acids with nectar carbohydrates (O'Brien et al. 2002).

As in a previous study (Cahenzli and Erhardt 2012b), the number of eggs produced was not significantly affected by any of the measured male reproductive parameters (Table 1). *C. pamphilus* females obviously used male-derived nutrients primarily to increase progeny's larval hatching mass rather than for increasing offspring number (Table 2; Cahenzli and Erhardt 2012b). In contrast, female larval reserves clearly affected egg number (Table 1; Cahenzli and Erhardt 2012a, b). Thus, nutrients acquired in the larval stage are also used as reproductive endowment for oocytes, as also, e.g., in *Heliconius charitonius* (Dunlap-Pianka 1979).

Our study shows for the first time that nectar amino acid uptake during the adult phase has fitness relevant effects for male reproduction, and that adult feeding does not only cover energy requirements for general maintenance, including flight expenditure (Willers et al. 1987). Thus, the results of the present study support previous findings suggesting a co-evolutionary pollination process between butterflies and flowers.

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References

Alm J, Ohnmeiss TE, Lanza J, Vriesenga L (1990) Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia 84:53–57

Baker HG, Baker I (1986) The occurrence and significance of amino acids in floral nectar. Plant Syst Evol 151:175–186

Bauerfeind SS, Fischer K (2009) Effects of larval starvation and adult diet derived amino acids on reproduction in a fruit-feeding butterfly. Entomol Exp Appl 130:229–237

Beck J (2007) The importance of amino acids in the adult diet of male tropical rainforest butterflies. Oecologia 151:741–747

Bissoondath CJ, Wiklund C (1996) Male butterfly investment in successive ejaculates in relation to mating system. Behav Ecol Sociobiol 39:285–292

Boggs CL (1981) Nutritional and life-history determinants of resource allocation in holometabolous insects. Am Nat 117:692–709

Boggs CL (1997) Dynamics of reproductive allocation from juvenile and

adult feeding: radiotracer studies. Ecology 78:192–202
Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. Funct Ecol 23:27–37
Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206:83–84
Boggs CL, Watt WB (1981) Population structure of Pierid butterflies. IV. Genetic and physiological investment in offspring by male *Colias*. Oecologia 50:320–324
Braby MF (1994) The significance of egg size variation in butterflies in relation to hostplant quality. Oikos 71:119–129
Cahenzli F, Erhardt A (2012a) Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. Oecologia 169: 1005-1014
Cahenzli F, Erhardt A (2012b) Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos 121: 1417-1423
Carvalho GB, Kapahi P, Benzer S (2005). Compensatory ingestion upon dietary restriction in *Drosophila melanogaster*. Nat Methods 2: 813–815
Crawley MJ (2007) The R book. Wiley, West Sussex
Dunlap-Pianka HL (1979) Ovarian dynamics in *Heliconius* butterflies: correlations among daily oviposition rates, egg weights, and quantitative aspects of oogenesis. J Insect Physiol 25, 741-749
Dunlap-Pianka H, Boggs CL & Gilbert LE (1977) Ovarian dynamics in Heliconiine butterflies: programmed senescence versus eternal youth. Science 197: 487–490
Ehrlich AH, Ehrlich PR (1978). Reproductive strategies in the butterflies: I. Mating frequency, plugging and egg number. J Kansas Entomol Soc 51:666–697
Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2003) Fitness consequences of temperature-mediated egg size plasticity in a butterfly. Funct Ecol 17:803–810
Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2006) Do mothers producing large offspring have to sacrifice fecundity? J Evolution Biol 19:380–391
Gilbert LE (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. Proc Natl Acad Sci USA 69: 1403–1407
Gilbert LE, Singer MC (1975) Butterfly ecology. Annu Rev Ecol Evol S 6:365–397
Goverde M, Erhardt A, Niklaus PA (2002) In situ development of a satyrid butterfly on calcareous grassland exposed to elevated carbon dioxide. Ecology 83:1399–1411
Goverde M, Erhardt A (2003) Effects of elevated CO2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyridae). Glob Change Biol 9:74–83
Hill CJ (1989) The effect of adult diet on the biology of butterflies. II. The common crow butterfly, Euploea core corinna. Oecologia 81:258–266
Hill CJ, Pierce NE (1989) The effect of adult diet on the biology of butterflies: the common imperial blue, *Jalmenus evagoros*. Oecologia 81:249–257
Jones KN, Odendall FJ, Ehrlich PR (1986) Evidence against the spermatophore as paternal investment in Checkerspot Butterflies (*Euphydryas*: Nymphalidae). Am Midl Nat 116:1–6
Karlsson B (1998) Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. Ecology 79:2931–2940
Lederhouse RC, Ayres MP, Scriber JM (1990) Adult nutrition affects male virility in *Papilio glaucus* L. Funct Ecol
Lepidopterologen-Arbeitsgruppe (1987) Tagfalter und ihre lebensräume, Band 1. Schweizerischer Bund für Naturschutz. Switzerland

Lewis Z, Wedell N (2007) Effect of adult feeding on male mating behaviour in the butterfly *Bicyclus anynana* (Lepidoptera: Nymphalidae). J Insect Behav 20:201–213

Lüttge U (1961) Über die zusammensetzung des nektars und den mechanismus seiner sekretion. I. Planta 56:189–212

Mevi-Schütz J, Erhardt A (2003) Effects of nectar amino acids on fecundity of the wall brown butterfly (*Lasiomata megera* L.). Basic Appl Ecol 4:413–421

Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long awaited link. Am Nat 165:411–419

Mevi-Schütz J, Goverde M, Erhardt A (2003) Effects of fertilization and elevated CO2 on larval food and butterfly nectar amino acid preference in Coenonympha pamphilus L. Behav Ecol Sociobiol 54:36–43

Moore RA, Singer MC (1987) Effects of maternal age and adult diet on egg weight in the butterfly Euphydryas editha. Ecol Entomol 12:401–408

Murphy DD (1983) Nectar sources as constraints on the distribution of egg masses by the checkerspot butterfly, *Euphydryas chalcedona* (Lepidoptera: Nymphalidae). Environ Entomol 12:463– 466

Murphy DD, Launer AE, Ehrlich PR (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly Euphydryas editha. Oecologia 56:257–263

Oberhauser KS (1988) Male monarch butterfly spermatophore mass and mating strategies. Anim Behav 36:1384–1388

Oberhauser KS (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. Behav Ecol Sociobiol 25:237–246

O'Brien DM, Boggs CL, Fogel ML (2002) Renewable and non-renewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. Proc Natl Acad Sci USA 99:4413–4418

O'Brien DM, Boggs CL & Fogel ML (2003) Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. P Roy So B-Biol Sci 270: 2631–2636

O'Brien DM, Schrag DP & Martínez del Rio C (2000) Allocation to reproduction in a hawkmoth: a quantitative analysis using stable carbon isotopes. Ecology 81: 2822–2831

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Romeis J, Wäckers FL (2002) Nutritional suitability of individual carbohydrates and amino acids for adult *Pieris brassicae*. Physiol Entomol 27: 148–156

Schoonhoven LM, Van Loon JJA, Dicke M (2006) Insect-plant biology, 2nd edn. Oxford University Press, Oxford

Seko T, Nakasuji F (2004) Effect of egg size variation on survival rate, development and fecundity of offspring in a migrant skipper, Parnara guttata guttata (Lepidoptera : Hesperiidae). Appl Entomol Zool 39:171–176

Simpson SJ, Raubenheimer ED (1993) A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. Philos Trans R Soc Lond B 342:381–402

Svärd L (1985) Paternal investment in a monandrous butterfly *Pararge aegeria*. Oikos 45:66–70

Svärd L, Wiklund C (1989) Mass and production-rate of ejaculates in relation to monandry polyandry in butterflies. Behav Ecol Sociobiol 24:395–402

Svärd L, Wiklund C (1991) The effects of ejaculate mass on female reproductive output in the European swallowtail butterfly, *Papilio machaon* (L.) (Lepidoptera: Papilionidae). J Insect Behav 4:33–42

Ward KE, Landolt PJ (1995) Influence of multiple matings on fecundity and longevity of female cabbage looper moths (Lepidoptera: Noctuidae). Ann Entomol Soc Am 88:768–772

Wedell N (1996) Mate quality affects reproductive effort in a paternally investing species. Am Nat 148:1075–1088

Wickman PO (1985) Territorial defense and mating success in males of the small heath butterfly *Coenonympha pamphilus* L. Anim Behav 33:1162–1168

Wiklund C (1984) Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. Oecologia 63:23–29

Wiklund C, Karlsson B (1984) Egg Size Variation in Satyrid Butterflies: Adaptive vs Historical, "Bauplan", and Mechanistic Explanations. Oikos 43:391–400

Wiklund C, Persson A (1983) Fecundity, and the relation of egg weight to offspring fitness in the speckled wood butterfly Pararge aegeria, or why don't butterfly females lay more eggs? Oikos 40:53–63

Wiklund C, Kaitala A, Lindfors V, Abenius J (1993) Polyandry and its effect on female reproduction in the green-veined white butterfly (Pieris napi L.). Behav Ecol Sociobiol 33:25–33

Willers JL, Schneider JC, Ramaswamy SB (1987) Fecundity, longevity and caloric patterns in female *Heliothis virescens*: changes with age due to flight and supplemental carbohydrate. J Insect Physiol 33:803–808

Ziegler H (1956) Untersuchungen über die leitung und sekretion der assimilate. Planta 47:447–500

Chapter 4

Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction

Abstract

The principal components of floral nectar are water and the sugars sucrose, fructose and glucose. Several studies have shown the importance of nectar sugars for female butterfly fecundity, whereas to date little attention has been paid to the effect of nectar sugars on male butterfly reproduction. Clear evidence for an effect of nectar sugars on male realized reproductive success is still missing.

In this study, we fed male *Coenonympha pamphilus* butterflies nectar mimics with low (5%), medium (20%) or high (30%) total sugar concentrations with a sucrose:glucose:fructose ratio of 2.7:1.1:1. Sugar solutions were made mimicking *Knautia arvensis*, an essential nectar plant for *C. pamphilus* and many other European butterflies. Realized male reproductive success for each treatment was measured indirectly via nuptial gifts, by recording reproductive parameters and by characterizing time patterns over the oviposition period of their female partner.

Male butterflies fed high-concentrated nectar sugars had a longer lifespan than males fed low-concentrated nectar sugars. In contrast, offspring of males fed medium-concentrated nectar sugars had a higher hatching mass than progeny of males fed low-concentrated nectar sugars, indicating a trade-off between somatic maintenance and reproduction in the use of nectar sugars. Thus, allocation patterns of nectar sugars differed according to sugar concentrations in adult food.

The method used in this experiment took into account the indispensable role of female butterflies in passing male nutrients to offspring. With this comprehensive approach, we can show the general importance of nectar sugars for male butterfly fitness and support previous findings suggesting a coevolutionary process between butterflies and flowers dependent on butterfly pollination.

Keywords: butterfly reproduction; Lepidoptera; pollination; resource allocation

Introduction

Floral nectar is the most common and widespread adult butterfly food source (Gilbert and Singer 1975). In contrast to the protein-rich larval diet, nectar is composed mainly of sugars, although other nectar constituents, in particular amino acids, can also play an important role for butterfly fitness (Watt et al. 1974; Baker and Baker 1983; O'Brien et al. 2004; Mevi-Schütz and Erhardt 2005). The concentration and composition of nectar sugars varies greatly between different plant species, but sucrose, fructose and glucose are the three main sugars found in nectar (Baker and Baker 1983). The sucrose: hexoses ratio in floral nectar correlates with different pollinator types, and butterfly-pollinated flowers have high proportions of sucrose in their nectar (Baker and Baker 1983). Correspondingly, butterflies seem to generally prefer sucrose over fructose and glucose as shown in several preference tests (Erhardt 1991,1992; Rusterholz and Erhardt 1997). However, demands for nectar may vary between different butterfly species due to different energy and nutritional needs (Boggs 1997).

In Lepidoptera, one role of adult feeding is to cover energy requirements for general maintenance. For example, sucrose is used as fuel for flying (Willers et al. 1987; O'Brien 1999), and nectar sugars influence longevity (Murphy et al. 1983). The

remaining sugars, those carried over from the larval stage and those acquired as adults, can be utilized in reproduction. Several studies have shown the importance of nectar sugars on female butterfly fecundity (Norris 1935; Stern and Smith 1960; Murphy et al. 1983; O'Brien et al. 2004). In contrast, to date little attention has been paid to the effect of nectar sugars on male butterfly reproduction.

Many insects have a mating system where the male transfers nutrients in spermatophores to the female during mating, often referred to as 'nuptial gifts' (Thornhill and Alcock 1983). Spermatophores of Lepidoptera can be quite large (Rutowski et al. 1983). Since butterfly spermatophores contain water, sugars, lipids, hydrocarbons, amino acids, hormones, and sperm (Marshall 1982, 1985), they are a costly physiological product composed of limited resources (Svärd 1985; Svärd and Wiklund 1986; Oberhauser 1988, Ferkau and Fischer 2006).

Furthermore, radiotracer studies demonstrated that female butterflies use sugars from the spermatophore for both egg production and incorporation into somatic tissue (Boggs 1997). This suggests that nectar sugars may also have an effect on male butterfly reproduction. Adult food limitation reduced the longevity and tended to reduce fresh and dry mass of spermatophores in *Pieris napi* and *Bicyclus anynana* (Ferkau and Fischer 2006). In contrast, *P. rapae* males fed on a 25% sugar solution showed neither negative effects of mating history, nor on the mass or protein content of ejaculates (Bissoondath and Wiklund 1996). So far, only one study has investigated the effect of varying sucrose concentrations on male reproductive output of nectar-feeding butterflies, reporting a positive relationship between the amount of sucrose ingested and the weight of the spermatophore ejaculated by virgin males (Watanabe and Hirota 1999). However, spermatophore mass is not a clear proof for realized male fecundity. For instance, neither total lipids, nor hydrocarbons (organic compounds consisting of chain lengths from C-21 to C-37) were related to spermatophore mass in *Pseudaletia unipuncta* (Marshall and McNeil 1989), and Bissoondath and Wiklund (1996) found no relationship between ejaculate mass and protein content of ejaculates produced by *P. rapae* and *P. brassicae* males mating for the first time. Furthermore, spermatophore mass did not influence female reproductive output in *Euphydryas editha*, *E. chalcedona* (Jones et al. 1986), *Danaus plexippus* (Oberhauser 1989), *Papilio machaon* (Svärd and Wiklund 1991) and *Trichoplusia ni* (Ward and Landolt 1995). In addition, nutrients invested into male's spermatophores and realized reproduction are not strictly equal, as amino acids from spermatophores can also be partially incorporated into female somatic tissue (Boggs 1997; Wedell 1996; Wiklund et al. 1993).

The objective of the present study was to investigate the effect of nectar sugars with a natural sucrose:glucose:fructose ratio and concentration on the longevity and the realized fecundity of male *Coenonympha pamphilus* butterflies via nuptial gifts. Rather than just measuring spermatophore mass, we measured the actual realized fecundity via female performance. This method takes the indispensible role of female butterflies into consideration and demonstrates how the male investment affects realized fecundity. Furthermore, the male butterflies were fed a true nectar mimic rather than just sucrose, resulting in more natural conditions than previous experiments. We expected that high nectar sugar concentrations benefit primarily male's somatic maintenance and accordingly increase male butterfly longevity, but that nectar sugars could also affect males' realized reproduction.

Material and methods

Study species

The small heath *C. pamphilus* L. (Lepidoptera: Satyrinae) is found on

different meadow types (Lepidopterologen-Arbeitsgruppe 1987) and is a common butterfly in Eurasia. We considered *C. pamphilus* as an appropriate study species, because females emerge with only 5-13% mature eggs of their total potential egg number (Goverde, Erhardt and Niklaus 2002; Goverde & Erhardt 2003). Thus, *C. pamphilus* females seem to have a particularly high potential to utilize male derived nutrients from nuptial gifts for egg production. On the other hand, *C. pamphilus* is monandrous and males of polyandrous species have a higher capacity for producing big ejaculates than males of monandrous species (Svärd and Wiklund 1989). However, *C. pamphilus* males occupying a territory can copulate even four times more than non-resident males (Wickman 1985), incurring non-trivial costs in producing ejaculates (Svärd 1985). Furthermore, monandry is a common mating strategy in butterflies and females of most butterfly species mate only once or just two to three times during their lifetimes (Ehrlich & Ehrlich 1978; Svärd and Wiklund 1989), suggesting that monandrous mating systems are well adapted. Hence, investigating a monandrous species as a first step is a conservative approach, and further investigations with polyandrous species may even show more pronounced effects on reproduction.

Seven *C. pamphilus* females were collected from an unfertilized meadow in the northern Jura Mountains (Nenzlingen BL, Switzerland, 47° 26′ 56″ N, 7° 33′ 48″ E) and kept in cages (20 cm x 20 cm x 40 cm) for oviposition. Eggs were carefully collected (we decided to cut the netting to collect the eggs in order to minimize egg handling, as females laid their eggs directly on the cage netting) and placed in Petri dishes.

Butterfly treatment

Larvae were raised on *Festuca rubra* host plants (seeds from UFA Samen Basel, Switzerland), grown on natural nutrient-poor soil (Plantania Ansaaterde). Pupal weight, a measure for nutrient reserves accumulated during larval stage, was recorded five days after pupation (Mettler Toledo Ab 204-S, Mettler Instruments, Switzerland). Hatched males were kept in plastic boxes (0.6 l), whereas females were placed in individual nylon mesh cages.

Male butterflies were randomly assigned to a nectar diet treatment consisting either of a nectar mimic with 30% (high), 20% (medium), or 5% (low) total sugar concentration (weight of sugar to total weight of nectar) and were fed three times before they were allowed to mate once with an unrelated, freshly emerged female. Females were fed medium-concentrated nectar, as direct effects from high-concentrated nectar could have masked potential effects from male-derived nutrients, and low-concentrated nectar could have limited female reproduction. Three female treatment groups resulted: Females mated to males fed with high-concentrated nectar (high, N = 14), with medium-concentrated nectar (medium, N = 11) and with low-concentrated nectar (low, N = 13).

In this experiment, we used a nectar mimic of the plant *Knautia arvensis*, an important nectar plant for *C. pamphilus* as well as for other butterflies in Swiss Jura Mountains. The nectar mimic contained sucrose (high-quality 242.24 g sucrose per 1000 g water, medium-quality 141.30 g sucrose per 1000 g water, low-quality 29.75 g sucrose per 1000 g water), glucose (high-quality 97.60 g glucose per 1000 g water, medium-quality 56.94 g glucose per 1000 g water, low-quality 11.99 g glucose per 1000 g water) and fructose (high-quality 88.73 g fructose per 1000 g water, medium-quality 51.76 g fructose per 1000 g water, low-quality 10.90 g fructose per 1000 g water) in a sucrose:glucose:fructose ratio of 2.7:1.1:1 (Rusterholz and Erhardt 1998), but no amino acids, since nectar amino acids can enhance fecundity in butterflies (Mevi-Schütz and Erhardt 2005). The test solutions were made with sodium free substances.

Preliminary experiments showed that *C. pamphilus* butterflies rejected a daily feeding (Cahenzli and Erhardt in press). In contrast to the varying availability of floral nectar quality and quantity in nature (Rusterholz and Erhardt 1998), nectar supply at the artificial feeding station was not limited. In the present experiment, the butterflies consumed obviously enough nutrients by feeding only every second day. Furthermore, the butterflies were kept in cages, reducing their mobility and diminishing their energy consumption. We therefore fed the butterflies their respective nectar diet every second day and allowed them to consume nectar until they voluntarily left the feeding station. *C. pamphilus* butterflies did not recognize the artificial feeding station as a natural nectar source. We therefore placed the butterflies beside the nectar filled tube and dipped the rolled out proboscis with the help of a needle into the nectar mimic to initiate feeding. To measure the amount of nectar consumed, we calculated the difference between the initial amount of nectar and the amount of nectar left by the butterflies after feeding, using a 100-µl Hamilton syringe.

Fitness and reproductive parameters

Larval host plants for oviposition were offered, but in our experiment *C. pamphilus* females laid their eggs mainly on the cage netting. We recorded the longevity of each butterfly (number of days from hatching out of the pupa to death) and collected all eggs laid by cutting them out of the cage netting. Longevity is a measure for nectar sugars invested in male's own somatic maintenance, whereas total number of eggs laid, egg duration (number of days from when the egg was laid to when the larva hatched), egg hatching success (number of eggs hatched per butterfly) and larval hatching mass (mg) are a measure for nectar sugars invested in male's realized reproduction. Freshly hatched larvae were weighed within 24 h (Mettler M3, Mettler Instruments, Switzerland, accuracy ± 0.001 mg). Furthermore, we characterized relationships between female butterfly age and number of eggs laid, larval hatching success and larval hatching mass. Eggs laid on the same day by the same butterfly were placed together in Petri dishes.

Statistical analysis

Longevity and the reproductive traits total number of eggs laid, egg duration, egg hatching success and larval hatching mass were analyzed with mixed-effects models. Because related individuals (individuals from the same paternal lineage) are not independent of each other, effects and interactions were tested against the random factor lineage. The models used male's nectar sugar concentration (high, medium, low) as a factor and male and female pupal mass (as an expression of resources acquired during larval phase) and the average amount of nectar consumed per feeding of male butterflies until mating as covariates. Male's average amount of nectar mimic consumed was tested against the factor nectar diet (high, medium, low) and male pupal mass as a covariate. There was no significant difference in average amount of nectar mimics consumed among females paired with males from different treatment groups ($F_{2,35}$ = 0.97, P = 0.4). The variable female's amount of consumed nectar was therefore excluded from further analyses. A stepwise model reduction of the applied models was employed, with the least significant interaction always removed first (Crawley 2007). Tukey-Kramer's HSD comparisons ($P < 0.05$) were performed between the levels of significant factors.

We used correlation analyses to detect a possible tradeoff between number of eggs and larval hatching mass, and to examine how covariates affected longevity and reproductive parameters.

Mixed-effects models with temporal pseudoreplication (repeated measures on the same females) were used to test if day of oviposition, adult diet and larval diet affected time patterns of reproductive traits.

Number of eggs and larval hatching success over time were analyzed with generalized linear mixed models with temporal pseudoreplication due to non-normal data structure (Crawley 2007). A stepwise model reduction of the applied models was employed, with the least significant interaction always removed first (Crawley 2007). Furthermore, quadratic regressions were used to characterize trends in the time pattern over the female oviposition period (Crawley 2007). All statistical analyses were calculated with R Statistical Software (Version 2.9.1; R Development Core Team 2009). All treatment means are indicated with standard errors.

Results

Effects of nectar sugar concentration on male butterflies

Male butterfly longevity differed among treatment groups ($F_{2,35}$ = 3.67, P = 0.038; Fig. 1). Adult diet quality affected longevity significantly, whereas lineage, male pupal mass and the amount of consumed nectar had no effect (Table 1). Tukey-Kramer's HSD comparisons revealed that high-quality males lived significantly longer than low-quality-males (Fig. 1). Medium-quality males lived not significantly longer than low-quality males or significantly shorter than high-quality males (Fig. 1).

Male butterflies fed low-concentrated nectar consumed significantly more nectar than males fed medium- or high-concentrated nectar ($F_{2,35}$ = 16.00, P < 0.001; Table 2). Lineage ($F_{1,5}$ = 0.06, P = 0.82) did not affect nectar consumption, whereas male pupal mass ($F_{1,28}$ = 6.28, P = 0.02) and nectar diet quality ($F_{1,28}$ = 12.83, P < 0.001) had significant effects. Male pupal mass tended to correlated positively with the amount of nectar consumed (R = 0.28; df = 36; P = 0.08).

Figure 1

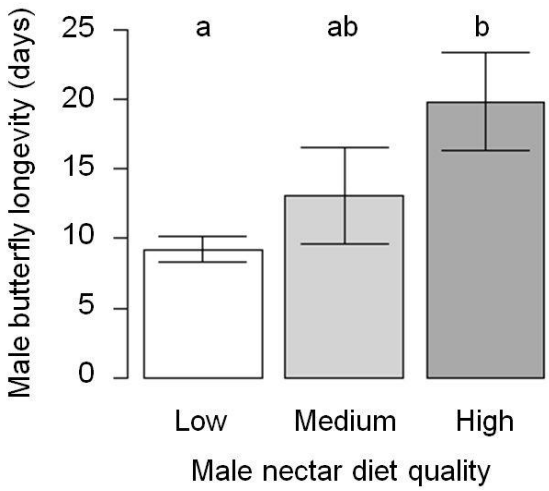

Fig. 1 Longevity of male *Coenonympha pamphilus* butterflies fed with low (5% total sugar; *white bar*), medium (20% total sugar; *light grey* bar) or high-quality (30% total sugar; *dark grey* bar) nectar. Mean ± SE. Different letters indicate significant differences for longevity.

Effects of nectar sugar concentration on reproduction

The total number of eggs laid did not differ between treatment groups ($F_{2,35}$ = 0.64, P = 0.54; Table 2). Total number of eggs laid was significantly affected by female pupal mass and male lineage, whereas male pupal mass had only a marginal effect (Table 1). Number of eggs laid differed between lineages, and female pupal mass correlated positively with egg number (R = 0.36; df = 36; P = 0.03). Male butterfly's nectar quality and the amount of nectar consumed had no significant effects (Table 1).

Egg duration ($F_{2,35}$= 0.05, P = 0.95; Table 2) and female longevity ($F_{2,35}$ = 0.77, P = 0.47; Table 2) did not differ significantly among treatment groups and were not influenced by lineage, male and female pupal mass, male's amount of nectar mimic consumed or male nectar diet quality (Table 1).

Table 1: Effects of pupal mass, male nectar diet quality, male's amount of nectar consumed and maternal lineage on reproduction of male *Coenonympha pamphilus* butterflies. *F* values, *P* values and the effect size (*R*) of the ANCOVA are presented.

	♂ Pupal mass (*df* = 1, 26)			♂ Nectar diet quality (*df* = 2, 26)			♂ Nectar amount (*df* = 1, 26)			Paternal lineage (*df* = 1, 5)			♀ Pupal mass (*df* = 1, 26)		
	F	*P*	*R*	*F*	*P*	*R*	*F*	*P*	*R*	*F*	*P*	*R*	*F*	*P*	*R*
Egg number	3.49	0.07	0.34	0.16	0.85	0.08	2.35	0.14	0.29	8.06	**0.036**	0.79	5.13	**0.03**	0.41
Egg duration	0.33	0.57	0.11	0.02	0.99	0.02	0.09	0.77	0.06	1.12	0.34	0.43	0.76	0.39	0.17
Hatching success	1.19	0.29	0.21	1.57	0.23	0.24	4.48	**0.044**	0.38	0.07	0.81	0.11	0.60	0.44	0.15
Hatching mass	0.33	0.57	0.09	3.73	**0.038**	0.35	0.66	0.43	0.16	0.21	0.67	0.20	0.94	0.34	0.19
♂ Longevity	0.004	0.95	0.01	11.09	**<0.001**	0.54	0.61	0.44	0.15	0.77	0.42	0.36	-	-	-
♀ Longevity	2.62	0.12	0.30	0.62	0.55	0.15	1.51	0.23	0.23	0.70	0.44	0.35	0.45	0.51	0.13

Note: Male nectar diet quality is shown as low (5%), medium (20%) or high (30%) total sugar concentration.
$R = \sqrt{(F)}\ (\sqrt{(F+df)})^{-1}$.

Table 2: Treatment means for reproduction of male *Coenonympha pamphilus* butterflies.

	Low (5%)			Medium (20%)			High (30%)		
Total no. eggs laid	79.62	±	7.73	89.09	±	9.18	92.21	±	7.41
Egg duration (days)	5.50	±	0.07	5.53	±	0.09	5.52	±	0.07
Hatching success	0.86	±	0.04	0.92	±	0.04	0.93	±	0.02
Nectar/feeding (µl)	8.71^{a}	±	0.82	5.22^{b}	±	0.50	5.65^{b}	±	0.31
Female Long. (days)	19.62	±	1.64	20.73	±	2.50	17.43	±	1.73

Note: Male butterflies were fed a nectar mimic with low (5% total sugar concentration), medium (20% total sugar concentration) or high (30% total sugar concentration) sugar concentration. Means ± SE. Different letters indicate significant differences among treatment groups (Tukey-Kramer HSD, $P < 0.05$).

Egg hatching success was the same for all treatment groups ($F_{2,35}$= 1.23, P = 0.31; Table 2) and was unaffected by lineage, male and female pupal mass and male nectar diet quality, whereas male butterfly's amount of nectar consumed until mating had a significant effect (Table 1). However, correlation analysis between the amount of consumed nectar and egg hatching success showed no correlation (R = 0.18; df = 36; P = 0.29).

Larval hatching mass differed significantly among treatment groups ($F_{2,35}$ = 3.94, P = 0.031; Fig. 2). Larvae from medium-quality males were significantly heavier than progeny from low-quality males (Fig. 2). Furthermore, there was no significant difference between larval hatching mass of offspring from high-quality males and medium-quality males, and between progeny from high-quality males and low-quality males (Fig. 2). Lineage, male's amount of nectar mimic consumed and male and female pupal mass did not affect mean larval hatching mass (Table 1). There was no trade-off between number of eggs and larval hatching mass (R = -0.10, N = 38, P = 0.57).

Figure 2

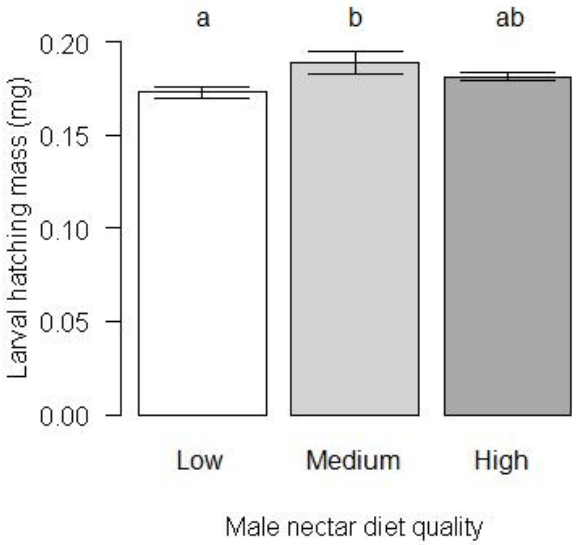

Fig. 2 Hatching mass of *Coenonympha pamphilus* larvae descending from male butterflies fed with low (5% total sugar; *white bar*), medium (20% total sugar; *light grey bar*) or high-quality (30% total sugar; *dark grey bar*) nectar. Mean ± SE. Different letters indicate significant differences in larval hatching mass.

Figure 3

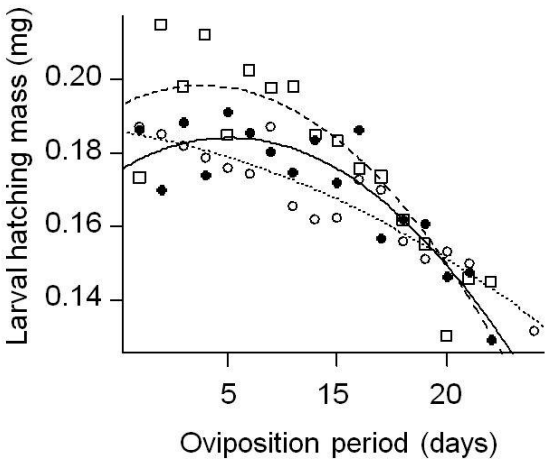

Fig. 3 Hatching mass of *Coenonympha pamphilus* larvae significantly decreases in all three paternity groups (males fed with nectar containing 30% total sugar *filled dots*, *solid line*, males receiving nectar with 20% total sugar *empty squares*, *dashed line*, males receiving nectar with 5% total sugar *empty dots*, *dotted line*) over female's oviposition period. Dots represent mean hatching mass of all larvae hatched at a certain day of the female oviposition period.

Time effects over oviposition period

Generalized linear mixed-model analysis revealed that day of oviposition, male diet quality and the interaction between male diet and day had a marginal effect on the time pattern of number of eggs laid, whereas individual had no effect (Table 3). Over the oviposition period, the number of eggs laid decreased significantly in all three treatment groups (high: R^2 = 0.67, N = 16, P < 0.001; medium: R^2 = 0.81, N = 18, P < 0.001; low: R^2 = 0.44, N = 21, P = 0.008), However, the number of eggs laid tended to decrease more slowly for females mated with medium-quality males than females mated with high-quality and low-quality males.

Table 3: Effects of day of oviposition, adult nectar diet and individual on time patterns of reproductive parameters of *Coenonympha pamphilus* butterflies. *Z* or *t* values, *P* values, degrees of freedom (*df*) and the effect size (*R*) of the ANCOVA are presented.

	Egg number				Larval hatching success				Larval hatching mass			
	z	*P*	*R*	*df*	*z*	*P*	*R*	*df*	*t*	*P*	*R*	*df*
Day of oviposition	-1.94	0.052	0.10	1,484	-1.58	0.11	0.08	1,357	11.78	**<0.001**	0.54	1,345
Individual	0.59	0.56	0.03	1,34	-0.82	0.41	0.04	1,34	1.58	0.12	0.26	1,34
♂ Adult diet	1.71	0.087	0.09	2,34	0.75	0.45	0.04	2,34	2.28	**0.01**	0.36	2,34
♂ Adult diet x day of oviposition	-1.84	0.065	0.10	2,484	-	n.s.	-		-	n.s.	-	-

Note: Male nectar diet quality is shown as low (5%), medium (20%) or high (30%) total sugar concentration.
$R = t\,(\sqrt{(t^2+df)})^{-1}$ or $z\,(\sqrt{(z^2+df)})^{-1}$.

Day of oviposition, adult diet and individual had no significant effects on the time pattern of egg hatching success over the oviposition period (Table 3). Egg hatching success was constant over oviposition period in the high- and medium-concentrated group (high: $R^2 = 0.002$, $N = 17$, $P = 1$; medium: $R^2 = 0.11$, $N = 17$, $P = 0.44$). In the low-concentrated group, egg hatching success decreased marginally ($R^2 = 0.17$, $N = 17$, $P = 0.096$).

Day of oviposition and male adult diet had a significant, whereas individual had no significant effect on time pattern of larval hatching mass (Table 3). Larval hatching mass significantly decreased over the oviposition period in the low-concentrated nectar diet group ($R^2 = 0.87$, $N = 17$, $P < 0.001$; Figure 3). In the medium- and high-concentrated group, larval hatching mass was constant until day 6, but decreased afterwards (high: $R^2 = 0.83$, $N = 17$, $P < 0.001$; medium: $R^2 = 0.80$, $N = 17$, $P < 0.001$; Figure 3).

Discussion

General effects of nectar sugars on male butterfly longevity

Male *C. pamphilus* butterflies fed with high-concentrated nectar (30%) benefited from nectar sugars by increasing their longevity (Fig. 1). Correspondingly, male *Papilio xuthus* fed with sucrose had a higher longevity than males fed only with water (Watanabe and Hirota 1999). Furthermore, food-restricted *Pieris napi* and *Bicyclus anynana* males had a significantly shorter life span than males fed with various flowers and a sucrose solution (Ferkau and Fischer 2006). As *C. pamphilus* males can mate several times throughout their life (Wickman 1985), a longer life span of males increases their chances for additional matings. Thus, increased longevity should have fitness relevant effects and could increase male butterfly reproductive success.

General effects of nectar sugars on male butterfly reproduction

Floral nectar provides primarily carbohydrates, although other nectar constituents such as amino acids can also play an important role for nectar feeding pollinators (Watt et al. 1974; Baker and Baker 1983; O'Brien et al. 2004). The results of our study clearly showed that *C. pamphilus* males used nectar sugars also directly for their reproduction (Fig. 2, Table 1). Correspondingly, spermatophore mass correlated with sucrose intake in *Papilio xuthus* (Watanabe and Hirota 1999). Furthermore, *C. pamphilus* males fed nectar containing only 5% total sugar concentration consumed significantly more nectar than males fed nectar with higher sugar concentrations (Table 2), emphasising the importance of nectar sugars for male butterflies. Increasing intake as a compensation for sugar deficient diets has also been found for other butterfly species (Hill 1989; Hill and Pierce 1989). Nevertheless, *C. pamphilus* males fed low-concentrated nectar obtained only a. 50 % (ca. 0.43 µg) of the amount of sugar compared with medium- and high-quality

males (ca. 1.04µg and 1.7 µg, respectively). Obviously, low-quality males had limited consumption abilities, restricting not only their energy budget but also their longevity and reproduction. In contrast, nuptial gifts from medium-quality males were obviously sufficient to increase egg provisioning, resulting in an increase in hatching mass of larvae, whereas number of eggs and egg hatching success were not increased (Table 1). Larvae from heavier eggs had a better survival rate and a shorter pupal period in *Parnara guttata* (Seko and Nakasuji 2004), and a better survival rate and a higher pupal mass in tropical Satyrinae (Braby 1994).

Tradeoff between direct reproduction and male longevity

Interestingly, longevity and larval hatching mass of offspring did not differ significantly between males fed with medium- or high-concentrated nectar (Fig. 1, 2). But in contrast to medium-quality males, high-quality males had a significantly longer life span than low-quality males (Fig. 1). Furthermore, in contrast to high-quality males, medium-quality males produced significantly heavier hatchlings than low-quality males (Fig. 2). Thus, it seems that moderate amounts of nectar sugars are primarily used to increase reproduction, whereas high sugar concentrations in nectar rather benefit male longevity. Consequently, individuals investing more into reproduction will have less energy available for their own somatic demands (Reznick 1985). In *P. napi,* males fed ad libitum with various flowers and sucrose lived significantly shorter after they had copulated once or twice compared to virgin males (Ferkau and Fischer 2006). On the other hand, the number of matings had no effect on the life span in male *Danaus plexippus* fed a 30% honey solution to satiation (Oberhauser 1989). However, the fact that male *C. pamphilus* butterflies increased either reproduction or longevity under an increased sugar supply in our experiment suggests a trade-off between somatic maintenance and reproduction for the use of carbohydrates from the adult diet. *C. pamphilus* butterflies visit a broad spectrum of nectar plants with varying total sugar concentrations, from medium (*Thymus serpyllum* 22.2 % weight sugar/ total weight nectar, i. e. ca. 242 g / 1000 g) to high (*Vicia cracca* 52.6 % weight sugar/ total weight nectar, i. e. ca 654 g / 1000 g; Rusterholz and Erhardt 1998), and can therefore vary the amount of sugars ingested from adult feeding according to different energy and nutritional needs (Boggs 1997).

Time effects over oviposition period

The number of eggs and the larval hatching mass decreased over the oviposition period in all three male sugar diet groups (Fig. 3). This result is in accordance with the decreasing larval hatching mass over female oviposition period in *C. pamphilus* females (Cahenzli and Erhardt in press). Egg mass also decreased with increasing female age in *Pararge aegeria* (Wiklund and Persson 1983), *Lasiommata megera* (Karlsson and Wiklund 1984) and *Araschnia levana* (Mevi-Schütz and Erhardt 2005). However, male nectar diet treatment significantly affected the time pattern of larval hatching mass. Hatching mass of larvae descending from medium- and high-quality fathers remained constant until day 6, and began decreasing afterwards, whereas the hatching mass of larvae from the low-quality fathers decreased from the beginning of the oviposition period (Fig. 3). Females used male-derived nutrients to increase larval hatching mass, but nuptial gifts from low-quality males were obviously too small to delay an immediate decline of larval hatching mass. However, the egg hatching success over the oviposition period was the same for all nectar diet treatments and did not decrease significantly over time.

Conclusion

This study shows that the uptake of sugars in floral nectar can enhance reproduction and longevity in male *C. pamphilus* butterflies, and that there is a tradeoff between these two traits. Furthermore, *C. pamphilus* females allocated additional resources derived from male nuptial gifts to increase larval hatching mass and to maintain hatching mass for almost a week.

Studies carried out so far have shown that nectar constituents like sugars, amino acids and salts can all solely enhance butterfly reproduction and fitness (Fig. 1, 2; Boggs and Jackson 1991; O'Brien et al. 2004; Mevi-Schütz and Erhardt 2005; Cahenzli and Erhardt in press). Hence, further investigations should examine if these nectar constituents interact, and how potential interactions affect male and female reproduction, as male and female reproductive budgets in butterflies are linked via nuptial gifts (Boggs 2009).

Thus, the results of the present study support previous findings suggesting a coevolutionary process between butterflies and flowers dependent on butterfly pollination.

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References

Baker HG, Baker I (1983) Floral nectar sugar constituents in relation to pollinator type. - In: Bentley, B. and Elias, T. (eds.), The biology of nectaries. Columbia University Press, New York, pp. 117–141

Bissoondath CJ, Wiklund, C. (1996) Male butterfly investment in successive ejaculates in relation to mating system. Behav Ecol Sociobiol 39:285–292

Boggs CL (1997) Reproductive allocation from reserves and income in butterfly species with differing adult diets. Ecology 78:181–191

Boggs CL (2009) Understanding insect life histories and senescence through a resource allocation lens. Funct Ecol 23:27–37

Boggs CL, Jackson LA (1991) Mud puddling by butterflies is not a simple matter. Ecol Entomol 16:123–127

Braby MF (1994) The significance of egg size variation in butterflies in relation to hostplant quality. Oikos 71:119–129

Cahenzli F, Erhardt A (2012) Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. Oecologia 169:1005-1014

Crawley MJ (2007) The R book. - John Wiley & Sons Ltd, West Sussex, England

Ehrlich AH, Ehrlich PR (1978) Reproductive strategies in the butterflies: I. mating frequency, plugging and egg number. J Kansas Entomol Soc 51:666–697

Erhardt A (1991) Nectar sugar and amino acid preferences of *Battus philenor* (Lepidoptera, Papilionidae). Ecol Entomol 16:425–434

Erhardt A (1992) Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). Oecologia 90:581–585

Ferkau C, Fischer K (2006) Costs of reproduction in male *Bicyclus anynana* and *Pieris napi* butterflies: Effects of mating history and food limitation. Ethology 112:1117–1127

Gilbert LE, Singer MC (1975) Butterfly ecology. Annl Rev Ecol Evol S 6:365–397

Goverde M, Erhardt A (2003) Effects of elevated CO_2 on development and larval food-plant preference in the butterfly

Coenonympha pamphilus (Lepidoptera, Satyridae). Global Change Biol 9:74–83

Goverde M, Erhardt A, Niklaus PA (2002) In situ development of a satyrid butterfly on calcareous grassland exposed to elevated carbon dioxide. Ecology 83:1399–1411

Hill CJ (1989) The effect of adult diet on the biology of butterflies. 2. The common crow butterfly, *Euploea core corinna*. Oecologia 81:258–266

Hill CJ, Pierce NE (1989) The effect of adult diet on the biology of butterflies 1. The common imperial blue, *Jalmenus evagoras*. Oecologia 81:249-257

Jones KN, Odendaal FJ, Ehrlich PR (1986) Evidence against the spermatophore as paternal investment in checkerspot butterflies (*Euphydryas*: Nymphalidae). Am Midl Nat 116:1–6

Karlsson B., Wiklund C (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly, *Lasiommata megera*. Oikos 43:376–385

Lepidopterologen-Arbeitsgruppe (1987) Tagfalter und ihre Lebensräume. 4. Auflage. Schweizerischer Bund für Naturschutz. Basel. Switzerland

Marshall LD (1982) Male nutrient investment in the Lepidoptera: what nutrients should males invest? Am Nat 120:273–279

Marshall LD (1985) Protein and lipid composition of *Colias philodice* and *C. eurytheme* spermatophores and their changes over time (Pieridae). J Res Lep 24:21–30

Marshall LD, McNeil JN (1989) Spermatophore mass as an estimate of male nutrient investment: A closer look in *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). Funct Ecol 3:605–612

Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long awaited link. Am Nat 165:411–419

Murphy DD, Launer AE, Ehrlich PR (1983) The role of adult feeding in egg production and population dynamics of the checkerspot butterfly *Euphydryas editha*. Oecologia 56:257–263

Norris MJ (1935) A feeding experiment on the adults of *Pieris rapae*. J Entomol Res Soc 68:125–127

Oberhauser KS (1988) Male monarch butterfly spermatophore mass and mating strategies. Anim Behav 36:1384–1388

Oberhauser KS (1989) Effects of spermatophores on male and female monarch butterfly reproductive success. Behav Ecol Sociobiol 25:237–246

O'Brien DM (1999) Fuel use in flight and its dependence on nectar feeding in the hawkmoth *Amphion floridensis*. J Exp Biol 202:441–451

O'Brien DM, Boggs CL, Fogel ML (2004) Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. Oikos 105:279–291

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0. URL http://www.R-project.org

Reznick D (1985) Costs of reproduction – an evaluation of the empirical evidence. Oikos 44:257–267

Rusterholz HP, Erhardt A (1997) Preferences for nectar sugars in the peacock butterfly, *Inachis io*. Ecol Entomol 22 220–224

Rusterholz HP, Erhardt A (1998) Flower preferences of butterflies in calcareous grasslands in the Swiss Jura mountains. - In: Rusterholz, H. P. and Erhardt A. (eds.), Interactions of butterflies with their nectar plants under present conditions and under elevated levels of atmospheric CO2. OKT Offset- und Kopierdruck, Basel, pp 59–80

Rutowski RL, Newton M, Schaefer J (1983) Interspecific variation in the size of the nutrient investment made by male butterflies during copulation. Evolution 37:708–713

Seko T, Nakasuji F (2004) Effect of egg size variation on survival rate, development

and fecundity of offspring in a migrant skipper, *Parnara guttata guttata* (Lepidoptera: Hesperiidae). Appl Entomol Zoo 39:171–176

Stern V, Smith, RF (1960) Factors affecting egg production and oviposition in populations of *Colias eurytheme* (Lepidoptera: Pieridae). Hilgardia 29:411–454

Svärd L (1985) Paternal investment in a monandrous butterfly *Pararge aegeria*. Oikos 45:66–70

Svärd L, Wiklund, C (1986) Different ejaculate delivery strategies in first versus subsequent matings in the swallowtail butterfly *Papilio machaon*. Behav Ecol Sociobiol 18:325–330

Svärd L, Wiklund (1989) Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. Behav Ecol Sociobiol 24:395–402

Svärd L, Wiklund C (1991) Effects of Sucrose Intake on Spermatophore Mass Produced by Male Swallowtail Butterfly *Papilio xuthus*. Zool Sci L 16:55–61

Thornhill R, Alcock, J (1983) The evolution of insect mating systems. London, UK: Harvard University Press

Ward KE, Landolt PJ (1995) Influence of multiple matings on fecundity and longevity of female cabbage looper moths (Lepidoptera: Noctuidae). Ann Entomol Soc Am 88:768–772

Watanabe M, Hirota M (1999) Effects of sucrose intake on spermatophore mass produced by male swallowtail butterfly *Papilio xuthus* L. Zool sci 16:55–61

Watt WB, Hoch PC, Mills SG (1974) Nectar resource use by *Colias* butterflies. Oecologia 14:353–374

Wedell N (1996) Mate quality affects reproductive effort in a paternally investing species. Am Nat 148:1075–1088

Wickman P-O (1985) Territorial defense and mating success in males of the small heath butterfly *Coenonympha pamphilus* L. Animl Behav 33:1162–1168

Wiklund C, Persson, A. (1983) Fecundity, and the relation of egg weight to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? Oikos 40:53–63

Wiklund C. et al. (1993) Polyandry and its effect on female reproduction in the greenveined white butterfly (*Pieris napi* L.). - Behav Ecol Sociobiol 33: 25–33.

Willers JL, Schneider JC, Ramaswamy SB (1987) Fecundity, longevity and caloric patterns in female *Heliothis virescens*: changes with age due to flight and supplemental carbohydrate. J Insect Physiol 33:803–808

Chapter 5

Transgenerational acclimatization in an herbivore-host plant relationship

Abstract

Twenty years ago, scientists began to recognize that parental effects are one of the most important influences on progeny phenotype. Consequently, it was postulated that herbivorous insects could produce progeny that are acclimatized to the host plant experienced by the parents to improve progeny fitness, because host plants vary greatly in quality and quantity, and can thus provide important cues about the resources encountered by the next generation. However, despite the possible profound implications for our understanding of host-use evolution of herbivores, host-race formation and sympatric speciation, intense research has been unable to verify transgenerational acclimatization in herbivore-host plant relationships.

We reared *Coenonympha pamphilus* larvae in the parental generation (P) on high- and low-quality host plants, and reared the offspring (F_1) of both treatments again on high- and low-quality plants. We tested not only for maternal effects, as most previous studies, but also for paternal effects. Our results show that parents experiencing predictive cues on their host plant can indeed adjust progeny's phenotype to anticipated host plant quality. Maternal effects affected female and male offspring, whereas paternal effects affected only male progeny. We here verify, for the first time, the long postulated transgenerational acclimatization in an herbivore-host plant interaction.

Key words: butterfly reproduction; larval feeding; Lepidoptera; maternal effects; paternal effects; phenotypic plasticity

Introduction

Over 20 years ago, scientists recognized parental effects as one of the most important influences on progeny phenotype with potential adaptive character (Kirkpatrick and Lande 1989; Mousseau and Dingle 1991) rather than as troublesome sources of variation in quantitative genetic studies (Falconer 1981). Consequently, herbivorous insects experiencing predictive cues on their host plants have been expected to produce offspring that are physiologically acclimatized to particular host plant characteristics (Via 1991; Fox et al. 1995; Spitzer 2004; Mc Lean et al. 2009). The acclimatized progeny would then benefit from parental host plant experience and improve their performance and hence increase its fitness. This theoretically postulated process is termed transgenerational conditioning or acclimatization, the most obvious way that parental effects based on environmental conditions could influence progeny in an adaptive way (Fox et al. 1995; Spitzer 2004). But although several studies found adaptive maternal effects in plants (Galloway and Etterson 2007; Sultan et al. 2009; Whittle et al. 2009), insects (Mousseau and Dingle 1991; Mousseau and Fox 1998a) and also in other animals (Yoder et al. 2006; Burges and Marshall 2011; Salinas and Munch 2012), transgenerational acclimatization of progeny to host plants has not been documented so far, in spite of many experimental attempts (Via 1991; Futuyma et al. 1993; Fox et al. 1995; Fox 1997; Spitzer 2004; Amarillo-Suarez and Fox 2006; Or and Ward 2007; Mc Lean et al. 2009). This is surprising, because host plants provide a wealth of cues about the resource quality and quantity encountered by the next insect generation (Spitzer

2004). Because transgenerational acclimatization would also have important biological implications, Mousseau and Fox (1998b) stated that more work is required in this area. For instance, transgenerational acclimatization could reduce the costs of using a novel host over several generations, affecting the evolution of host range for an insect (Spitzer 2004). Furthermore, selection for host fidelity could be increased, possibly influencing host-use evolution of herbivores, host-race formation and sympatric speciation (Fox et al. 1995; Mousseau and Fox 1998a, 1998b; Spitzer 2004).

So far, there is only limited evidence from herbivorous insects that parental host plants could have substantial effects on progeny performance (Mc Lean et al. 2009). For example, it is known that insects can alter egg provisioning on the basis of maternal host plant experience and provide their progeny with increased resources for early larval development (Awmack and Leather 2002). *Pieris rapae* females alter patterns of egg size and possibly egg provisioning based on nitrogen content in artificial diet, and offspring reared on the same nitrogen concentration as their mother had a higher larval mass at day four after eclosion (Rotem et al. 2003). However, after day four, this difference was no longer evident, and no positive effects on fitness could be detected thereafter (Rotem et al. 2003). There are also several other studies documenting adaptive maternal effects based on host plant experience, but these effects did not acclimatize offspring to specific anticipated host plant conditions (Futuyma et al. 1993; Fox et al. 1995; Amarillo-Suarez and Fox 2006). For example, larvae of the seed beetle *Stator limbatus* whose mothers were reared on *Pseudosamanea guachapele* developed faster than offspring from mothers that were raised on *Acacia greggii*, regardless of whether progeny was reared on *P. guachapele* or *A. greggii* (Amarillo-Suarez and Fox 2006). Thus, maternal experience did not adjust progeny phenotype to an anticipated host plant type, but affected offspring encountering any haphazard host plant type. Maternal effects therefore generally enhanced offspring performance, but this is not acclimatization to specific host plants, as the interaction between parental and progeny host plant effects was not significant. So far, only in the special case of aphids, which are thought to be particularly prone for trans-generational effects, because several generations are "telescoped" within a developing aphid as embryos and may therefore be affected directly by the food of their mother or grandmother (Spitzer 2004), some evidence for transgenerational acclimatization was found (De Barro et al. 1995). By contrast, other studies found no evidence for transgenerational acclimatization in aphids (Via 1991; Mc Lean et al. 2009).

We reared *Coenonympha pamphilus* larvae in the parental generation (P) on nitrogen-rich (high-quality) and nitrogen-poor (low-quality) host plants, and reared the offspring (F_1) of both treatments again on high- and low-quality plants. In two separate experiments, we tested whether males (P) and females (P) adjusted progeny performance to anticipated larval host conditions. *C. pamphilus* used in this study, as well as many other phytophagous insects, have to cope with varying host plant quality (high- versus low-nitrogen levels), nitrogen being a key nutrient for development and fitness in insects (Schoonhoven et al. 2006. The nutritional quality experienced by the parental generation (P) could therefore prepare the progeny (F_1) to optimally use the resources on similar nutritional plant quality and to improve performance and fitness.

Materials and methods

Study species

Coenonympha pamphilus L. (Lepidoptera: Satyrinae), the small heath, is a common butterfly in Eurasia and is found on various meadow types

(Lepidopterologen-Arbeitsgruppe 1987). The larvae feed on a variety of grass species (Koch 1991), differing in nutritional quality, but *Festuca rubra* is favoured (Goverde and Erhardt 2003). Seven C. pamphilus females were collected from an unfertilized meadow in the northern Jura Mountains (Nenzlingen BL 47° 26′ N, 7° 33′ E, Switzerland). The eggs laid were sorted by maternal lineage and placed in separately marked Petri dishes.

Host plants

Larval food plants of *F. rubra* were grown in 750 ml plastic pots filled with untreated calcareous soil from a nutrient-poor meadow near Liesberg BL in Switzerland. Each pot was planted with 450 seeds (UFA Samen Basel, Switzerland). Plants were grown in a greenhouse at the University of Basel, with ambient sun light in summer and supplement light (1000 W broad spectrum, light period from 06.00 to 20.00) during cloudy weather conditions and a day/night cycle of 25ºC/19 ºC. High-quality larval food plants were fertilized once a week (N : P : K = 1 : 1 : 1). The low-quality larval food plants received only water. Plant nitrogen content was analysed from eight week old grass samples using a CHN analyzer.

Silica is the main anti-herbivore defence in grasses and is more important than chemical defences in deterring herbivory on grasses (Vicari and Bazely 1993; Massey et al. 2007). Fertilization can slightly decrease the silica content in *F. rubra*, but this decrease in foliar silica content has no significant effect on larval performance in *C. pamphilus* (Cahenzli and Erhardt 2012a).

Larval rearing in the parental generation (P)

Larvae (P) descending from each of the seven wild-caught females were randomly divided into two groups, whereby one-half of the larvae was assigned to the high-quality host plants and the other half to the low-quality host plants. All lineages were included in the analysis. Larvae from different wild caught-females were reared separately in linegroups of 10 and kept singly in Petri dishes after two weeks. Light period was set from 06.00 to 20.00 with a day/night cycle of 25ºC/19 ºC. Larvae reared on high-quality host plants (High-P) received high-quality host plants ad libitum in Petri dishes, whereas food quantity of last instar larvae of the parental generation raised on low-quality host plants (Low-P) was limited to the amount ingested by High-P larvae. This approach was chosen to ensure low-nitrogen availability in Low-P larvae, as we concentrated on effects of nitrogen supply in this study. In nature, *C. pamphilus* occurs in various meadow types and feeds on a variety of grass species differing greatly in nutritional quality (Lepidopterologen-Arbeitsgruppe 1987; Koch 1991; Goverde and Erhardt 2003). Furthermore, high-silica content in low-quality host plants limits larval nitrogen acquisition (Cahenzli and Erhardt 2012a), and low-nitrogen concentration in host plants can also cause compensatory feeding in *C. pamphilus* (Cahenzli and Erhardt 2012a), resulting in underestimating effects of low-nitrogen supply (Carvalho et al. 2005).

To generate auxiliary butterflies for mating, additional larvae from the seven wild-caught females were reared on low-quality host plants fed ad libitum.

Approach to test for maternal effects

We mated 12 High-P and 12 Low-P females with auxiliary males to test for maternal effects. Twenty larvae (F_1) from each mother were assigned randomly to the high- and low-quality plants (Figure 1). All F_1 larvae were fed ad libitum, allowing them to respond unconstrained to their respective diet. Female or male offspring (F_1) from a single mother were pooled. We therefore obtained a sample size of n = 48

female offspring (F_1) and n = 48 male offspring (F_1), resulting in a total sample size of n = 96 to test for maternal effects.

Approach to test for paternal effects

We mated 12 High-P and 12 Low-P males with auxiliary females to test for paternal effects. Twenty larvae (F_1) from each father were assigned randomly to the high- and low-quality plants (Figure 1). All F_1-larvae were fed ad libitum, allowing them to respond unconstrained to their respective diet. Female or male offspring (F_1) from a single father were pooled. We therefore obtained a sample size of n = 47 female offspring (F_1) and n = 47 male

Figure 1

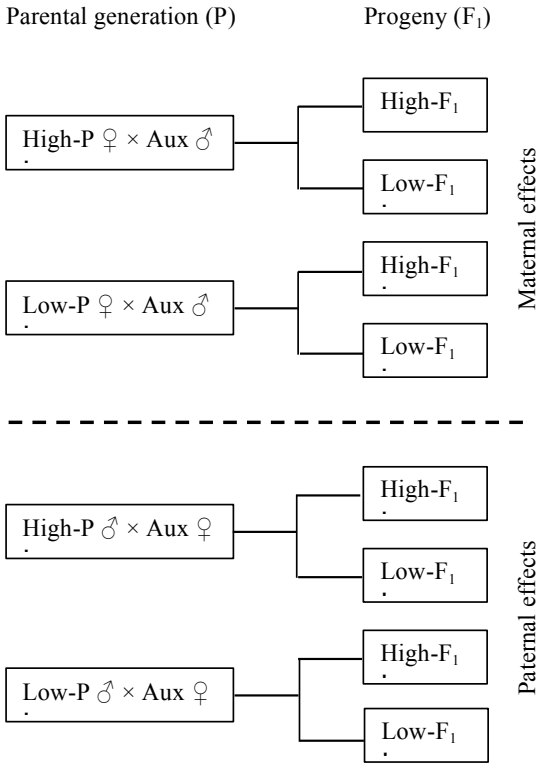

Fig. 1 Experimental flow chart. Larvae of the parental generation (P) and their progeny (F_1) were raised on high- and low-quality host plants. Adult butterflies of the parental generation were mated with auxiliary males or females (Aux) reared on low-quality host plants and fed ad libitum. Progeny (F_1) descending either from treated males or females (P) was treated and analysed separately.

offspring (F_1), resulting in a total sample size of n = 94 to test for paternal effects. The smaller sample size to test for paternal effects is due to one damaged, low-quality pot from which the larvae escaped.

Fitness parameters

We recorded larval hatching mass within 24 hours (mg), and the fitness traits larval duration (number of days from eclosion to pupation), pupal mass on the fifth day after pupation (mg) and forewing length within 24h after emergence (mm; lateral wingspan of the left forewing). We measured larval hatching mass instead of egg mass because *C. pamphilus* females frequently laid their eggs on the cage netting, although larval host plants for oviposition were offered. Weighing eggs without destroying them was therefore difficult. However, previous studies with other butterfly species showed that egg mass and larval hatching mass are tightly correlated (Karlsson and Wiklund 1984). Thus, the measured larval hatching mass in this study is a good substitute for egg mass to assess parental provisioning to offspring. Larval duration was measured because prolonged larval duration may increase the exposure time to predators, parasites and other adverse factors (Clancy and Price 1987; Williams 1999). Furthermore, pupal mass is an appropriate indicator for butterfly fitness, because male (Ferkau and Fischer 2006) and female (Cahenzli and Erhardt 2012b) pupal mass is correlated with fecundity. We measured also forewing length, because thorax mass and forewing geometry affect flight performance (Berwaerts et al. 2002), which can be important for dispersal, foraging, reproduction and predator avoidance.

Statistical analysis

Host plant nitrogen level and fitness traits in the parental generation (P) were analysed with two-sided *t*-tests. Fitness traits of progeny (F_1) were analysed with

mixed-effects models (Crawley 2007) with the categorical variables sex, host plant quality (P, F_1) and the continuous variable larval hatching mass (F_1). To account for inherited parental characteristics, the models testing for maternal effects included also the random factor maternal lineage (Crawley 2007) and the continuous covariates pupal mass or forewing length of untreated fathers, depending on the analysed fitness trait (Table 1). The models testing for paternal effects additionally included the random factor paternal lineage (Crawley 2007) and the continuous covariates pupal mass or forewing length of untreated mothers, depending on the analysed fitness trait (Table 2). A stepwise model reduction was used, with the least

Figure 2

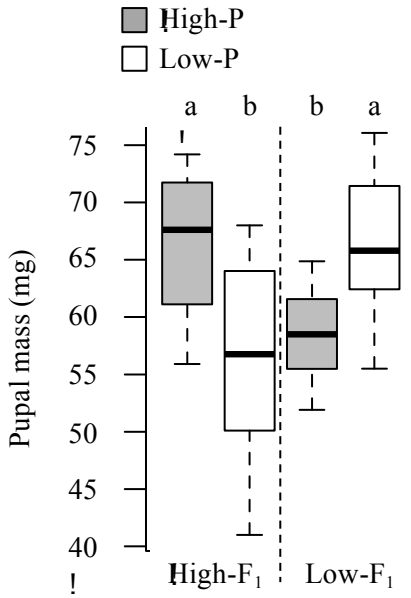

Fig. 2 Pupal mass of *Coenonympha pamphilus* females (F_1). Progeny (High-F_1 versus Low-F_1) was raised on high- and low-quality host plants and descended from mothers (High-P versus Low-P) reared on high- (grey bars) and low-quality (white bars) host plants. The box represents the interquartile range from first to third quartile; the line across the box indicates the median and the whiskers show maximum and minimum values. Different alphabets indicate significant differences between treatment groups (Tukey multiple comparison, $P < 0.05$, offspring descending from a single mother were pooled, see methods, $n = 12$ for each treatment group).

significant interactions always removed first (Crawley 2007). Larval duration and forewing length (F_1) were analysed with generalized linear mixed-effects models owing to non-normal data structure (Crawley 2007, Pinheiro and Bates 2000). Two sided *t*-tests between pooled treatment groups (progeny reared on the same versus offspring reared on the opposite host plant quality from their parents) and Tukey multiple comparisons between unpooled treatment groups (High-P/High-F_1, Low-P/High-F_1, High-P/Low-F_1, Low-P/Low-F_1) were performed. Correlation analyses were used to test for relationships between fitness traits. All statistical analyses were two-sided and *P*-values < 0.05 were considered statistically significant.

Results

Host plant quality

Fertilized *F. rubra* host plants (3.16 ± 0.05 g nitrogen per 100 gram dry weight) had significantly higher leaf nitrogen content than unfertilized host plants (1.67 ± 0.09 g nitrogen per 100 gram dry weight; $t = 15.2$, $n = 16$, $P < 0.001$).

Parental generation

Females as well as males (P) reared on high-quality host plants had a significantly shorter larval duration (females: high-quality 24.67 ± 0.64 days; low-quality 38.78 ± 1.42 days; $t = 9.1$, $n = 24$, $P < 0.001$; males: high-quality 21.23 ± 0.67 days; low-quality 30.46 ± 0.92 days; $t = 8.2$, $n = 24$, $P < 0.001$), higher pupal mass (females: high-quality 67.62 ± 1.44 mg; low-quality 45.79 ± 1.17 mg; $t = 11.8$, $n = 24$, $P < 0.001$; males: high-quality 51.69 ± 1.07 mg; low-quality 37.17 ± 0.78 mg; $t = 10.9$, $n = 24$, $P < 0.001$) and longer forewings (females: high-quality 13.11 ± 0.16 mm; low-quality 10.89 ± 0.14 mm; $t = 10.6$, $n = 24$, $P < 0.001$; males: high-quality 12.56 ± 0.14 mm; low-quality 10.36 ± 0.14 mm; $t = 8.4$, $n = 24$, $P <$

0.001) than females and males raised on low-quality host plants.

Effects of maternal experience on progeny performance

Larval duration (F_1) was significantly affected by sex and host plant quality (F_1), whereas host plant quality of mothers (P), maternal lineage and larval hatching mass had no significant effects (Table 1). Larvae reared on high-quality host plants developed faster (females: 25.66 ± 0.43 days; males: 21.51 ± 0.47 days) than larvae raised on low-quality host plants (females: 31.39 ± 1.05 days; t = 5.5, n = 48, P < 0.001; males: 25.87 ± 0.87 days; t = 4.6, n = 47, P < 0.001) irrespective of maternal host plant quality.

Pupal mass (F_1) was significantly affected by sex, maternal lineage and pupal mass of untreated fathers (P), whereas host plant quality (maternal, F_1) and larval hatching mass had no significant effects (Table 1). There was a significant maternal-host-by-progeny-host interaction (Table 1). High-P/High-F_1 and Low-P/Low-F_1 females had a significantly higher pupal mass than Low-P/High-F_1 and High-P/Low-F_1 females (Figure 2). Pupal mass of male offspring was affected less clearly by maternal experience, but pooled male progeny reared on the same (Low-P/Low-F_1 plus High-P/High-F_1) rather than the opposite (Low-P/High-F_1 plus High-P/Low-F_1) host plant quality as their mother had a higher pupal mass (t = 3.48, n = 48, P = 0.001; Figure 3a). Larval duration and pupal mass did not significantly correlate (females: R^2 = 0.02, n = 48, P = 0.39; males: R^2 = 0.00, n = 48, P = 0.73).

Forewing length (F_1) was significantly affected by sex, host plant quality (maternal, F_1) and forewing length of untreated fathers (P), whereas maternal lineage and larval hatching mass had no significant effects (Table 1). There was a significant maternal-host-by-progeny-host interaction (Table 1). Pooled progeny reared on the same (Low-P/Low-F_1, plus High-P/High-F_1) rather than the opposite (Low-P/High-F_1, plus High-P/Low-F_1) host plant quality as their mother had significantly longer forewings (females: t = 3.1, n = 48, P = 0.004; males: t = 3.1, n = 48, P = 0.003; Figure 3b). Pupal mass and forewing length were positively correlated (females: R^2 = 0.81, n = 48, P < 0.001; males: R^2 = 0.86, n = 48, P < 0.001).

Effects of paternal experience on progeny performance

Larval duration (F_1) was significantly affected by sex, host plant quality (paternal, F_1) as well by larval hatching mass, whereas paternal lineage had no significant effect (Table 2). Larvae reared on high-quality host plants developed faster (females: 25.66 ± 0.43 days; males: 21.51 ± 0.47 days) than larvae raised on low-quality host plants (females: 30.12 ± 0.73 days; t = 5.5, n = 48, P < 0.001; males: 25.24 ± 0.59 days; t = 4.6, n = 47, P < 0.001) irrespective of paternal host plant quality. Furthermore, Low-P/Low-F_1 females developed faster (28.33 ± 0.97 days) than High-P/Low-F_1 females (31.58 ± 0.85 days; Tukey multiple comparison: P = 0.03), causing a significant paternal-host-by-progeny-host interaction (Table 2).

Pupal mass (F_1) was significantly affected by sex and pupal mass of untreated mothers (P), whereas host plant quality (paternal, F_1) and larval hatching mass had no significant effects (Table 2). Paternal lineage had a marginal effect (Table 2). There was a significant paternal-host-by-progeny-host interaction (Table 2). Pupal mass of pooled male progeny reared on the same (Low-P/Low-F_1 plus High-P/High-F_1) rather than the opposite (Low-P/High-F_1 plus High-P/Low-F_1) host plant quality as their fathers was significantly higher in male offspring (t = 2.4, n = 47, P = 0.02), whereas there was no significant difference in pooled female progeny (t = 0.9, n = 47, P = 0.36; Figure 3a). Larval duration and pupal mass did not significantly correlate (females: R^2 = 0.13,

Figure 3

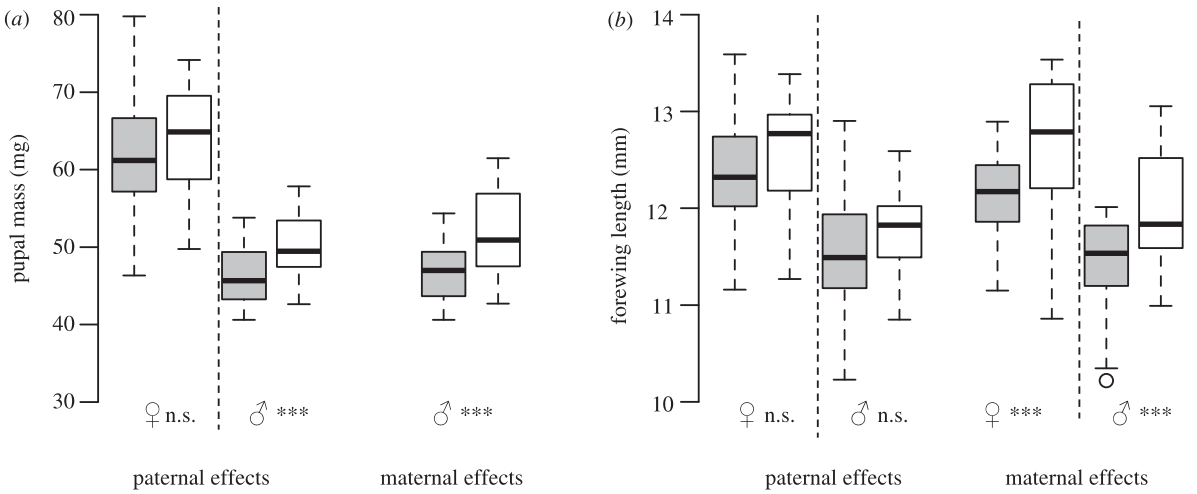

Fig. 3 Pupal mass (a) and forewing length (b) of *Coenonympha pamphilus* males and females of the F_1 generation descending from males and females (P). Progeny was raised on high- and low-quality host plants and descended from mothers (maternal effects) or fathers (paternal effects) reared on high- and low-quality host plants. The box represents the interquartile range from first to third quartile; the line across the box indicates the median and the whiskers show maximum and minimum values; asterisks indicate statistical differences between larvae reared on the same (white bar) and the opposite (grey bar) host plant quality as their father or mother (ns $P > 0.05$, *** $P < 0.001$, two-sided *t*-test, offspring descending from a single mother or father were pooled, see methods, $n = 24$ for each treatment group, except for groups reared on the same host plants as their father $n = 23$ for each treatment group). Data for pupal mass of females (F_1) descending from mothers (P) are shown in figure 2.

$n = 47$, $P = 0.37$; males: $R^2 = 0.17$, $n = 47$, $P = 0.26$).

Forewing length (F_1) was significantly affected by sex and forewing length of untreated females (P), whereas paternal lineage, larval hatching mass and host plant quality (F_1) had no significant effects (Table 2). Larval host quality of fathers had a marginal effect (Table 2). There was a significant paternal-host-by-progeny-host interaction (Table 2). However, forewing length did not differ significantly between pooled progeny reared on the same (Low-P/Low-F_1 plus High-P/High-F_1) rather than the opposite (Low-P/High-F_1 plus High-P/Low-F_1) host plant quality as their father (females: $t = 0.9$, $n = 47$, $P = 0.35$; males: $t = 1.7$, $n = 47$, $P = 0.10$; Figure 3b). Pupal mass and forewing length were positively correlated (females: $R^2 = 0.88$, $n = 47$, $P < 0.001$; males: $R^2 = 0.66$, $n = 47$, $P < 0.001$).

Discussion

Maternal experience

Progeny reared on the same (Low-P/Low-F_1, High-P/High-F_1) rather than the opposite (Low-P/High-F_1, High-P/Low-F_1) host plant quality as their mother performed significantly better (Figure 2, 3, Table 1). This clearly shows that mothers acclimatized their progeny to the anticipated host plant quality. Highly significant maternal-host-by-progeny-host interactions emphasized these findings (Table 1). Without transgenerational acclimatization, our results should have shown the negative effects of nitrogen-poor larval food on all Low-F_1 larvae, because High-P parents had a higher pupal mass and forewing length than Low-P parents. However, the nitrogen-poor larval food had no negative effects on Low-P/Low-F_1 larvae, and Low-P/Low-F_1 females performed also better than High-P/Low-F_1 females. Our results show not

Table 1: Effects of host plant quality of females of the parental generation (P) on progeny (F_1) in *Coenonympha pamphilus*. The effect size (R^2), *F* values, *P* values and degrees of freedom (*df*) are presented

	Larval duration (F_1)				Pupal mass (F_1)				Forewing length (F_1)			
	P	*F*	R^2	*df*	*P*	*F*	R^2	*df*	*P*	*F*	R^2	*df*
Sex	**<0.001**	49.7	0.37	1,85	**<0.001**	131.1	0.61	1,83	**<0.001**	33.8	0.29	1,83
Host plant quality (F_1)	**<0.001**	55.6	0.40	1,85	0.78	0.1	0.00	1,83	**0.003**	9.4	0.10	1,83
Host plant quality (P)	0.20	1.7	0.02	1,85	0.41	0.7	0.01	1,83	**0.012**	6.6	0.07	1,83
					0.02	5.6	0.06	1,83				
Aux pupal mass (P)												
									0.008	7.4	0.08	1,83
Aux forewing length (P)												
Maternal lineage	0.33	1.6	0.19	1,5	**0.03**	8.3	0.62	1,5	0.77	0.1	0.02	1,5
Larval hatching mass (F_1)	0.31	1.0	0.01	1,85	0.61	0.3	0.00	1,83	0.56	0.3	0.00	1,83
Host plant quality (P) × host plant quality (F_1)					**<0.001**	39.4	0.32	1,83	**<0.001**	15.6	0.16	1,83

Note: Host plant quality (P, F_1) is high-nitrogen versus low-nitrogen level. Females (P) were mated with unrelated auxiliary males (Aux) fed ad libitum with low-quality host plants. $R^2 = F(F+df)^{-1}$. *P* values <0.05 are bold. A stepwise model reduction of all models was used, with the least significant interactions always removed first (Crawley 2007).

Table 2: Effects of host plant quality of males of the parental generation (P) on progeny (F_1) in *Coenonympha pamphilus*. The effect size (R^2), *F* values, *P* values and degrees of freedom (*df*) are presented

	Larval duration (F_1)				Pupal mass (F_1)				Forewing length (F_1)			
	P	*F*	R^2	*df*	*P*	*F*	R^2	*df*	*P*	*F*	R^2	*df*
Sex	**<0.001**	71.2	0.46	1,82	**<0.001**	115.2	0.59	1,81	**<0.001**	42.7	0.35	1,80
Host plant quality (F_1)	**<0.001**	14.8	0.15	1,82	0.13	2.4	0.03	1,81	0.20	1.7	0.02	1,80
Host plant quality (P)	**0.027**	5.1	0.06	1,82	0.50	0.5	0.01	1,81	0.09	3.0	0.04	1,80
					0.01	7.6	0.09	1,81				
Aux pupal mass (P)												
									<0.001	12.6	0.14	1,80
Aux forewing length (P)												
Paternal lineage	0.72	0.1	0.03	1,5	0.09	4.3	0.46	1,5	0.13	3.3	0.40	1,5
Larval hatching mass (F_1)	**0.032**	4.8	0.05	1,82	0.17	1.9	0.02	1,81	0.19	1.8	0.02	1,80
Host plant quality (P) × host plant quality (F_1)	**0.01**	6.6	0.07	1,82	**0.04**	4.5	0.05	1,81	**0.02**	5.7	0.04	1,80

Note: Host plant quality (P, F_1) is high-nitrogen vs. low nitrogen-level. Males (P) were mated with unrelated auxiliary females (Aux) fed ad libitum with low-quality host plants. $R^2 = F(F+df)^{-1}$. *P* values <0.05 are bold. A stepwise model reduction of all models was employed, with the least significant interactions always removed first (Crawley 2007).

only that Low-P/Low-F_1 larvae can compensate for low-quality larval food owing to maternal experience, but also that the High-P/High-F_1 females performed significantly better than the Low-P/High-F_1 females, although both groups were raised on nitrogen-rich host plants. Furthermore, contrary to intuition, Low-P/Low-F_1 larvae achieved the same pupal mass as High-P/High-F_1 larvae, and Low-P/Low-F_1 females even had a higher pupal mass than Low-P/High-F_1 females, despite the low nitrogen content in low-quality host plants. Obviously, mothers adjusted the ability of their progeny's food intake or efficiency of larval nitrogen utilization according to their own experienced host plant quality, thereby adjusting their progeny also to the anticipated future host plant quality encountered by their offspring. Thus, maternal experience primarily determined larval performance in our experiment. By contrast, other studies that attempted to show transgenerational acclimatization often found strong effects of the progeny's immediate host plant instead of transgenerational acclimatization owing to parental experience (Via 1991; Spitzer 2004; Amarillo-Suarez and Fox 2006; Or and Ward 2007; Mc Lean et al. 2009).

Furthermore, pupal mass of female offspring was more strongly affected by maternal transgenerational acclimatization than pupal mass of male progeny, as can be seen in the distinct difference between treatment groups (Figure 2, 3a). Pupal mass of males (F_1) either was less variable than female pupal mass, because *C. pamphilus* males are generally smaller than females and may have a smaller range to vary their pupal mass, or maternal experience was more strongly mediated within the same sex than from females (P) to males (F_1).

Paternal experience

While maternal effects are widely recognized as important contributors to offspring phenotypes, transgenerational acclimatization to host plants based on paternal experience has been little studied (Futuyma et al. 1993; Fox et al. 1995). However, many insects have a mating system in which males transfer nutrients and other chemical compounds such as hormones and enzymes to females at mating, which may affect reproduction and accordingly offspring (Bissoondath and Wiklund 1996; Cahenzli and Erhardt 2013). Experiments to find transgenerational acclimatization in *S. limbatus* beetles showed symmetry between the magnitude of paternal and maternal host effects on survivorship of progeny (Fox et al. 1995). In our study, similarly to maternal effects, paternal host quality experience increased pupal mass of male offspring reared on the same host quality as their fathers (Figure 3a). However, in contrast to male offspring, paternal experience had no detectable effect on female progeny (Figure 3), and paternal effects were weaker than maternal effects (Table 1, 2). This suggests that maternal effects are generally more important than paternal effects. However, paternal experience could compensate for the limited effect of maternal experience on male progeny, as the expression of genes or chromosomes can depend on the sex through which the chromosome is transmitted (Jablonka and Lamb 1989). Correspondingly, maternal and paternal effects of *Ophraella notulata* beetles reared on different host plants were not independent of each other, but transgenerational acclimatization was not found in that species (Futuyma et al. 1993).

Biological implications

Previous studies that have attempted to show transgenerational acclimatization found only direct effects of progeny host (Via 1991; Spitzer 2004; Amarillo-Suarez and Fox 2006; Or and Ward 2007; Mc Lean et al. 2009) or parental effects independent from progeny's rearing host on offspring performance (Futuyma et al.

1993; Fox et al. 1995; Fox 1997; Amarillo-Suarez and Fox 2006). Transgenerational acclimatization to host plants possibly occurs primarily within a host species rather than between different host species with differing chemical and physiological attributes. This is supported by a hint for transgenerational acclimatization found in *P. rapae* females, which were reared on artificial diets differing only in nitrogen concentration (Rotem et al. 2003). Females altered patterns of egg size and possibly egg provisioning based on their food quality and influenced progeny, even though only early during its development and without ultimate positive effects on fitness (Rotem et al. 2003).

Of course we cannot neglect the general role of random genetic inheritance, as lineage and maternal and paternal characteristics of the auxiliary parental mating partners also significantly affected progeny (Table 1, 2). Adaptive maternal effects and genetic inheritance act together (Fox et al. 1995). Genetic fixation of a trait is appropriate in stable environments with a constant quantity and quality of resources, but resource-based transgenerational acclimatization is favourable for quick adaptation from one generation to the next. For example, host plant quality can vary over time, and when host plant quality changes with a higher rate than genetic adaptation to altered host conditions occurs in herbivores, transgenerational acclimatization could enable temporary adaptation to the altered host plant quality. Furthermore, *C. pamphilus* lives on spatially patchy distributed meadow types and small fragmented habitats, all differing considerably in nutritional quality (Lepidopterologen-Arbeitsgruppe 1987; Koch 1991). In addition, *C. pamphilus* males often reproduce in restricted territories (Wickman 1985), and mated females are relatively immobile and often walk on the ground for oviposition instead of flying long distances (Wickman 1986). It is therefore likely that offspring develop within the same patch and accordingly on the same host plant quality as their parents. Thus, transgenerational acclimatization may enhance local adaptation to greatly diverse habitats of *C. pamphilus*. Clearly, transgenerational acclimatization is of immense ecological interest, because improved adaptation of an organism to its resources increases its fitness. This, in turn, influences population size and mortality, and finally even alters dynamics of entire populations (Rossiter 1991).

Coenonympha pamphilus females as well as females of other Satyrid butterflies often do not discriminate between oviposition sites (Wiklund and Karlsson 1984), presumably because they reproduce within spatially restricted habitats with a consistent host plant quality. By contrast, oviposition choice could be an important factor for transgenerational acclimatization in other insect species, because acclimatized progeny located on a different host plant quality as their parents suffers from decreased fitness (Figures 2, 3). Contrary to intuition, Low-P females should therefore oviposit on low-quality host plants rather than on high-quality plants. Several theories, like the 'Hopkins host selection principle' (Hopkins 1917), the 'neo- Hopkins principle' (Jaenike 1983) and 'chemical legacy' (Corbet 1985), actually suggest that maternal host plants can influence oviposition choice, but evidence for this effect is controversial (Janz et al. 2009; Facknath and Wright 2007; Barron 2001). If females lay their eggs indeed on the same host plant type they were acclimatized to and on which they acclimatize their progeny, host-race formation could be influenced when correlations between oviposition choices and larval performance are maintained in a randomly mating population through maternal host experience (Fox et al. 1995). Such interactions could result in a runaway process that facilitates sympatric speciation in systems where oviposition choices determine the environment for offspring development (Diehl and Bush 1989; Wade 1998). Divergent host plant use can also cause assortative mating by phenotypically

altering traits involved in mate recognition. For instance, male mustard leaf beetles *Phaedon cochleariae* preferred to mate with females reared on the same rather than a different host plant species (Geiselhardt et al. 2012).

Transgenerational acclimatization to host plants in herbivorous insects was postulated for a long time, but has not been shown to date despite intense research and its profound implications for ecological and evolutionary processes (Spitzer 2004; Mc Lean et al. 2009). This process, verified for the first time in this study, requires more investigation in other insect-host plant interactions, because transgenerational acclimatization could generally affect the coexistence between plants and insects, two of the most diverse groups of living organisms united by intricate relationships.

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References

Amarillo-Suarez AR, Fox CW (2006) Population differences in host use by a seed-beetle: local adaptation, phenotypic plasticity and maternal effects. Oecologia 150:247–258

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Ann Rev Entomol 47:817–844

Barron AB (2001) The life and death of Hopkins' host-selection prinicple. J Insect Behav 14:725–737

Berwaerts K, Van Dyck H, Aerts P (2002) Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Funct Ecol 16:484–491

Bissoondath CJ, Wiklund C (1996) Male butterfly investment in successive ejaculates in relation to mating system. Behav Ecol Sociobiol 39:285–292

Burgess SC, Marshall DJ (2011) Temperature-induced maternal effects and environmental predictability. J Exp Biol 214:2329–2336

Cahenzli F, Erhardt A (2012a) Host plant defence in the larval stage affects feeding behaviour in adult butterflies. Anim Behav 84:995–1000

Cahenzli F, Erhardt A (2012b) Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos 121:1417–1427

Cahenzli F, Erhardt A (2013) Nectar amino acids enhance reproduction in male butterflies. Oecologia 171:197–205

Carvalho GB, Kapahi P, Benzer S (2005) Compensatory ingestion upon dietary restriction in *Drosophila melanogaster*. Nat Methods 2:813–815

Clancy KM, Price PW (1987) Rapid herbivore growth enhances enemy attack − sublethal plant defenses remain a paradox. Ecology 68:733–737

Corbet SA (1985) Insect chemosensory responses: A chemical legacy hypothesis. Ecol Entomol 10:143–153

Crawley MJ, ed. (2007) The R Book. John Wiley & Sons Ltd, West Sussex, England

De Barro PJ, Sherratt TN, David O, MacLean M (1995) An investigation of the differential performance of clones of the aphid *Sitobion avenae* on two host species. Oecologia 104:379–385

Diehl SR, Bush GL (1989) Speciation and its Consequences. Sinauer Associates Inc., U.S.

Facknath S, Wright DJ (2007) Is host selection in leafminer adults influenced by pre-imaginal or early adult experience? J Appl Entomol 131:505–512

Falconer DS (1981) Introduction to quantitative genetics. Longman.

Ferkau C, Fischer K (2006) Costs of Reproduction in Male *Bicyclus anynana* and *Pieris napi* Butterflies: Effects of Mating History and Food Limitation. Ethology 112:1117–1127

Fox CW, K. Waddell KJ, Mousseau TA (1995) Parental host plant affects offspring life histories in a seed beetle. Ecology 76:402–411

Fox CW (1997) The ecology of body size in a seed beetle, *Stator limbatus:* persistence of environmental variation across generations? Evolution 51:1005–1010

Futuyma D J, Herman C, Milstein S, Keese MC (1993) Apparent transgenerational effects of host-plant in the leaf beetle *Ophraella notulata* (Coleoptera, Chrysomelidae). Oecologia 96:365–372

Galloway LF, Etterson JR (2007) Transgenerational plasticity is adaptive in the wild. Science 318:1134–1136

Geiselhardt S, Otte T, Hilker M (2012) Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. Ecol Let 15:971–977

Goverde M, Erhardt A (2003) Effects of elevated CO_2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyridae). Global Change Biol 9:74–83

Hopkins AD (1917) A discussion of H.G. Hewitt's paper on "Insect behavior." J Econ Entomol 10:92–93

Jablonka E, Lamb MJ (1989) The inheritance of acquired epigenetic variations. J Theor Biol 139:69–83

Jaenike J (1983) Induction of host preference in *Drosophila melanogaster*. Oecologia 58:320– 325

Janz N, Söderlind L, Nylin S (2009) No effect of larval experience on adult host preferences in *Polygonia c-album* (Lepidoptera: Nymphalidae): on the persistence of Hopkin's host selection principle. Ecol Entomol 34, 50–57

Karlsson B, Wiklund C (1984) Egg weight variation and lack of correlation between egg weight and offspring fitness in the wall brown butterfly, *Lasiommata megera*. Oikos 43:376–385

Kirkpatrick M, Lande R (1989) The evolution of maternal characters. Evolution 43:485–503

Koch, M. ed. 1991. Schmetterlinge. Neumann Verlag, Radebeul, Germany

Lepidopterologen-Arbeitsgruppe (1987) Tagfalter und ihre Lebensräume, Band 1. Schweizerischer Bund für Naturschutz, Basel, Switzerland

Massey FP, Ennos AR, Hartley SE (2007) Grasses and the resource availability hypothesis: the importance of silica-based defences. J Ecol 95:414–424

Mc Lean AC, Ferrari J, Godfray HJC (2009) Effects of the maternal and pre-adult host plant on adult performance and preference in the pea aphid, *Acyrthosiphon pisum*. Ecol Entomol 34:330–338

Mousseau TA, Dingle H (1991) Maternal effects in insect life histories. Annu Rev Entomol 36:511–534

Mousseau TA, Fox CW, eds. (1998a) Maternal effects as adaptations. Oxford Univ. press

Mousseau TA, Fox CW (1998b) The adaptive significance of maternal effects. Trends Ecol Evol 13:403–407

Olson P-O C, Anderbrant O, Löfstedt C. (2006) Experience influences oviposition behaviour in two pyralid moths, *Ephestica cautella* and *Plodia interpunctella*. Anim Behav 72:545–551

Or K, Ward D (2007) Maternal effects on the life histories of bruchid beetles infesting *Acacia raddiana* in the Negev desert, Israel. Entomol Exp Appl 122:165–170

Pinheiro JC, Bates DM, eds. (2000) Mixed Effects-Models in S and S-Plus. Springer

Rossiter MC (1991) Environmentally-based maternal effects: a hidden force in

insect population dynamics? Oecologia 7:288–294
Rotem K, Agrawal AA, Kott L (2003) Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? Ecol Entomol 28:211–218
Salinas S, Munch SB (2012) Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. Ecol Lett 15:159–163
Schoonhoven LM, Van Loon JJA, Dicke M, eds. (2006) Insect – Plant Biology Vol. 2. Oxford Univ. Press, Oxford
Spitzer BW (2004) Maternal effects in the soft scale insect *Saissetia coffeae* (Hemiptera: Coccidae). Evolution 58:2452–2461
Sultan SE, Barton K, Wilczek AM (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. Ecology 90:1831–1839
Via S (1991) Specialized host plant performance of pea aphid clones is not altered by experience. Ecology 72:1420–1427
Vicari M, Bazely DR (1993) Do grasses fight back – the case for antiherbivore defenses. Trends Ecol Evol 8:137–141
Wade M (1998) Maternal Effects as Adaptations. Oxford Univ. Press, NewYork
Whittle CA, Otto SP, Johnston MO, Krochko JE (2009) Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana*. Botany 87:650–657
Wickman P-O (1985) Territorial defence and mating success in males of the small heath butterfly *Coenonympha pamphilus* L. Anim Behav 33:1162–1168
Wickman P-O (1986) Courtship solicitation by females of the small heath butterfly, *Coenonympha pamphilus (*L.) (Lepidoptera: Satyridae) and their behaviour in relation to male territories before and after copulation. Anim Behav 34:153–157
Wiklund C, Karlsson B (1984) Egg size variation in Satyrid butterflies: adaptive vs historical, "Bauplan", and mechanistic Explanations. Oikos 43:391–400
Williams IS (1999) Slow-growth, high-mortality - a general hypothesis, or is it? Ecol Entomol 24:490–495
Yoder JA, Tank JL, Rellinger EJ (2006) Evidence of a maternal effect that protects against water stress in larvae of the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). J Insect Physiol 52:1034–1042.

Chapter 6

Female butterflies adapt and allocate their progeny to the host plant quality of their own larval experience

Abstract

Recent studies with diverse taxa have shown that parents can utilize their experience of the environment to adapt their offspring's phenotype to the same environmental conditions. Thus, offspring would then perform best under environmental conditions experienced by their parents due to transgenerational phenotypic plasticity. Such an effect has been dubbed transgenerational acclimatization. However, evidence that parents can subsequently ensure the appropriate environmental conditions in order that offspring benefit from transgenerational acclimatization has never been demonstrated. We reared *Pieris rapae* larvae in the parental generation on high- and low-nitrogen host plants, and reared the offspring (F_1) of both treatments again on high- and low-nitrogen plants. Furthermore, we tested if females prefer to oviposit on high- or low-nitrogen host plants in two-way choice tests. We here show not only that females adapt their offspring's phenotype to the host plant quality that they themselves experienced, but that females also mainly oviposit on the host quality to which they adapt their offspring. Moreover, effects of larval host plant on oviposition preference of females increased across two generations, showing an adaptive host-shift from one generation to the next. These findings may have profound implications for host-race formation and sympatric speciation.

Key words: butterfly reproduction; Hopkins' host selection principle; host-shift; maternal effects; oviposition; transgenerational acclimatization; phenotypic plasticity

Introduction

In some ecological circumstances the current environment can provide cues about the environment that will be encountered by the next generation (Mousseau and Fox 1998a). We might expect parents to utilize this information to produce offspring through transgenerational phenotypic plasticity that are adapted to the specific conditions that their own experience predicts in order to increase offspring fitness (Mousseau and Fox 1998b). Such an effect has been dubbed transgenerational conditioning or acclimatization (Fox et al. 1995; Mousseau and Fox 1998a, 1998b; Spitzer 2004). When parental and offspring environmental conditions are positively correlated, transgenerational acclimatization would augment offspring performance by adapting those offspring to the environment experienced by their parents. It should be detectable as a two-way parental-environment-by-progeny-environment interaction on progeny performance (Fox et al. 1995; Spitzer 2004; Salinas and Munch 2012).

There is a growing number of studies with diverse taxa showing that parents do indeed utilize their experience of the environment to produce offspring that are phenotypically adapted to the same environmental conditions (Bashey et al. 2006; Plaistow et al. 2006; Yoder et al. 2006; Blödner et al. 2007; Galloway and Etterson 2007; Marshall 2008; Sultan et al. 2009; Salinas and Munch 2012; Cahenzli and Erhardt 2013; Latzel et al. 2014). Good examples come from the bryozoan *Bugula neritina* (Marshall 2008) and from sheepshead minnows *Cyprinodon variegatus* (Salinas and Munch 2012). In the bryozoans, there was a strong interaction between the copper

concentrations in the maternal and larval environment in their effects on the survival of offspring in copper-polluted water. Offspring from toxicant-exposed mothers had lower mortality in water with high levels of copper than progeny of toxicant-naïve mothers, whereas there was no effect on mortality when offspring were grown in unpolluted water. In the minnows, there was a significant interaction between parental and offspring water temperature in their effect on offspring growth. Progeny from parents kept in high and low temperature water grew best at high and low temperatures, respectively.

Transgenerational acclimatization is only adaptive when parental and offspring environments are positively correlated. Therefore in species that undergo such acclimatization, we might also expect to see the evolution of traits that generate or enhance such a correlation. This raises the question as to whether or not females might prefer to place their progeny in environments to which their offspring will be acclimatized, perhaps even if those environments are suboptimal in some overall sense. Maternal behavior of this nature–could have profound implications for our understanding of evolutionary and ecological processes (Fox et al. 1995; Mousseau and Fox 1998a; Spitzer 2004).

Relationships between phytophagous insects and their host plants are ideal to investigate this question for two reasons. First, because generation times are sufficiently short that maternal experience can often predict offspring environment (Spitzer 2004), and, second, because it is possible to measure maternal oviposition preferences and offspring performances independently of each other. These features of herbivorous insects allow investigation of the potential roles of transgenerational acclimatization, in coordination with selective oviposition behavior, in host-race formation, host-shifts and sympatric speciation. We already know that some herbivorous insects prefer to oviposit on the host quality that they themselves experienced as larvae (Akhtar and Isman 2003; Chow et al. 2005; Moreau et al. 2008), even though the underlying mechanisms of this behavior and the plausibility for a general hypothesis for host selection are debated (Janz et al. 2008). Our own prior work shows that females can, through transgenerational acclimatization, produce offspring that are phenotypically adapted to the host quality experienced by their parents (Cahenzli and Erhardt 2013). If we consider these results together, we see that, both oviposition choice and offspring performance, can be affected by maternal host plant experience. However, no studies have yet addressed both traits in a conclusive experimental design. When females prefer to lay their eggs on the same host plant type to which they were acclimatized and to which they acclimatize their progeny (Fox et al. 1995), the possibility arises of a runaway process that facilitates sympatric speciation (Diehl and Bush 1989; Wade 1998).

Previous studies investigating relationships between female oviposition choice and offspring performance have not considered potential transgenerational acclimatization (see references in Gripenberg et al. 2010). Neither have studies testing for transgenerational acclimatization asked whether offspring are selectively placed on resources to which they are acclimated. Here, we combine these questions, for what we believe is the first time, by asking whether mothers can indeed align both the adaptation and allocation of their progeny to the environmental conditions that their own experience predicts those progeny to encounter. We expect (1) that females prefer to oviposit on the host plant quality that they themselves experienced, and (2) that larval performance of offspring (F_1) on high- and low-nitrogen plants depends on maternal experience, resulting in significant maternal-host-by-progeny-host interactions on progeny's larval performance.

Materials and methods

Study species

Our prior demonstration of transgenerational acclimation used the grass-feeding butterfly *Coenonympha pamphilus* (Cahenzli and Erhardt 2013). Unfortunately, these insects were not suitable for our current incorporation of oviposition host preferences into transgenerational studies, since it is not clear that they respond primarily to host quality when choosing oviposition sites (Wiklund 1984). For this reason we switched study species to *Pieris rapae* (Lepidoptera: Pieridae), a multivoltine butterfly that undergoes up to four generations per year in central Europe (Lepidopterologen-Arbeitsgruppe 1987). *P. rapae* larvae feed on various wild and cultivated Brassicaceae (Lepidopterologen-Arbeitsgruppe 1987). The large variety of larval host plants, intraspecific differences in host plant nitrogen content and multiple generations per year render *P. rapae* a suitable candidate to investigate potential effects of transgenerational acclimatization, including effects of larval host plant experience on adult oviposition choice.

As larval host plants for *P. rapae* we used *Brassica oleracea* convar. *capitata*. It is known that fertilization increases foliar nitrogen content of host plants and generally enhances larval development in butterflies (e. g. Slansky and Feeny 1977; Schoonhoven et al. 2006; Cahenzli and Erhardt 2013). Therefore, we focused on nitrogen levels as a measure of host plant quality.

Larval rearing

Larval food plants were grown in 1 litre plastic pots filled with nutrient-poor soil (seeding compost, Compo Sana, Switzerland). Plants were grown in a greenhouse with a day/night cycle of 25ºC/20 ºC. High-nitrogen larval food plants (high) were fertilized once a week (N:P:K = 1:1:1). The low-nitrogen larval food plants (low) received only water. Plant nitrogen content was analyzed using a CHN analyzer.

Five *P. rapae* females were caught in the wild between Biel-Benken, Allschwil and Oberwil BL 47° 31′ N, 7° 33′ E, Switzerland. Their offspring comprised the parental generation in our experiments. Offspring of each wild-caught female were randomly divided into two groups, with one group assigned to the high-nitrogen host plants and the other to the low-nitrogen host plants. Larvae from each wild-caught female were reared in groups of ten until they were four days old and thereafter kept singly in individual Petri dishes. Larvae reared on high-nitrogen host plants (High-P) received high-nitrogen host plants ad libitum in Petri dishes, whereas food quantity of last instar larvae of the parental generation raised on low-nitrogen host plants (Low-P) was limited to the amount ingested by High-P larvae. This approach was chosen to ensure low-nitrogen availability to Low-P larvae, because larvae given food ad libitum may compensate for low nitrogen by ingesting higher quantities of food (Slansky and Feeny 1977; Rotem et al. 2003). Pupae were detached from plant material and were kept in individual cups until emergence.

We mated 11 freshly emerged high-P and 10 low-P females with unrelated males from the same larval treatment. From each mother (P), fifteen larvae (F_1) were randomly chosen for assignment to high-nitrogen hosts (High-F_1) and fifteen to low-nitrogen hosts (Low-F_1). These larvae constituted the F_1 generation. Data from same-sex F_1 offspring of a single mother (P) were pooled. By this means we obtained a sample size of $n = 42$ female F_1-offspring (11 High-P/High-F_1, 11 High-P/Low-F_1, 10 Low-P/High-F_1, 10 Low-P/Low-F_1) and $n = 42$ male F_1-offspring (11 High-P/High-F_1, 11 High-P/Low-F_1, 10 Low-P/High-F_1, 10 Low-P/Low-F_1), resulting in a total sample size of $n = 84$

(42 F_1-females plus 42 F_1-males) to test for maternal effects on F_1-offspring.

Oviposition choice

Mated females were placed in individual nylon mesh cages (20 cm x 60 cm x 40 cm), each containing a single potted low-nitrogen host and a single high-nitrogen plant of similar size. A potted *Aster* spp. was also provided in each cage for adult nectar feeding. The low- and high-nitrogen plants were placed as far as possible from each other and the nectar plants were positioned in the middle. Each test lasted 48 hours and positions of high- and low-nitrogen host plants were exchanged within each cage at the beginning of the second day to control for possible position effects. Oviposition choice of each female was calculated as the number of eggs laid on high-nitrogen host plants divided by total number of eggs laid.

Performance parameters

We recorded larval duration (number of days from eclosion to pupation), pupal mass on the day after pupation (mg) and forewing length within 24h after emergence (mm; lateral wingspan of the left forewing). Larval duration was measured because prolonged larval duration may increase the exposure time to predators, parasites and other adverse factors (Clancy and Price 1987; Williams 1999). Furthermore, pupal mass is an appropriate indicator for butterfly fitness, since male (Ferkau and Fischer 2006) and female (Cahenzli and Erhardt 2012) mass is correlated with fecundity. We also measured forewing length, since forewing geometry affects flight performance (Berwaerts et al. 2002), which can be important for dispersal, foraging, reproduction and predator avoidance.

Statistical analysis

Effects of nutrient-level treatment on host plant nitrogen level and fitness-traits in the parental generation (P) were analyzed with two-tailed t-tests. Two-tailed t-tests were also used to test for differences between the number of eggs laid on high- and low-nitrogen host plants. Furthermore, we used generalized linear mixed-effects models (Crawley 2007, Pinheiro and Bates 2000) to test for factors affecting maternal oviposition choice (P). The model used the categorical variable host plant quality of mothers (P) and the continuous variables female pupal mass and pupal mass of mating partners. Because related individuals (individuals from the same maternal lineage) are not independent of each other, effects and interactions were tested against the random factor lineage (Crawley 2007). Correlation analyses were used to test if maternal pupal mass (P) and female oviposition choice (P) were correlated within maternal feeding treatments.

Generalized linear mixed-effects models were used to test for effects of transgenerational acclimatization on fitness-traits in the offspring generation (F_1). The models used the categorical variables sex, host plant quality of mothers (P) and host plant quality of progeny (F_1). To account for inherited parental characteristics, the models also included maternal lineage as a random factor (Crawley 2007), plus the continuous covariates pupal mass or forewing length of fathers, depending on the fitness-trait analyzed (Table 1).

A stepwise model reduction was employed, with the least significant interactions always removed first (Crawley 2007). Tukey-Kramer's HSD comparisons were performed to test for differences among the four treatment groups (High-P/High-F_1, High-P/Low-F_1, Low-P/High-F_1, Low-P/Low-F_1) in larval duration, pupal mass or forewing length. P values < 0.05 were considered as statistically significant. All statistical analyses were calculated with R Statistical Software version 2.14.1 (R Development Core Team 2011).

Figure 1

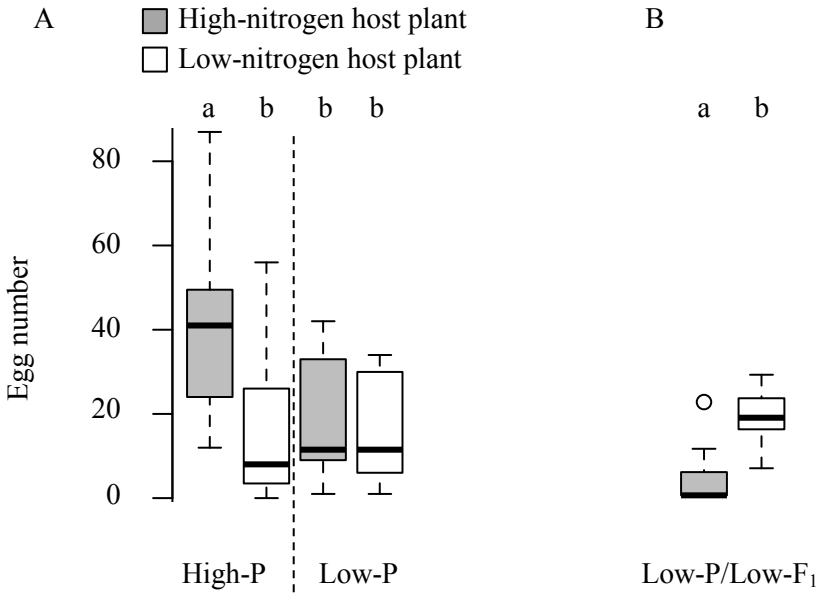

Fig. 1 Number of eggs laid on high-nitrogen (grey bars) or low-nitrogen host plants (white bars) by *Pieris rapae* females of the parental generation (A) and females of the F_1 generation raised on low-nitrogen larval host plants and descending from mothers reared on low-nitrogen host plants (Low-P/Low-F_1) (B). Females of the parental generation (High-P vs. Low-P) were raised on high- and low-nitrogen host plants. The box represents the interquartile range from first to third quartile, the line across the box indicates the median and the whiskers show the maximum and minimum values. Different letters indicate significant differences between treatment groups ($P < 0.05$, $n = 11$ for High-P females, $n = 10$ for Low-P females; $n = 13$ for Low-P/Low-F_1 females).

Results

Host plant quality

Fertilized *B. oleracea* plants (5.59 ± 0.31 g nitrogen / 100 g dry weight) had a significantly higher leaf nitrogen content than unfertilized plants (0.67 ± 0.09 g nitrogen / 100 g dry weight; $t = 15.13$, $n = 20$, $P < 0.001$).

Butterfly parental generation

Female as well as male butterflies reared on high-nitrogen host plants had a significantly shorter larval duration, higher pupal mass and longer forewings than butterflies raised on low-nitrogen host plants (Table 2).

Oviposition choice was significantly affected by maternal larval host plant nitrogen level ($F_{1,13} = 9.92$, $P = 0.008$). Females reared on high-nitrogen host plants laid significantly more eggs on high-nitrogen host plants than on low-nitrogen host plants (Fig. 1a). In contrast, females reared on low-nitrogen host plants accepted high- and low-nitrogen plants equally (Fig. 1a).

We also found an apparent effect on host choice of female pupal mass ($F_{1,13} = 7.44$, $P = 0.02$). However, this effect disappeared when we analyzed it separately within low-nitrogen ($R^2 = 0.17$, $n = 10$, $P = 0.24$) and high-nitrogen treatments ($R^2 = 0.04$, $n = 11$, $P = 0.56$). Finally, we found no significant effects on host choice of maternal lineage ($F_{1,3} = 0.02$, $P = 0.89$) or pupal mass of mating partners ($F_{1,13} = 2.01$, $P = 0.18$).

Figure 2

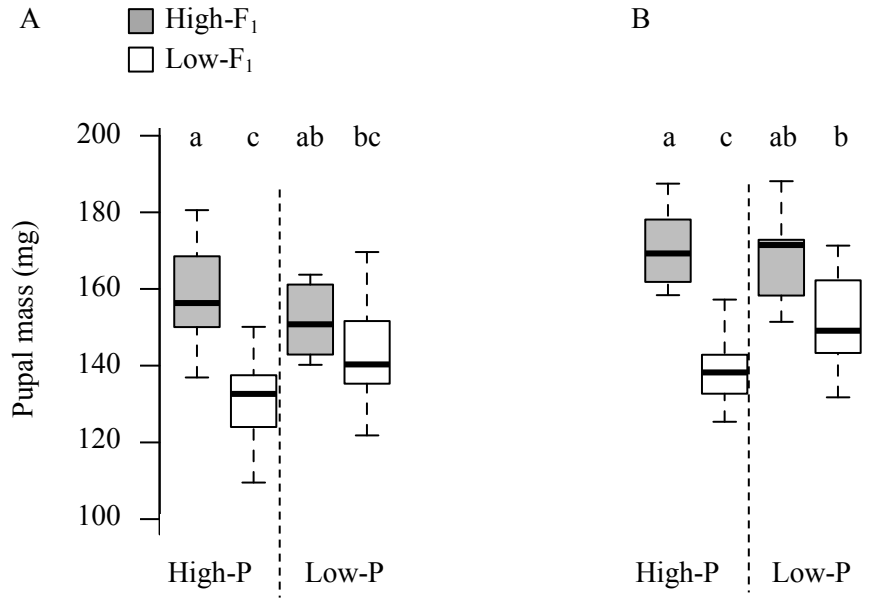

Fig. 2 Pupal mass of *Pieris rapae* females (A) and males (B) of the F_1 generation. Progeny (F_1) was raised on high-nitrogen (grey bars) and low-nitrogen host plants (white bars) and descended from mothers reared on high-nitrogen (High-P) and low-nitrogen host plants (Low-P). The box represents the interquartile range from first to third quartile, the line across the box indicates the median and the whiskers show the maximum and minimum values; different letters indicate significant differences between treatment groups ($P < 0.05$, $n = 11$ for each High-P treatment group, $n = 10$ for each Low-P treatment group).

F_1 generation

Larval duration (F_1) was significantly affected by host plant nitrogen level (F_1), whereas sex, host plant nitrogen level of mothers (P) and maternal lineage had no significant effects (Table 1). Tukey multiple comparisons revealed that High-P/High-F_1 larvae (14.75 ± 0.39 days) developed significantly faster than High-P/Low-F_1 larvae (18.62 ± 0.67 days, $P < 0.001$), and Low-P/High-F_1 larvae (16.09 ± 0.66 days) developed significantly faster than Low-P/Low-F_1 larvae (18.46 ± 0.66 days; $P = 0.047$).

Pupal mass (F_1) was significantly affected by sex and host plant nitrogen level (F_1), whereas maternal host plant nitrogen level (P), maternal lineage and pupal mass of fathers had no significant effect (Table 1). There was a significant maternal-host-by-progeny-host interaction (Table 1). High-P/High-F_1 larvae had a significantly higher pupal mass than High-P/Low-F_1 larvae, whereas Low-P/Low-F_1 offspring achieved the same pupal mass as Low-P/High-F_1 progeny (Fig. 2). Furthermore, Low-P/Low-F_1 males had a significantly higher pupal mass than High-P/Low-F_1 males (Fig. 2b).

Forewing length (F_1) was significantly affected by host plant nitrogen level (F_1), whereas sex, maternal host plant nitrogen level (P), maternal lineage and forewing length of fathers had no significant effect (Table 1). There was a significant maternal-host-by-progeny-host interaction (Table 1). Tukey multiple comparisons revealed that High-P/High-F_1 butterflies had significantly longer forewings (20.77 ± 0.18 mm) than High-P/Low-F_1 butterflies (19.19 ± 0.15 mm; $P < 0.001$), whereas Low-P/Low-F_1 butterflies (19.83 ± 0.18 mm) achieved the same forewing length as Low-P/High-F_1 offspring (20.42 ± 0.13 mm, $P = 0.18$). Low-P/Low-F_1 females laid

Table 1: Effects of host plant nitrogen level of females of the parental generation (P) on progeny (F_1) in *Pieris rapae*. The effect size (R^2), *F* values, *P* values and degrees of freedom (*df*) are presented

	Larval duration (F_1)				Pupal mass (F_1)				Forewing length (F_1)			
	P	*F*	R^2	*df*	*P*	*F*	R^2	*df*	*P*	*F*	R^2	*df*
Sex	0.76	0.08	0.00	1,75	**<0.001**	22.25	0.23	1,73	0.12	2.51	0.00	1,73
*Host plant quality (F_1)	**<0.001**	48.60	0.39	1,75	**<0.001**	59.54	0.45	1,73	**<0.001**	48.00	0.40	1,73
*Host plant quality (P)	0.18	1.87	0.02	1,75	0.10	2.83	0.04	1,73	0.35	0.87	0.01	1,73
Paternal pupal mass					0.16	2.03	0.03	1,73				
Paternal forewing length									0.10	2.79	0.04	1,73
Maternal lineage	0.63	0.23	0.08	1,3	0.45	0.76	0.20	1,3	0.80	0.08	0.02	1,3
*Host plant quality (P) × host plant quality (F_1)					**0.001**	11.49	0.14	1,73	**0.002**	10.0	0.12	1,73

*Host plant nitrogen level (P, F_1) is high-nitrogen vs. low-nitrogen level. $R^2 = F(F+df)^{-1}$. *P* values <0.05 are bold. A stepwise model reduction of all models was employed, with the least significant interactions always removed first (Crawley 2007).

Table 2: Effects of host plant nitrogen level on *Pieris rapae* butterflies in the parental generation (P).

	Low-nitrogen		High-nitrogen		*n*	*P*	*t*
♀ Larval duration (days)	24.00	± 0.33	20.00	± 0.42	21	<0.001	7.47
♂ Larval duration (days)	21.50	± 0.37	15.55	± 0.25	21	<0.001	13.31
♀ Pupal mass (mg)	96.79	± 1.83	149.58	± 3.22	21	<0.001	15.68
♂ Pupal mass (mg)	110.14	± 5.44	180.19	± 2.48	21	<0.001	11.71
♀ Forewing length (mm)	17.42	± 0.19	20.49	± 0.20	21	<0.001	11.08
♂ Forewing length (mm)	17.29	± 0.38	21.44	± 0.14	21	<0.001	10.29

Butterflies were raised on high-nitrogen or low-nitrogen host plants.

significantly more eggs on low-nitrogen host plants than on high-nitrogen plants (t = 5.66, n = 13, $P < 0.001$; Fig. 1b).

Discussion

Maternal experience enhances offspring fitness

According to our knowledge this study shows for the first time that *P. rapae* females can indeed align the adaptation and allocation of their progeny to the environmental conditions that their own experience predicted in order to realize maximum offspring fitness. Oviposition choices of parental generation females reared on different host plant qualities differed significantly (Fig. 1a). Females reared on high-nitrogen host plants (High-P) preferred to oviposit on high-nitrogen plants, whereas Low-P females equally accepted high- and low-nitrogen host plants for oviposition. Correspondingly, these females acclimatized their progeny to the different host plants based on their own larval host experience (Fig. 2), indicated by highly significant maternal-host-by-progeny-host interactions on progeny performance (Table 1). Low-P/Low-F_1 larvae performed as well as Low-P/High-F_1 larvae, despite the lower nutritional quality of low-nitrogen host plants in the F_1 generation (Fig. 2). Furthermore, Low-P/Low-F_1 males performed significantly better than High-P/Low-F_1 males (Fig. 2b), although both treatment groups were reared on low-nitrogen host plants. Thus, patterns of larval performance (F_1) reflected patterns of maternal oviposition choice (P) (Fig. 1, 2). Moreover, effects of larval host plant on oviposition preference increased across the two generations of our experiment. In contrast to Low-P females, Low-P/Low-F_1 females that were acclimatized to low-nitrogen host plants clearly prefered to oviposit on low-nitrogen plants (Figure 1), indicating an adaptive process.

Hopkins' host selection principle and transgenerational acclimatization

It has long been known that herbivorous insect larvae can become conditioned to perform well on their hosts and also learn to prefer them, or that adults learn to find and/or prefer particular hosts for oviposition (review in Jermy 1987). A few exceptions exist, in which feeding on one host causes acceptability for that host to decline with experience (e. g. Lee and Bernays 1988; Mader et al. 2012). As early as 1917, Hopkins suggested that adult herbivorous insects should prefer to oviposit on the plants they had encountered as larvae ("Hopkins' host selection principle" HHSP) (Hopkins 1917). In contrast to the HHSP, one of the major well-documented assumptions in the theory on the evolution of insect oviposition behavior is that females select suitable host plants that maximize offspring fitness (Thompson & Pellmyr 1991). Females should therefore oviposit on the best available host species and not rely on their larval experience for oviposition choices (Janz et al. 2008). Most studies actually failed to support the HHSP (Wiklund 1974; Tabashnik et al. 1981; Williams 1983; Rojas and Watt 1999; Liu and Liu 2006; Murphy 2007; Janz et al. 2008; but see Akhtar and Isman 2003; Chow et al. 2005; Moreau et al. 2008). However, if mothers adapt their offspring to the environmental conditions that their own experience predict, it is essential that they also allocate their progeny accordingly. Indeed, in contrast to most previous studies that failed to support the HHSP, we can clearly demonstrate this process, coordinated with transgenerational acclimatization in *P. rapae* (Fig. 1, 2). Generally, both processes, HHSP and transgenerational acclimatization, are only adaptive in accordance with each other. Neither unadapted offspring located on the parental host, nor progeny adapted to the parental host, but located randomly on host plants will have a fitness advantage.

As few authors have sought effects of larval experience on oviposition in alignment with transgenerational acclimatization, this aspect of our experimental design is novel and we cannot know whether our result would apply in many cases. In the only comparable study of which we know (Akhtar and Isman 2003), maternal and offspring diet differed, restricting conclusions regarding potential transgenerational acclimatization.

Interestingly, transgenerational acclimatization to host plants was only hinted at (Rotem et al. 2003) and finally verified in trials with different qualities within one host species rather than between different host species (Cahenzli and Erhardt 2013). The few studies actually showing evidence for the HHSP were also conducted with different host qualities within one host plant species, however without taking transgenerational acclimatization into account (Akhtar and Isman 2003; Chow et al. 2005; Moreau et al. 2008). In contrast, all studies using different plant species failed to support the HHSP (Wiklund 1974; Tabashnik et al. 1981; Williams 1983; Rojas and Watt 1999; Liu and Liu 2006; Murphy 2007; Janz et al. 2008). These findings suggest that females may only prefer to oviposit on the host quality that they themselves experienced as larvae (HHSP) when they can also adapt their offspring to this host (transgenerational acclimatization), emphasizing an alignement of the HHSP with transgenerational acclimatization in phytophagous insects.

Host shift

P. rapae and *P. brassicae* females show reduced oviposition on plants that already carry conspecific eggs (Schoonhoven et al. 2006), most likely to reduce the risk of inter- or intraspecific competition and parasitism, and to avoid harmful plant defence triggered by prior conspecific larvae (Geiselhardt et al. 2013). Thus, acceptance of low-nitrogen hosts for larval development due to transgenerational acclimatization as found in our study greatly broadens the range of suitable host plants.

The expansion of Low-P females to low-nitrogen host plants could represent a first step in the formation of a new host race, since effects of larval host plant on oviposition preference increased across the two generations of our experiment (Fig. 1). In a second step, females that prefer and perform well on low-nitrogen plants could shift to other host plant species providing similar characteristics. Both finding and being able to develop on new host species seem more likely if the new host species has similar characteristics as hosts within the former host range of a phytophagous insect (Nylin and Janz 2009). Indeed, host shifts between more closely related plants seem to happen more frequently (Janz and Nylin 1998). Host shifts in turn are viewed as an important step in sympatric speciation (Drès and Mallet 2002). Divergent host plant use can also cause assortative mating by phenotypically altering traits involved in mate recognition. For instance, male mustard leaf beetles *Phaedon cochleariae* preferred to mate with females reared on the same rather than a different host plant species (Geiselhardt et al. 2012).

Conclusion

Our results show that mothers experiencing predictive cues in their environment can indeed select the correct environmental conditions for their offspring and adjust progeny's phenotype to the conditions that their own experience predicts. Transgenerational acclimatization in coordination with selective offspring allocation, in this case the larval performance and the oviposition behavior of phytophagous insects, is of essential ecological interest because improved adaptation of an organism to its resources increases its fitness. This in turn influences population size and mortality, alters dynamics of entire populations (Rossiter

1991) and may lead to sympatric speciation.

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References

Akhtar Y, Isman MB (2003) Larval exposure to oviposition deterrents alters subsequent oviposition behavior in generalist, *Trichoplusia ni* and specialist, *Plutella xylostella* moths. J Chem Ecol 29:1853–1870

Bashey F (2006). Cross-generational environmental effects and the evolution of offspring size in the Trinidadian guppy *Poecilia reticulata*. Evolution 60:348-361

Berwaerts K, Van Dyck H, Aerts P (2002) Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Funct Ecol 16:484–491

Blödner C, Goebel C, Feussner I, Gatz C, Polle A (2007). Warm and cold parental reproductive environments affect seed properties, fitness, and cold responsiveness in *Arabidopsis thaliana* progenies. Plant Cell Environ 30:165-175

Cahenzli F, Erhardt A (2012) Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. Oecologia 169:1005-1014

Cahenzli F, Erhardt A (2013) Transgenerational acclimatization in a herbivore-plant relationship. Proc R Soc B 280:1756

Chow JK, Akhtar Y, Isman MB (2005) The effects of larval experience with a complex plant latex on subsequent feeding and oviposition by the cabbage looper moth: *Trichoplusia ni* (Lepidoptera: Noctuidae). Chemoecology 15:129-133.

Clancy KM, Price PW (1987) Rapid herbivore growth enhances enemy attack − sublethal plant defenses remain a paradox. Ecology 68:733–737

Crawley MJ (2007) The R book. John Wiley & Sons Ltd, West Sussex, England

Diehl SR, Bush GL (1989) Speciation and its Consequences. Sinauer Associates Inc., U.S.

Drès M, Mallet J (2002) Host races in plant-feeding insects and their importance in sympatric speciation. Proc R Soc B 357:471-492

Ferkau C, Fischer K (2006) Costs of Reproduction in male *Bicyclus anynana* and *Pieris napi* butterflies: Effects of mating history and food limitation. Ethology 112:1117–1127

Fox CW, Waddell KJ, Mousseau TA (1995) Parental host plant affects offspring life histories in a seed beetle. Ecology 76:402–411

Galloway LF, Etterson JR (2007) Transgenerational plasticity is adaptive in the wild. Science 318:1134-1136

Geiselhardt S, Otte T, Hilker M (2012) Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. Ecol Lett 15:971–977

Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference–performance relationships in phytophagous insects. Ecol Lett 13: 383–393

Hopkins AD (1917) A discussion of H.G. Hewitt's paper on "Insect behavior." J Econ Entomol 10:92–93

Jaenike J (1983) Induction of host preference in *Drosophila melanogaster*. Oecologia 58:320– 325

Janz N, Nylin S (1998) Butterflies and plants: a phylogenetic study. Evolution 52:486–502

Janz N, Söderlind L, Nylin S (2009) No effect of larval experience on adult host

preferences in *Polygonia c-album* (Lepidoptera: Nymphalidae): on the persistence of Hopkin's host selection principle. Ecol Entomol 34:50–57

Jermy T (1987) The role of experience in the host selection of phytophagous insects. In: perspectives in chemoreception and behavior. Chapman RF, Bernays EA, Stoffolano JG (eds.). Springer-Verlag, New York, pp. 143-157

Latzel V, Janecek S, Dolezal S, Klimesova J, Bossdorf O (2014) Adaptive transgenerational plasticity in the perennial *Plantago lanceolata*. Oikos 123:41-46

Lee JC, Bernays EA (1988) Declining acceptability of a food plant for the polyphagous grasshopper *Schistocerca americana*: the role of food aversion learning. Physiol Entomol 13:291-301

Lepidopterologen-Arbeitsgruppe (1987) Tagfalter und ihre lebensräume, Band 1. Schweizerischer Bund für Naturschutz. Switzerland

Liu S-S, Liu T-X (2006) Preimaginal conditioning does not affect oviposition preference in the diamondback moth. Ecol Entomol 31:307-315

Mader BJ, Daoust SP, Cardinal-Aucoin M, Bauce E, Despland E (2012) Larval experience induces adult aversion to rearing host plants: a novel behaviour contrary to Hopkins' host selection principle. Ecol Entomol 37:204-211

Marshall DJ (2008) Transgenerational plasticity in the sea: context-dependent maternal effects across the life history. Ecology 89:418-427

Moreau J, Rahme J, Benrey B, Thiery D (2008) Larval host plant origin modifies the adult oviposition preference of the female European grapevine moth *Lobesia botrana*. Naturwissenschaften 95:317-324

Mousseau TA, Fox CW (1998a) Maternal effects as adaptations. Oxford University Press, Oxford, UK.

Mousseau TA, Fox CW (1998b) The adaptive significance of maternal effects. Trends Ecol Evol 13:403-407

Murphy SM (2007) Inconsistent use of host plants by the Alaskan swallowtail butterfly: adult preference experiments suggest labile oviposition strategy. Ecol Entomol 32:143-152

Nylin S, Janz S (2009) Butterfly host plant range: an example of plasticity as a promoter of speciation? Evol Ecol 23:137-146

Pinheiro JC, Bates DM (2000) Mixed Effects-Models in S and S-Plus. Springer.

Plaistow SJ, Lapsley CT, Benton TG (2006) Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. Am Nat 167:206–215

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rojas JC, Wyatt TD (1999) The role of pre- and post-imaginal experience in the host-finding and oviposition behaviour of the cabbage moth. Physiol Entomol 24:83-89

Rossiter MC (1991) Environmentally-based maternal effects: a hidden force in insect population dynamics? *Oecologia* 7:288–294

Rotem K, Agrawal AA, Kott L (2003) Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? Ecol Entomol 28:211–218

Salinas S, Munch SB (2012) Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. Ecol Lett 15:159-163

Schoonhoven LM, Van Loon JJA, Dicke M (2006) Insect – plant biology. 2nd edition, Oxford University Press, Oxford, UK

Slansky F, Feeny P (1977) Stabilization of rate of nitrogen accumulation by larvae of cabbage butterfly on wild and cultivated food plants. Ecol Monogr 47:209–228

Spitzer BW (2004) Maternal effects in the soft scale insect *Saissetia coffeae*

(Hemiptera: Coccidae). Evolution 58:2452–2461
Sultan SE, Barton K, Wilczek AM (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. Ecology 90:1831–1839
Tabashnik BE, Wheelock H, Rainbolt JD, Watt WB (1981) Individual variation in oviposition preference in the butterfly *Colias eurytheme*. Oecologia 50:225-230
Thompson JN, Pellmyr O (1991) Evolution of oviposition behavior and host preferences in Lepidoptera. Ann Rev Entomol 36:65–89
Wade M (1998) Maternal Effects as Adaptations. Oxford Univ. Press, NewYork
Wiklund C (1974) Oviposition preferences in *Papilio machaon* in relation to the host plants of the larvae. Entomol Exp Appl 17:189-198
Wiklund C (1984) Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. Oecologia 63:23-29
Williams IS (1999) Slow-growth, high-mortality - a general hypothesis, or is it? Ecol Entomol 24:490-495
Yoder JA, Tank, JL, Rellinger EJ (2006). Evidence of a maternal effect that protects against water stress in larvae of the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). J Insect Physiol 52:1034-1042

Summary and general conclusions

Chapters one to three and chapter five and six emphasize the importance of larval nitrogen acquisition. In butterflies, nitrogen is mainly acquired during the larval stage (Boggs 1981), since larval host plants have a significantly lower carbon-nitrogen ratio than adult food sources like floral nectar (Schoonhoven et al. 2006). Resources acquired during the larval stage directly affect fecundity in butterfly species emerging with all eggs yolked, but larval-derived nutrients can also indirectly affect butterfly fecundity through stored fat bodies in species that have only a few or no eggs ready upon emergence (Boggs 1997b). Chapters one to three and chapter five and six showed that males and females reared with limited amounts of nitrogen in their larval food suffered from a lower pupal mass and forewing length, and females emerging with restricted larval reserves laid fewer eggs than females raised with an optimal nitrogen supply. Furthermore, males reared on nitrogen-poor larval host plants provided poorer nuptial gifts, since females mated to low-quality males were obviously faced with a trade off between offspring quality and quantity. In contrast, females mated to males reared under unconstrained larval food conditions could use male-derived nuptial gifts to increase offspring hatching mass while progeny number did not decrease. These results support previous studies on effects of larval nitrogen acquisition for larval development and reproduction in butterflies (e. g. Bissoondath and Wiklund 1996; Boggs 1997a; Mevi-Schütz et al. 2003; Mevi-Schütz and Erhardt 2005). Thus, butterflies have developed several strategies to compensate for suboptimal larval food conditions (Bink and Siepel 1996; Lavoie and Oberhauser 2004). For example, chapters one to three and chapter five and six show that larvae reared on nitrogen-poor host plants increased larval development time to prolong their feeding period in order to acquire sufficient amounts of nitrogen. Furthermore, numerous other studies showed that herbivorous insects can also increase the amount of food ingested per time, enhancing the conversion of nitrogen into body mass or enhancing the efficiency of nitrogen absorption (Schoonhoven et al. 2006). For example in chapter six, transgenerationally acclimatized larvae reared on low-nitrogen host plants and descending from mothers reared also on nitrogen-poor plants achieved the same pupal mass as larvae reared on nitrogen-rich host plants. Obviously, these acclimatized larvae could more efficiently utilize nitrogen from the low-nitrogen supply.

Furthermore, chapter one shows that host plant defence is an important factor determining larval performance and nutrient acquisition in butterflies. In accordance to previous studies (review in Reynolds et al. 2009), chapter one also shows that silica as physical host plant defence deterred *Coenonympha pamphilus* larvae from acquiring sufficient nitrogen during larval feeding. However, in spite of physical host plant defence, other plant families than grasses use primarily chemical secondary plant metabolites to deter herbivores from feeding (Schoonhoven et al. 2006). Nevertheless, food specialists are often adapted to chemical plant defences and may even benefit from secondary plant metabolites (Schoonhoven et al. 2006). For instance, larvae of the common blue butterfly *Pollyomatus icarus* can metabolize the surplus of nitrogen in cyanogenic plants for growth (Goverde et al. 2008). Thus, in other butterfly species than the grass-feeding *C. pamphilus*, secondary plant metabolites may play an important role in determining larval nutrient reserves.

Butterflies are holometabolous insects and have the ability to compensate for a nitrogen-poor diet both as herbivorous larvae as well as nectar-feeding adults. A compensatory interaction between larval

and adult nitrogen uptake was therefore expected when larval resources were restricted. Chapter one shows that *C. pamphilus* males and females reared on nitrogen-poor host plants containing high silica levels to deter herbivores from feeding increased their relative consumption of amino acid-rich nectar. This is in accordance with the nectar amino acid preference found in *C. pamphilus* and *Araschnia levana* females raised on nitrogen-poor larval host plants (Mevi-Schütz et al. 2003; Mevi-Schütz and Erhardt 2003). Furthermore, irrespective of larval food resources, females of several other butterfly species show a distinct preference for nectar containing amino acids, suggesting some form of dependency and utilization of this nectar constituent (Alm et al. 1990; Erhardt and Rusterholz 1998; Rühle 1999; Rusterholz and Erhardt 2000; Mevi-Schütz and Erhardt 2004). Furthermore, as shown in chapter three, *C. pamphilus* males consumed more nectar with amino acids than nectar without amino acids irrespective of larval food conditions, supporting the tentative evidence in chapter one that also male butterflies, which have so far been thought to be indifferent to nectar amino acids, can show a preference for nectar containing amino acids over nectar lacking amino acids.

Chapter two confirms that nectar amino acids can increase female butterfly reproduction. *C. pamphilus* females fed with amino acid-rich nectar produced heavier offspring and increased egg hatching success over the oviposition period compared to females fed with nectar lacking amino acids. Furthermore, in contrast to females reared on quantitatively limited, nitrogen-poor larval host plants and fed with nectar lacking amino acids, females reared under the same restricted larval food conditions, but fed with nectar containing amino acids, tended to lay as many eggs as females reared on quantitatively unlimited nitrogen-rich larval host plants. Hence, *C. pamphilus* females used nectar amino acids to compensate for nitrogen deficiencies acquired during the larval feeding period. These results support previous findings that have shown that female butterflies benefit from nectar amino acids (Mevi-Schütz and Erhardt 2005; Bauerfeind and Fischer 2009). However, there are different strategies in butterflies for the use of nectar amino acids in order to increase reproduction and accordingly fitness. For example, *A. levana* females primarily increase egg quantity, whereas females of *C. pamphilus* primarily increase offspring quality. *A. levana* larvae live on patchily distributed *Urtica dioica*, a plant species with a high nitrogen content early in the season but lower nitrogen levels later in the summer, thereby reducing female emergence mass and number of eggs laid. In contrast, *C. pamphilus* larvae live on locally restricted meadows with a consistent host plant quality within one patch, since grasses regenerate over the season. Furthermore, *C. pamphilus* acclimatizes larval performance and accordingly larval nutrient acquisition to host plants. Thus, larval food conditions for all generations of *C. pamphilus* are similar. Therefore, female emergence mass should also be rather similar over the different generations, as should be the number of offspring. Thus, differently to *A. levana*, the number of eggs in *C. pamphilus* might be less variable under natural conditions. Hence, *C. pamphilus* might have evolved a strategy utilizing nectar amino acids to primarily increase offspring quality rather than quantity.

Chapter three provides the first evidence that also male butterflies can use nectar amino acids to enhance their reproduction. *C. pamphilus* females mated with males fed with amino acid-rich nectar produced heavier offspring than females mated with males that were fed nectar without amino acids. However, *C. pamphilus* males are monandrous and produce relatively small spermatophores (Svärd and Wiklund 1989), and larval hatching mass of progeny was only increased during the first six days of the female oviposition period,

suggesting that females had depleted potential male resources. The present findings are all the more relevant because they show that nectar-derived nitrogen can improve male reproductive success even in a species where males deliver small nuptial gifts, documenting a nutritional pathway likely also present in other butterfly species producing bigger spermatophores than *C. pamphilus* and potentially relying more on nuptial gifts. For example, in polyandrous *Pieris napi* butterflies, mating frequency influences nectar amino acid preference of females (Mevi-Schütz et al. 2004). However, increased size of ejaculates in polyandrous species is also attributed to the male's higher risk to face sperm competition (Wiklund and Forsberg 1991). Thus, a monandrous mating system would require fewer male resources because it is not necessary to exceed a competitor's ejaculate size. On the other hand, males of monandrous species may still invest highly in their nuptial gifts, as they have certainty about their paternity. In contrast, nutrients of a male's nuptial gift in polyandrous species benefit often the progeny of subsequently mating males (Wickler 1985). Thus, although nuptial gifts in monandrous species are smaller than in polyandrous butterflies (Svärd & Wiklund 1989), they are still an important resource for female reproduction. Nevertheless, further studies should investigate the role of nectar amino acids in polyandrous butterfly species. However, irrespectively of the question of polyandry versus monandry, chapter three and four emphasize the importance of nuptial gifts for female reproduction in butterflies and show the potential of male nutritional investment into reproduction.

Apart from positive effects of nectar amino acids, chapter four shows that *C. pamphilus* males can use also nectar sugars to enhance their reproduction. This corresponds well to several previous findings having shown that female butterflies benefit from nectar sugars (Norris 1935, Stern and Smith 1960, Murphy et al. 1983, O'Brien et al. 2004), and that there is a positive relationship between the amount of sucrose ingested by virgin male butterflies and the weight of the spermatophore ejaculated (Watanabe and Hirota 1999). However, carbohydrates are also important for somatic maintenance. This is emphasized with the finding that *C. pamphilus* males used nectar sugars to either increase reproduction or their longevity, suggesting a tradeoff between somatic maintenance and reproduction for the use of carbohydrates from the adult nectar diet. Nevertheless, increased longevity can prolong the reproductive period and accordingly increase the reproductive success of an individual. For instance, male butterflies require a recovery period between multiple matings, and the extent of the intermediate interval has an effect on mass and protein content of spermatophores (Oberhauser 1992; Bissoondath and Wiklund 1996). Thus, increased longevity enhances the number of matings and prevents a decrease in spermatophore quality in subsequent matings by longer recovery periods. Furthermore, also female butterflies can benefit from an increased longevity, since multiple matings can enhance female fecundity (Wiklund et al. 1993; Stjernholm & Karlsson 2000), and chapter two shows that egg hatching success can be increased to the end of female oviposition period by nectar amino acids. However, depending on external circumstances, butterflies can invest resources either directly into reproduction, or indirectly via a prolongation of their longevity. This emphasizes the benefit of variable resource allocation strategies in butterflies.

Butterflies do not visit flowers randomly, but often show distinct flower preferences (Erhardt 1995). Furthermore, butterflies have different innate color preferences (Faegeri and van der Pijl 1979), and floral colors may correlate with different types of nectar quality. Chapters two to four clearly show the benefit of floral nectar on butterfly reproduction. Butterflies can also show high degrees of flower constancy

(Faegeri and van der Pijl 1979), possibly due to learned correlations between optical attraction cues and nectar quality. Furthermore, butterflies and butterfly-pollinated flowers show a number of features that are adjusted to each other, summarized in a 'syndrome' (Faegeri and van der Pijl 1979). Thus, the findings of chapters one to four strongly suggest a coevolutionary process between butterflies and flowers dependent on butterfly pollination.

C. pamphilus males and females used nectar amino acids and male-derived nectar sugars to increase provisioning to offspring and accordingly increased progeny's hatching mass. Several previous studies with other butterfly species showed benefits of increased egg and larval size (Murphy et al. 1983; Braby 1994; Fischer et al. 2003; Seko and Nakasuji 2004; Fischer et al. 2006; but see Wiklund and Persson 1983; Wiklund and Karlsson 1984). However, further studies should test if heavier *C. pamphilus* hatchlings have an increased fitness. *C. pamphilus* females lay their eggs not only on benign larval food plants, but also on dead plant material, from which freshly hatched larvae must find new host plants (Wiklund 1984). Thus, if larger hatchling larvae can travel longer distances, they have an increased likelihood to find fresh larval food plants.

Chapter five and six show that offspring benefits not only from increased provisioning of nutrients, but also from parental experience by transgenerational acclimatization to host plant quality. In *C. pamphilus*, offspring reared on the same rather than the opposite host plant quality as their parents increased their larval performance and achieved a heavier pupal mass and longer forewings. Furthermore, *Pieris rapae* females acclimated their offspring to the host plant quality they selected for oviposition based on their own larval host experience. This shows that parents experiencing predictive cues on their host plant can indeed adjust progeny's phenotype to anticipated host plant quality and accordingly increase offspring fitness on that host. Generally, transgenerational acclimatization could lead to an evolutionary change without involving genetic variation since positive selection of epigenetic variants that have improved performance is plausible. Furthermore, chapter six shows that females oviposit on the host plant they acclimatize their offspring. Thus, host-race formation could be influenced when non-genetic correlations between these oviposition choices and larval performance are maintained in a randomly mating population through maternal host experience (Fox et al. 1995). Moreover, offspring reared on low-quality host plants (and descending from mothers reared on low-quality plants) that were acclimatized to low-quality hosts preferred to oviposit on unfavorable low-quality host plants. However, due to transgenerational acclimatization, larvae can perform well on low-quality host plants. This shows an adaptive host-shift from one generation to the next based on the correlation between transgenerational acclimatization and oviposition choice. Ultimately, selection for host fidelity could be increased, possibly influencing host-use evolution of herbivores, host-race formation and sympatric speciation (Fox et al. 1995; Mousseau and Fox 1998a, 1998b; Spitzer 2004). Although host plants vary greatly in quality and quantity and can thus provide important cues about the resources encountered by the next generation, chapter five and six provide the first proofs of transgenerational acclimatization to host plants in herbivorous insects.

Butterfly reproduction and fitness significantly correlate with food quality and quantity. Thus, there are diverse strategies over the whole butterfly life cycle to deal with suboptimal food sources in order to maximize reproduction and fitness. First, during the larval stage, larval feeding duration can be prolonged and relative consumption rates and/or efficiency of larval nitrogen utilization could be increased to compensate for low-quality larval host plants. Second, adult

nectar diet can compensate for deficiencies acquired during larval stage and generally enhance reproduction. Furthermore, female butterflies can use male-derived nuptial gifts for egg production. Third, parents can increase provisioning to offspring and acclimatize and oviposit progeny to anticipated host plants by transgenerational acclimatization to increase offspring performance and fitness. These diverse strategies during larval and adult stage and even trans-generationally to maximize reproduction and fitness show that butterflies are well adapted to quickly changing host plant supplies. Furthermore, it shows that the holometabolous life cycle, using different food sources during larval and adult stage, is a favorable surviving strategy.

References

Alm J, Ohnmeiss TE, Lanza J, Vriesenga L (1990) Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia 84:53–57.

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Ann Rev Entomol 47:817–844

Baker HG, Baker I (1973) Amino acids in nectar and their evolutionary significance. Nature 241:543–545

Baker HG, Baker I (1986) Floral nectar sugar constituents in relation to pollinator type. The Biology of Nectaries. Columbia University Press, New York

Baker HG, Baker I (1986) The occurrence and significance of amino acids in floral nectar. Plant Syst Evol 151:175–186

Bazin A, Goverde M, Erhardt A, Shykoff J (2002) Influence of atmospheric carbon dioxide enrichment on induced response and growth compensation after herbivore damage in *Lotus corniculatus*. Ecol Entomol 27:271–278

Bernays EA, Graham M (1988) On the evolution of host specificity in phytophagous arthropods. Ecology 69:886–892

Bink FA, Siepel H (1996) Nitrogen and phosphorus in Molinia caerulea (Gramineae) and its impact on larval development in the butterfly-species *Lasiommata megera*. Entomol Gen 20:271–280

Bissoondath CJ, Wiklund C (1996) Male butterfly investment in successive ejaculates in relation to mating system. Behav Ecol Sociobiol 39:285–292

Boggs CL (1981) Nutritional and life-history determinants of resource allocation in holometabolous insects. Am Nat 117:692–709

Boggs CL (1997a) Dynamic of reproductive allocation from juvenile and adult feeding: radiotracer studies. Ecology 78:192–202

Boggs CL (1997b) Reproductive allocation from reserves and income in butterfly species with differing adult diets. Ecology 78:181-191

Boggs CL, Gilbert LE (1979) Male contribution to egg production in butterflies : first evidence for transfer of nutrients at mating. Science 206:83–84

Braby MF (1994) The significance of egg size variation in butterflies in relation to hostplant quality. Oikos 71:119–129

Cyr H, Pace ML (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature 361:148–150

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 44:1389–1403

Erhardt A (1995) Ecology and conservation of butterflies. Chapman and Hall, London

Erhardt A, Rusterholz HP (1998) Do peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds? Oecologia 117:536–542

Faegeri K, van der Pijl L (1979) The principles of pollination acology, 3th ed. Pergamon Press, Oxford

Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2003) Fitness consequences of temperature-mediated egg size

plasticity in a butterfly. Funct Ecol 17:803–810
Fischer K, Bot ANM, Brakefield PM, Zwaan BJ (2006) Do mothers producing large offspring have to sacrifice fecundity? J Evolution Biol 19:380–391
Fox CW, Waddell KJ, Mousseau TA (1995) Parental host plant affects offspring life histories in a seed beetle. Ecology 76:402–411
Gilbert LE, Singer MC (1975) Butterfly ecology. Annu Rev Ecol Evol S 6:365–397
Goverde M, Erhardt A (2003) Effects of elevated CO_2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyridae). Global Change Biol 9:74–83
Goverde M, Bazin A, Kéry M, Shykoff J A, Erhardt A (2008) Positive effects of cyanogenic glycosides in food plants on larval development of the common blue butterfly. Oecologia 157:409–418
Huges PR, Voland ML (1988) Increase in feeding stimulants as the primary mechanism by which SO_2 enhances performance of Mexican bean beetle on soybean. Entomol Exp Appl 48:257–262
Karlsson B (1998) Nuptial gifts, resource budgets, and reproductive output in a polyandrous butterfly. Ecology 79:2931–2940
Labandeira CC (1998) Early history of arthropod and vascular plant associations. Annu Rev Earth Pl SC 26:329–377
Labandeira CC, Sepkoski JJ (1993) Insect diversity in the fossil record. Science 261:310-315
Lavoie, B. and K. S. Oberhauser. (2004) Compensatory Feeding in *Danaus plexippus* (Lepidoptera:Nymphalidae) in Response to Variation in Host Plant Quality. Environ Entomol 33:1062–1069
Lüttge U (1961) Über die zusammensetzung des nektars und den mechanismus seiner sekretion. I. Planta 56:189–212
Massey FP, Ennos AR, Hartley SE (2007) Grasses and the resource availability hypothesis: the importance of silica-based defences. J Ecol 95:414–424
May RM (1988) How many species are there on earth? Science 241: 1441–1449.
Mevi-Schütz J, Goverde M, Erhardt A 2003. Effects of fertilization and elevated CO_2 on larval food and butterfly nectar amino acid preference in *Coenonympha pamphilus* L. Behav Ecol Sociobiol 54:36–43
Mevi-Schütz J, Erhardt A (2003) Larval nutrition affects female nectar amino acid preference in the map butterfly (*Araschnia levana*). Ecology 84:2788–2794
Mevi-Schütz J, Erhardt A (2004) Mating frequency influences nectar amino acid preference of *Pieris napi*. Proc Roy Soc B 271:153–158
Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long awaited link. Am Nat 165:411–419
Mousseau TA, Fox CW (1998a) The adaptive significance of maternal effects. Trens Ecol Evol 13:403–407
Mousseau TA, Fox CW (1998b) Maternal effects as adaptions. Oxford Univ. press, Oxford, UK
Mousseau TA, Fox CW (1998b) The adaptive significance of maternal effects. Trens Ecol Evol 13:403–407
Myers JH (1985) Effect of physiological condition of the host plant on the oviposition choice of the cabbage white butterfly, *Pieris rapae*. J Anim Ecol 54:193–204
Norris MJ (1935) A feeding experiment on the adults of Pieris rapae. J Entomol Res Soc 68:125–127
Oberhauser KS (1988) Male monarch butterfly spermatophore mass and mating strategies. Anim Behav 36:1384–1388
Oberhauser KS (1992) Rate of ejaculate breakdown and intermating intervals in monarch butterflies. Behav Ecol Sociobiol 31:367–373
O'Brien DM, Boggs CL, Fogel ML (2002) Renewable and nonrenewable resources: amino acid turnover and allocation to reproduction in Lepidoptera. P Natl Acad

Sci USA 99:4413–4418
O'Brien DM, Boggs CL, Fogel ML (2004) Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. Oikos 105:279–291
Pimentel D, Andow DA (1984) Pest management and pesticide impacts. Insect Sci Appl 5:141–149
Poulin R, Thomas F (2008) Epigenetic effects of infection on the phenotype of host offspring: parasites reaching across host generations. Oikos 177:331–335
Price PW (1997) Insect ecology. John Wiley & Sons, New York
Reynolds OL, Keeping MG, Meyer JH (2009) Silicon-augmented resistance of plants to herbivorous insects: a review. Ann Appl Biol 155:171–186
Rodrigues D, Moreira GRP (2004) Seasonal variation in larval host plants and consquences for *Heliconius erato* (Lepidoptera: Nymphalidae) adult body size. Austral Ecol 29:437–445
Rusterholz H-P, Erhardt A (2000) Can nectar properties explain sexspecific flower preferences in the adonis blue butterfly *Lysandra bellargus*? Ecol Entomol 25:81–90
Rutowski RL, Gilchrist GW, Terkanian B (1987) Female butterflies mated with recently mated males show reduced reproductive output. Behav Ecol Sociobiol 20:319–322
Rühle C (1999) Preferences for nectar amino acids and nectar sugars in different Lepidoptera species: *Lysandra bellargus, Pollyommatus icarus, Maniola jurtina* and *Autographa gamma*. Diplomarbeit thesis, University of Basel
Schoonhoven LM, Van Loon JJA, Dicke M (2006) Insect – plant biology. Oxford Univ. Press, Oxford, UK
Seko T, Nakasuji F (2004) Effect of egg size variation on survival rate, development and fecundity of offspring in a migrant skipper, Parnara guttata guttata (Lepidoptera : Hesperiidae). Appl Entomol Zool 39:171–176
Simpson SJ, Raubenheimer ED (2007) Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. J Gerontol 62A:707–713
Spitzer BW (2004) Maternal effects in the soft scale insect *Saissetia coffeae* (Hemiptera: Coccidae). Evolution 58:2452–2461
Stern VM, Smith RF (1960) Factors affecting egg production and oviposition in populations of *Colias eurytheme* (Lepidoptera: Pieridae). Hilgardia 29:411–454
Stjernholm F, Karlsson B (2000) Nuptial gifts and the use of body resources for reproduction in the green-veined white butterfly *Pieris napi*. Proc Roy Soc B 267:807–811
Strong DR, Lawton JH, Southwood TRE (1984) Insects on plants: community patterns and mechanisms. Harvard University Press, Cambridge, MA
Svärd L (1985) Paternal investment in a monandrous butterfly *Pararge aegeria*. Oikos 45:66–70
Svärd L, Wiklund C (1989) Mass and production-rate of ejaculates in relation to monandry polyandry in butterflies. Behav Ecol Sociobiol 24:395–402
Thornhill R (1976) Sexual selection and paternal investment in insects. Am Nat 110: 153–163
Vicari M, Bazely DR 1993. Do grasses fight back – the case for antiherbivore defenses. Trends Ecol Evol 8:137–141
Wickler W (1985) Stepfathers in insects and their pseudoparental investment. Zeitschrift für Tierpsychologie 69:72–78
Wiklund C, Forsberg J (1991) Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. Oikos 60:373–381
Wiklund C, Kalrsson B (1984) Egg Size Variation in Satyrid Butterflies: Adaptive vs Historical, "Bauplan", and Mechanistic Explanations. Oikos 43:391–400
Wiklund C, Kaitala A, Lindfors V, Abenius J (1993) Polyandry and its effect on female reproduction in the

greenveined white butterfly (*Pieris napi* L.). Behav Ecol Sociobiol 33:25–33
Wiklund C, Karlsson B (1984) Egg Size Variation in Satyrid Butterflies: Adaptive vs Historical, "Bauplan", and Mechanistic Explanations. Oikos 43:391–400
Wiklund C, Persson A (1983) Fecundity, and the relation of egg weight to offspring fitness in the speckled wood butterfly Pararge aegeria, or why don't butterfly females lay more eggs? Oikos 40:53–63
Willers JL, Schneider JC, Ramaswamy SB (1987) Fecundity, longevity and caloric patterns in female *Heliothis virescens*: changes with age due to flight and supplemental carbohydrate. J Insect Physiol 33:803–808
Ziegler H (1956) Untersuchungen über die leitung und sekretion der assimilate. Planta 47:447–500

Illustrations in figure 1

Ecosystema (2012) Published online 30. October 2012 on http://www.ecosystema.ru
MacArthur W (1843) Published online 16. February 2010 on http://hortuscamden.com
Soreng JS, Peterson PM (2012) Revision of Poa L. (Poaceae, Pooideae, Poeae, Poinae) in Mexico: new records, re-evaluation of *P. ruprechtii*, and two new species, *P. palmeri* and *P. wendtii)*. Phyto Keys 15:1-104
Watson L, Dallwitz MJ (2008) British insects: butterflies. Version: 29th December 2011. http://delta-intkey.com

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Review für das Fachjournal 'Functional Ecology'	2012
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Review für das Fachjournal 'Oikos'	2014
Review für das Fachjournal 'Ecological Entomology'	2014

Wissenschaftliche Arbeiten

Cahenzli, F. & Erhardt, A. 2013. Transgenerational acclimatization in an herbivore-host plant relationship. Proceedings of the Royal Society B, 280: 1756 20122856.

Cahenzli, F. & Erhardt, A. 2013. Nectar amino acids enhance reproduction in male butterflies. Oecologia, 171: 197-205.

Cahenzli, F. & Erhardt, A. 2012. Enhancing offspring quality or quantity? Different ways for using nectar amino acids in female butterflies. Oecologia, 169: 1005-1014.

Cahenzli, F. & Erhardt, A. 2012. Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos, 121: 1417-1423.

Cahenzli, F. & Erhardt, A. 2012. Host plant defence in the larval stage affects feeding behaviour in adult butterflies. Animal Behaviour, 84: 995-1000.

Cahenzli, F. & Erhardt, A. 2008. The effect of nectar amino acids on female fecundity in the butterfly *Coenonympha pamphilus* (Lepidoptera, Satyrinae): Consideration of good- and bad larval food treatments. Unpublished MSc Thesis

Cahenzli, F., Bonetti, C. & Erhardt, A. Divergent strategies in pre- and postzygotic reproductive isolation between two closely related *Dianthus* species. In preparation

Cahenzli, F., Wenk, B. A. & Erhardt, A. Female butterflies adapt and allocate their progeny to the host plant quality of their own larval experience. In review

Bloch, D., Cahenzli, F. & Erhardt, A. Frequency-dependent pollinator preferences and ethological isolation of two closely related sympatric carnations. In review

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