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Response of the copper butterfly *Lycaena tityrus* to increased leaf nitrogen in natural food plants: evidence against the nitrogen limitation hypothesis

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Abstract This study examined the effects of increased leaf N in natural food plants on oviposition, preimaginal survival, growth, and adult size of the butterfly *Lycaena tityrus*. Female butterflies did not discriminate between leaves of high and low N content. In accordance with previous studies, we found higher growth rates and concomitantly decreased development times at a high N level. However, because of high pupal (and larval) mortality (overall 73.0%) as well as a reduction in adult size (by ca. 8%) this was, overall, not beneficial to the butterflies. Thus, our results were not consistent with the broad interspecific trend that insect herbivore performance is positively correlated with leaf N. These findings undermine the general applicability of the N limitation hypothesis. As the detrimental effects were largely confined to the pupal and adult stages, results obtained from the larval phase only may not yield reliable results and must therefore be interpreted with caution. If negative effects of N enrichment are found more frequently in declining species inhabiting nutrient poor grassland, this will have major implications for the conservation of these species.

Key words Lepidoptera · Herbivore performance · Host-plant quality · Fertilisation

Introduction

The presence and concentration of N in food plants is considered to be one of the most important factors affecting the performance in herbivorous insects. In general, higher N levels increase developmental rates, as was found in quite a number of lepidopterans (e.g. Slansky and Feeny 1977; Tabashnik 1982; Taylor 1984; Myers 1985; Cates et al. 1987; Estiarte et al. 1994; Hunter and

McNeil 1997; Grundel et al. 1998) as well as in other insect groups (e.g. Ohmart et al. 1985; Obermaier and Zwölfer 1999). Low N levels were found to result in poor larval survival (Myers and Post 1981; Myers 1985; Cates et al. 1987; Taylor 1988; Clancy 1992), and larvae may have to compensate for lowered N concentrations by increasing food intake or concentrating their feeding on the most N-rich parts of plants (Slansky and Feeny 1977; Mattson 1980; Ravenscroft 1994; Obermaier and Zwölfer 1999). Thus, it has been concluded that insect herbivores are limited by low N concentrations in food plants (e.g. Slansky and Feeny 1977; Tabashnik 1982; White 1984, 1993; Ravenscroft 1994), and that herbivore performance is generally positively related to increases in N content of foliage (Clancy 1992; White 1993). Less conspicuous effects may further arise from interactions with other animals, for example in ant-tended lycaenids (Baylis and Pierce 1991).

Although many publications (see above) report on positive correlations between insect performance and host-plant N concentrations, there is also substantial contradictory evidence (e.g. Schroeder 1986; Joern and Behmer 1998). In particular, a recent study on grasshoppers revealed that three species responded in different ways to increased levels of N (Joern and Behmer 1998). Regarding adult survival, one species showed a positive response, one a negative and another no reaction at all. The authors concluded that, although host-plant quality can contribute significantly to population responses, a uniform explanation like the “N limitation hypothesis” (White 1993) is not likely. However, grasshoppers compared to butterflies have the striking advantage of being able to acquire N from adult food resources, whereas the latter rely (regarding N) almost exclusively on reserves accumulated during the larval stage (e.g. Wheeler 1996; but see Beck et al. 1999). Therefore, finding negative effects of high N levels on fitness in a butterfly would even more strongly undermine the generality of the N limitation hypothesis.

In contrast to the vast majority of previous studies on butterflies, which focused mostly on pest species, the

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subject of the present study is declining the sooty copper, *Lycaena tityrus* (Poda 1761). Although still fairly widespread in parts of Europe, notable declines and local extinctions due to intensification of grassland management were recorded in recent decades and are still under way (e.g. Van Swaay 1990; Ebert and Rennwald 1991). Within Europe, this butterfly is now stated to be "vulnerable" in Austria, Belgium, Luxembourg, The Netherlands and Romania, and it is extinct in Denmark (Van Swaay and Warren 1999). In such species inhabiting unimproved grassland biotopes, effects of food-plant fertilisation may not follow the broad trend summarised above. Possibly, alterations in landuse practices yield not only straightforward effects, for example an increased mortality due to high mowing frequencies (cf. Erhardt and Thomas 1991; Tscharrtker and Greiler 1995), but also indirect ones owing to the change of food-plant quality as a consequence of recurrent applications of fertilisers. However, in contrast to the considerable knowledge about effects of fertilisation on vegetation (e.g. Van Duren et al. 1997; Bakker and Berendse 1999), those on co-occurring herbivorous insects remain still largely unclear. Hence, the primary objective of this study was to examine responses of *L. tityrus* to an increased leaf N content in its principal host-plant, *Rumex acetosa* (L.). Therefore, we address the following issues here:

1. Do ovipositing *L. tityrus* females discriminate between host plants of different N concentrations?
2. Do larvae grow better on leaves with higher N content?
3. Does differential mortality occur during development?
4. Are there any effects on fitness-relevant parameters such as adult size?

Materials and methods

Study organism

L. tityrus is a widespread temperate zone butterfly, ranging from western Europe to central Asia (Ebert and Rennwald 1991). The species is bivoltine with two discrete generations per year in most parts of its range (Ebert and Rennwald 1991; Tolman and Lewington 1998). Larvae of the last brood enter diapause, overwintering half grown in the third instar. The principal larval host plant is *R. acetosa*, but some congeneric plant species such as *Rumex acetosella* (L.) and *Rumex scutatus* (L.) are utilised as well (Schweizerischer Bund für Naturschutz 1987; Ebert and Rennwald 1991; Tolman and Lewington 1998). The animals for this study belonged to the alpine subspecies *L. tityrus subalpinus* (Speyer 1851). Seven freshly emerged females were caught in the summer of 1999 (6 August) in the central Alps (Senales valley, northern Italy) at an altitude of about 1800 m above sea level. In this region, *L. tityrus* is monovoltine with adults being on the wing from mid-July through late August. However, as our own rearing experiments showed, even these alpine populations are potentially multivoltine.

Host-plant treatments

The principal host plant, *R. acetosa*, was used for the experiments. Plants were reared from seed under greenhouse conditions. Older

seedlings were potted individually and randomly assigned to one of two treatments. Every 2 days, plants were given either water alone or water containing nitrogenous fertiliser (NH_4NO_3). During hot periods, when there was a risk of dehydration, all plants were given additional water on the intervening days. The two treatments were designed to establish differences in leaf N levels. In order to control for the effectiveness of treatments, standard CHN analyses (element analyser, CHNO-Rapid) were carried out. For these, random samples of fresh leaf cuttings were gathered in both groups in the first and second half of the feeding experiments. Afterwards, cuttings were dried to a constant weight at 70°C for 2 days, ground to a homogeneous powder, and finally analysed. From each of the four samples, five analyses were drawn.

Oviposition experiments

L. tityrus females were kept in an environment cabinet (L18:D6). Experiments were done in another cabinet under warmer conditions (30°C). On 3–4 days with at least 1 intervening day, the seven females were placed individually in cages and given a simultaneous choice between fertilised and unfertilised *R. acetosa* leaves. Leaf cuttings in each pair were (as far as possible) of the same size and shape. The position of plants within cages was switched every 1 to 2 h to control for position effects. The plant bunches were removed daily; afterwards the eggs were counted. All eggs laid on fertilised and unfertilised *R. acetosa*, respectively, during the experiment were pooled for each female. Data were analysed with the non-parametric Wilcoxon test (cf. Sachs 1997).

Rearing experiments

Eggs of three females were exposed to a constant temperature of 20°C (L18:D6). To investigate the effect of host-plant quality, hatchlings of every female were randomly divided among two groups, one reared on fertilised, the other on unfertilised *R. acetosa* (20°C; L18:D6 throughout). In order to minimise all other causes of variability, only full sibs were compared (split-brood design) within the three replicates (families). Young hatchlings were placed group-wise (10–20) in transparent plastic boxes (250 ml) containing moistened filter paper and fresh cuttings of *R. acetosa* in ample supply. Later on, larvae were separated into small groups (2–3 larvae) or reared singly. The boxes were checked daily and supplied with new food when needed. To even out minor temperature differences within cabinets, the boxes were shifted around daily. For each individual we measured the following traits: development time from hatching to pupation and adult eclosion, pupal and adult weight, forewing length, growth rate, and proportional weight loss at metamorphosis. Pupae were weighed on the day following pupation, adults on the day of eclosion after having excreted meconium. The individual growth rate of each larvae used in the experiments was calculated according to Gotthard et al. (1994) (see also Nylin 1992; Nylin et al. 1993):

Growth rate = $[\ln(\text{pupal weight}) - \ln(\text{hatchling weight})] / \text{larval time}$.
This formula gives a relative growth rate representing the mean weight gain per day. To assess the weight of newly hatched larvae, a sample of 30 larvae were weighed soon after hatching, their weight being 0.076 mg on average. Due to their low weight, larvae could only be weighed as a group, and hence no SD can be given. As variance in egg weight seems to be small (e.g. Jones et al. 1982; Wiklund et al. 1991), and since a newly hatched larva increases its weight some 50% after a few hours of feeding (Wiklund et al. 1991), the above-mentioned value was used as a standardised weight for newly hatched larvae to calculate individual growth rates (cf. Wiklund et al. 1991; Gotthard et al. 1994). Proportional weight loss between pupation and adult eclosion was measured using the formula given in Gotthard et al. (1994):
Proportion weight loss = $1 - (\text{adult weight} / \text{pupal weight})$.

Statistical analysis

Differences between groups of larvae, pupae or butterflies were localised using the least significant difference post hoc comparison (significance threshold $P < 0.05$ throughout) after a two-way analysis of co-variance (ANCOVA), with plant quality and family as factors and sex as covariate, or, in the case of a comparison between successful and unsuccessful individuals reared on fertilised host-plants (see below), after a two-way ANOVA. In the latter, a distinction between sexes was not appropriate, because the “unsuccessful” individuals died during the pupal phase. In the ANCOVAs, sex was controlled for because of unbalanced sample sizes due to differential mortality. Nevertheless, results in tables and figures are predominantly shown separately for sexes, because there clearly are differences, for example in development times owing to protandry (cf. Fischer and Fiedler in press).

All data on development times were log-transformed before analysis to achieve normality and homogeneity of variances. Because there were no significant statistical interactions between factors in any of the cases, these results are not shown in detail. Throughout the text all means are given ± 1 SD.

Results

Host-plant quality

The leaves of the fertilised plants had a darker colour and higher N content than those of unfertilised plants (fertilised plants, $5.61 \pm 0.06\%$ N and $6.21 \pm 0.03\%$ N of dry mass; unfertilised plants, $3.59 \pm 0.06\%$ N and $3.89 \pm 0.06\%$ N of dry mass; data from first and second half of the experiment, respectively; $n=5$ measurements each). Hence, the two treatments were successful in establishing differences in plant quality. In contrast to N, values for C (fertilised plants, $42.10 \pm 0.00\%$ C

and $42.96 \pm 0.06\%$ C compared to $44.20 \pm 0.07\%$ C and $43.36 \pm 0.09\%$ C in unfertilised plants) and hydrogen (fertilised plants, $5.73 \pm 0.11\%$ H and $5.87 \pm 0.10\%$ H compared to $5.74 \pm 0.08\%$ H and $5.80 \pm 0.06\%$ H in unfertilised plants) were more or less constant throughout.

Oviposition experiment

On average, females laid slightly more eggs on the unfertilised (96 ± 38 ; $n=672$ eggs) compared to the fertilised host plants (80 ± 24 ; $n=559$ eggs). However, there was no significant effect of host-plant quality (Wilcoxon test, $Z_{n=7}=0.68$, $P=0.50$). Hence, females did not discriminate between fertilised and unfertilised *R. acetosa* plants during oviposition.

Effects of fertiliser application on survival rates

Overall, host-plant quality led to a highly divergent mortality of larvae and pupae, which exceeded 73.0% on fertilised compared to only 33.3% on unfertilised host plants ($n=222$ in both groups). During larval development the difference was 46.4% mortality on fertilised plants compared to 27.9% mortality on unfertilised plants. However, this was caused by only one family, which performed particularly badly on fertilised *R. acetosa* (Table 1). In contrast, pupal mortality was greatly increased in all three families (overall 49.6% on fertilised compared to 8.1% on unfertilised plants).

For larvae reared on fertilised host plants a comparison was carried out between the individuals which died during the pupal stage and those which completed development successfully (a similar comparison for larvae reared on unfertilised plants was not appropriate due to low mortality during the pupal stage). Individuals which later died exhibited a significantly longer larval development (29.3 ± 3.3 days) than successful ones (26.7 ± 2.7 days; ANOVA, $F_{1,113}=22.0$, $P < 0.0001$), without a significant difference between families (ANOVA, $F_{2,113}=0.13$, $P=0.88$; Table 2). Furthermore, pupal weight was lower in the moribund individuals (123.3 ± 16.0 mg) compared to the survivors (129.6 ± 12.7 mg; ANOVA $F_{1,113}=7.49$, $P=0.007$). There were also less pronounced family effects (ANOVA $F_{2,113}=3.24$, $P=0.043$) regarding pupal weight.

Table 1 Mortality (%) in three families of *Lycaena tityrus* on fertilised (N_+) compared to unfertilised (N_0) host plants (*Rumex acetosa*). Figures of a pair (N_+ , N_0) followed by the same letter do not differ significantly (Bonferroni corrected χ^2 -tests; null hypothesis: equal distribution of mortality among treatments, threshold for significance $P < 0.006$)

	Family no. 1		Family no. 2		Family no. 3	
	N_+	N_0	N_+	N_0	N_+	N_0
<i>n</i> (initial)	81	80	78	79	63	63
Larvae	43.2 a	40.0 a	60.3 a	13.9 b	33.3 a	30.2 a
Pupae	41.3 a	18.8 a	58.1 a	2.9 b	52.4 a	2.3 b
Total	66.7 a	51.3 a	83.3 a	16.5 b	68.3 a	31.7 b

Table 2 Development time and pupal weight of successful and unsuccessful (died in the pupal stage) individuals of *L. tityrus* reared on fertilised *R. acetosa*. Figures within a row followed by

the same letter do not differ significantly [Least significant difference (LSD) post hoc comparison after two-way ANOVA, threshold for significance $P < 0.05$]

	Family no. 1		Family no. 2		Family no. 3	
	Alive	Dead	Alive	Dead	Alive	Dead
<i>n</i>	27	19	13	18	20	22
Larval time (days)	26.5 \pm 3.1 a	29.3 \pm 3.9 b	26.6 \pm 2.4 a	29.3 \pm 4.0 b	26.9 \pm 2.5 a	29.3 \pm 2.0 b
Pupal weight (mg)	129.3 \pm 10.8 a,c	118.2 \pm 16.9 b	137.0 \pm 12.4 c	126.7 \pm 17.6 a,b	125.2 \pm 13.6 a,b	124.8 \pm 13.5 a,b

Table 3 Life history data (mean±1 SD) for male and female *L. tityrus* of three different families in relation to host-plant quality (N+ and N₀ *R. acetosa*). Figures of a pair (N+, N₀) followed by the same letter do not differ significantly [LSD post hoc comparison after two-way analysis of covariance (ANCOVA), threshold for significance $P<0.05$]. For abbreviations, see Tables 1 and 2

Males	Family no. 1		Family no. 2		Family no. 3	
	N+ (n=21)	N ₀ (n=21)	N+ (n=8)	N ₀ (n=39)	N+ (n=13)	N ₀ (n=20)
Larval time (days)	25.9±3.0 a	29.4±4.3 b	25.8±1.3 a	30.9±3.5 b	27.5±2.9 a	30.8±4.7 b
Pupal time (days)	12.8±0.9 a	12.0±1.1 b	11.8±0.7 a	11.8±1.1 a	12.7±1.0 a	12.5±1.1 a
Larval+pupal time (days)	38.7±2.8 a	41.4±4.1 b	37.5±1.4 a	42.8±3.2 b	40.2±3.2 a	43.3±4.6 b
Pupal weight (mg)	127.5±9.0 a	136.3±14.2 b	137.2±14.3 a	138.6±11.6 a	121.2±14.9 a	133.5±10.2 b
Adult weight (mg)	53.9±7.7 a	54.4±7.0 a	50.4±5.3 a	53.0±7.7 a	46.9±4.8 a	52.4±7.1 a
Forewing length (mm)	16.3±0.6 a	16.6±0.6 a	16.1±0.4 a	16.2±0.4 a	15.8±0.6 a	16.3±0.6 b
Weight loss (%)	58.8±4.8 a	60.1±4.7 a	63.2±2.7 a	61.8±3.6 a	61.0±4.3 a	60.8±3.9 a
Growth rate (%/day)	29.0±2.9 a	25.9±3.6 b	29.2±1.6 a	24.6±2.7 b	27.0±3.0 a	24.7±3.4 b

Females	Family no. 1		Family no. 2		Family no. 3	
	N+ (n=6)	N ₀ (n=18)	N+ (n=5)	N ₀ (n=27)	N+ (n=7)	N ₀ (n=23)
Larval time (days)	28.7±2.3 a	30.6±3.4 a	28.0±3.2 a	33.6±3.8 b	25.7±1.0 a	28.9±3.5 b
Pupal time (days)	12.7±0.5 a	12.5±1.5 a	12.0±0.7 a	11.8±1.1 a	12.7±1.1 a	13.1±0.7 a
Larval+pupal time (days)	41.3±2.7 a	43.1±3.1 a	40.0±2.9 a	45.4±3.4 b	38.4±1.4 a	42.0±3.1 b
Pupal weight (mg)	135.6±14.6 a	137.9±13.1 a	136.7±10.3 a	154.5±18.5 b	132.6±6.5 a	139.8±18.4 a
Adult weight (mg)	61.1±4.2 a	63.7±8.2 a	63.1±5.8 a	69.9±9.7 a	58.9±4.9 a	61.0±10.2 a
Forewing length (mm)	16.2±0.4 a	16.5±0.6 a	15.8±0.5 a	16.6±0.6 b	16.1±0.5 a	16.2±0.9 a
Weight loss (%)	55.7±3.2 a	53.9±3.0 a	53.8±2.4 a	54.8±2.6 a	55.6±2.9 a	56.6±4.2 a
Growth rate (%/day)	26.2±1.8 a	24.8±2.4 a	27.0±2.9 a	22.9±2.5 b	29.1±0.9 a	26.3±3.1 b

Table 4 Two-way ANCOVA for effects of plant quality and families on life history traits in *L. tityrus*. Sex was controlled for as covariate

Trait	Source	n	df	F	P
Larval time (days)	Plant quality	208	1, 201	45.2	<0.0001
	Family	208	2, 201	1.3	0.3
	Plant quality×family	208	2, 201	1.8	0.2
Pupal time (days)	Plant quality	208	1, 201	1.1	0.3
	Family	208	2, 201	9.8	<0.0001
	Plant quality×family	208	2, 201	1.6	0.2
Larval+pupal time (days)	Plant quality	208	1, 201	46.1	<0.0001
	Family	208	2, 201	0.2	0.8
	Plant quality×family	208	2, 201	2.6	0.08
Pupal weight (mg)	Plant quality	208	1, 201	12.2	0.0006
	Family	208	2, 201	7.0	0.0012
	Plant quality×family	208	2, 201	0.4	0.7
Adult weight (mg)	Plant quality	208	1, 201	4.8	0.029
	Family	208	2, 201	5.0	0.008
	Plant quality×family	208	2, 201	1.0	0.36
Forewing length (mm)	Plant quality	207	1, 200	14.0	0.0002
	Family	207	2, 200	6.0	0.003
	Plant quality×family	207	2, 200	0.2	0.8
Weight loss (%)	Plant quality	208	1, 201	0.6	0.4
	Family	208	2, 201	7.5	0.0007
	Plant quality×family	208	2, 201	0.8	0.4
Growth rate (%/day)	Plant quality	208	1, 201	43.8	<0.0001
	Family	208	2, 201	0.9	0.4
	Plant quality×family	208	2, 201	2.1	0.1

Effects of fertiliser application on development time and adult size

Total development time (from hatching to adult) strongly depended on whether food plants were fertilised or not (Table 3; for statistics see Table 4). This was true for all

three replicates, and significant family effects did not occur. Overall, mean development time was about 4 days longer on unfertilised compared to fertilised *R. acetosa* (43.0±3.7 compared to 39.2±2.8 days). This was due to a longer duration of the larval stage, whereas the duration of the pupal stage did not contribute in a significant way

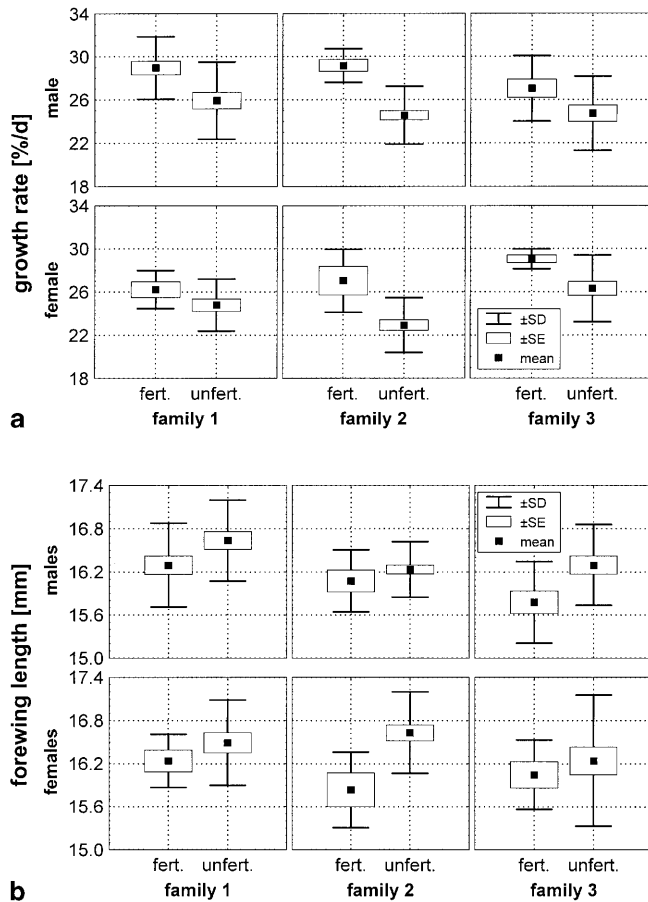


Fig. 1 Growth rate (a) and forewing length (b) in three families of *Lycaena tityrus* in relation to host-plant quality. Throughout, growth rate of larvae reared on fertilised (*fert.*) *Rumex acetosa* was higher compared to those of larvae reared on unfertilised (*unfert.*) plants. However, this was accompanied by a reduction in adult size. *d* Day

to differences in mean development time. Regarding duration of the larval stage, again no family effects were present, but duration of the pupal stage varied considerably between families.

Shorter development times of larvae reared on fertilised host plants corresponded with invariably higher growth rates (Fig. 1a). Furthermore, they were accompanied by a reduction in pupal and adult weight as well as forewing length (Fig. 1b). In the latter three traits there were also family effects found (Table 4). Weight loss at metamorphosis was not affected by host-plant quality. However, differences between families were notable.

Discussion

According to our results, *L. tityrus* females were not able to discriminate between fertilised and unfertilised *R. acetosa*. However, the ability of ovipositing butterflies to select food plants most suitable for their offspring has been demonstrated for other species (e.g. Rausher 1981; Williams 1983; Myers 1985; Ng 1988; Janz and Nylin

1997). Oviposition in relation to nutrient contents was also found in another lycaenid (Baylis and Pierce 1991). A possible explanation for the lack of discrimination in *L. tityrus* might be that butterflies have encountered high N levels in their host plant, like in our experiment, only recently, due to the intensification of grassland management, and therefore have not yet been selected for avoiding detrimentally high N concentrations.

This leads to the question, is the experimental design suitable to match relevant conditions for this butterfly? We definitely do think so. For example, Joern and Behmer (1998) used a range of 1–7% N dry weight (compared to a maximum of about 6% in the present study) to span the scope reported for some grasses and forbs under natural conditions. Thus, it seems plausible that similar or even higher amounts can be reached by recurrent applications of fertilisers on intensified grassland. Furthermore, other experiments with artificial as well as natural diets still confirmed positive effects on lepidopterans at similar or even higher N concentrations [up to 7.6% and 7.0% N dry mass (Tabashnik 1982; Clancy 1992); see also Slansky and Feeny 1977]. These species clearly were able to benefit from food plants particularly rich in N.

In accordance with previous studies (e.g. Slansky and Feeny 1977; Tabashnik 1982; Taylor 1984; Ohmart et al. 1985; Cates et al. 1987; Estiarte et al. 1994; Hunter and McNeil 1997; Grundel et al. 1998), increased growth rates and a concomitantly shorter development time were found on food plants with higher N levels. The latter might be due to a determination of the amount of food ingested by the amount of N, which in turn should affect the amounts of other nutrients, as was hypothesised by Clancy (1992). However, in the particular case of *L. tityrus*, the far higher mortality as well as the reduced adult size (pupal and adult weight, forewing length) in individuals reared on fertilised food plants leads to an alternative explanation, suggesting that larvae simply had severe physiological problems caused by the high N concentration. Therefore, they might have shortened their development times to escape food stress. In contrast to the former explanation, this could possibly explain the increase in growth rates as well, for many butterflies are able to speed up their development when needed (cf. Nylin and Gotthard 1998; Gotthard et al. 1999). Support for this explanation comes from the observation that those individuals which developed quickly and simultaneously reached higher weights were more likely to survive until adulthood, whereas those which died as pupae had achieved significant smaller weights, but had required more time to reach these weights.

Irrespective of the precise physiological mechanism, our data clearly demonstrate that increased leaf N is, overall, not beneficial to *L. tityrus*. The higher mortality (a six-fold increase in the pupal stage) as well as the reduced adult size (about 8% in pupal and adult weight) contrast with previous studies on lepidopterans showing positive effects (e.g. Myers and Post 1981; Taylor 1984; Myers 1985; Ohmart et al. 1985; Baylis and Pierce

1991). In grass-feeding *Carterocephalus palaemon*, dependency on plants of increased nutrient status is so strong that larvae develop slowly and eventually die on nutrient-poor plants (Ravenscroft 1994). Thus, our results give substantial support to the view of Joern and Behmer (1998), who doubted uniform explanations, and further undermine the general applicability of the N limitation hypothesis (White 1993). Moreover, differences between families in adult size and mortality rates confirm that there must be a considerable genetic variation in the ability to cope with differential plant quality, suggesting that even intraspecific variability has to be considered.

However, total leaf N may not be the only controlling factor for the observed phenomena, but may be correlated with other factors which were not measured in this study. Changes in the level of N in a plant tend to be accompanied by changes in the level of many other nutrients, water, and numerous allelochemicals (e.g. Mattson 1980; Scriber and Slansky 1981; Taylor 1984; Schroeder 1986). Nevertheless, the extraordinary importance of N as compared to other resources (e.g. carbohydrates, water) was confirmed in other studies (Tabashnik 1982; Joern and Behmer 1998). The purpose of this study was not to uncover the real mechanistic causes of the shown effects. Rather, because fertilisation of grassland will always result in complex reactions of plants instead of a change in but one parameter, we tried to mimic exactly these plant responses and their consequences for the butterfly under study.

In this study, sex effects were largely ignored as both sexes obviously reacted in a similar manner to differences in plant quality (cf. Fig. 1; for detailed information on sex-related differences in reaction norms see Fischer and Fiedler in press). However, it is worth mentioning that, in general, protandry persisted in our experiments except in family no. 3, again showing a considerable degree of genetic variability.

In conclusion, the responses of *L. tityrus* to variation in leaf N of the larval host plant were not consistent with the broad interspecific trend that insect herbivore performance is positively correlated with N content. As the detrimental effects were largely confined to the pupal and adult stages, results obtained from the larval phase only (cf. Slansky and Feeny 1977; Tabashnik 1982; Baylis and Pierce 1991; Estiarte et al. 1994), where even in the present study a higher mortality was found in only one family, may not always yield reliable results and should therefore be interpreted with strict caution. Whenever possible one has to trace the whole life cycle of the studied insect.

If such detrimental effects are found more frequently in declining herbivore species inhabiting nutrient poor grassland, this will be of great importance to the conservation of such species. Threats do not only emerge from the application of fertilisers on grassland, but from atmospheric N depositions concerning whole landscapes as well (e.g. Berendse et al. 1993; Bakker and Berendse 1999). Thus, we feel that less conspicuous effects on

host-plant quality should receive more attention. In the case of *L. tityrus*, the larval host plant *R. acetosa* is still extremely widespread and abundant, and is therefore not limiting the occurrence and population size of this species. Changes in the nutrient balance of the host plant may well play a role in the ongoing and regionally dramatic decline of this once abundant herbivorous insect in intensively managed landscapes of Europe.

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