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Nitrogen enrichment of host plants has mostly beneficial effects on the life-history traits of nettle-feeding butterflies

Susanne Kurze[a], Thilo Heinken[a], Thomas Fartmann[b,c,*]

[a] Institute of Biochemistry and Biology, General Botany, University of Potsdam, Maulbeerallee 3, 14469 Potsdam, Germany
[b] Department of Biodiversity and Landscape Ecology, Faculty of Biology and Chemistry, Osnabrück University, Barbarastraße 13, 49076 Osnabrück, Germany
[c] Institute of Biodiversity and Landscape Ecology (IBL), Hafenweg 31, 48155 Münster, Germany

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ABSTRACT

Butterflies rank among the most threatened animal groups throughout Europe. However, current population trends differ among species. The nettle-feeding butterflies *Aglais io* and *Aglais urticae* cope successfully with the anthropogenic land-use change. Both species are assumed to be pre-adapted to higher nitrogen contents in their host plant, stinging nettle (*Urtica dioica*). However, it is currently unknown, whether this pre-adaptation enables both *Aglais* species to cope successfully or even to benefit from the excessive nitrogen availabilities in nettles growing in modern farmlands. For this reason, this study focused on the response of both *Aglais* species to unfertilized nettles compared to nettles receiving 150 or 300 kg N ha^{-1} yr^{-1} (i.e., common fertilizer quantities of modern-day agriculture). Fertilized nettles were characterized by higher nitrogen concentrations and lower C:N ratios compared to the control group. In both *Aglais* species, the individuals feeding on fertilized nettles had higher survival rates, shorter larval periods and heavier pupae and, in *A. urticae* also longer forewings. All these trait shifts are beneficial for the individuals, lowering their risk to die before reproduction and increasing their reproductive potential. These responses agree with the well-accepted nitrogen-limitation hypothesis predicting a positive relationship between the nitrogen content of the diet and the performance of herbivorous insects. Furthermore, our findings suggest that the increasing abundance of both *Aglais* species may result not only from the increasing spread of nettles into the farmland but also from changes in their quality due to the eutrophication of the landscape during recent decades.

1. Introduction

Butterflies rank among the most threatened animal groups throughout Europe (Thomas et al., 2004) and are declining across multiple habitat types (Fartmann et al., 2013; Schirmel and Fartmann, 2014). However, current population trends among species differ largely (Öckinger et al., 2006; van Swaay et al., 2015). Trait-based approaches show that butterfly species with high mobility, wide niche breadth, low host-plant specificity, and long flight period experience lower extinction risks than butterflies with contrasting characteristics (Kotiaho et al., 2005; Ekroos et al., 2010). However, famous exceptions are monophagous butterflies with a nitrogen-rich diet (Betzholtz et al., 2013). *Aglais io* and *Aglais urticae* are commonly mentioned as typical winners of recent anthropogenic land-use change since the increasing eutrophication of the landscape results in an enormous spread of their host plant, stinging nettle (*Urtica dioica*) (Öckinger et al., 2006; Betzholtz et al., 2013; Serruys and van Dyck, 2014; Merckx et al., 2015). This assumption is supported by several studies documenting an increase in abundance of *A. io* and *A. urticae* (Öckinger et al., 2006; Nilsson et al., 2008; but see van Dyck et al., 2009).

Both species are assumed to be pre-adapted to higher nitrogen contents in their diet since they prefer nitrophilous stinging nettle as their host plant (Serruys and van Dyck, 2014), which has very high nitrogen contents compared with other herbaceous plants (Müllerová et al., 2014). Indeed, both *Aglais* species positively respond to re-grown nettles with higher water and nitrogen contents and to fertilized nettles (Pullin, 1986, 1987; Audusseau et al., 2015). However, these studies did not consider fertilizer quantities now regularly used in agriculture. Therefore, it is currently unknown, whether this pre-adaptation enables both *Aglais* species to cope successfully or even to benefit from higher nitrogen contents in nettles inhabiting nitrogen-rich sites in modern farmlands.

Indeed, the relationship between the increasing spread of nettles from their natural habitats such as nitrogen-rich woodland gaps and river banks into the intensive farmland and the increasing abundance of both *Aglais* species seems less straightforward. The performance of *A.*

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[*] Corresponding author. Department of Biodiversity and Landscape Ecology, Faculty of Biology and Chemistry, Osnabrück University, Barbarastraße 13, 49076 Osnabrück, Germany.
E-mail addresses: lilium.brevis@googlemail.com (S. Kurze), heinken@uni-potsdam.de (T. Heinken), t.fartmann@uos.de (T. Fartmann).

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urticae individuals increased when larvae fed on nettles growing in field margins compared to woodland gaps, but the higher survival rates and faster development were rather caused by the warmer microclimate in field margins than the different host plant qualities in both habitats (Merckx et al., 2015). In contrast, in *A. io* the utilization of nettles in field margins and woodland gaps resulted in a trade-off between offspring quality and quantity (Serruys and van Dyck, 2014). Individuals developing on nitrogen-richer nettles in woodland gaps reached higher survival rates, but individuals developing on nettles in warmer field margins had higher fitness predictions (Serruys and van Dyck, 2014). Thus, both studies (Serruys and van Dyck, 2014; Merckx et al., 2015) highlight the interaction of host plant quality and microclimate on the performance of both *Aglais* species, but provide only limited support that the assumed pre-adaptation to higher nitrogen contents helps the individuals to successfully utilize nettles growing in highly eutrophic sites in intensive farmland.

However, to answer this question unambiguously is of increasing importance (cf. Nijssen et al., 2017), because the eutrophication of the landscape through the application of enormous fertilizer quantities (Liu et al., 2015) and atmospheric nitrogen deposition (Bobbink et al., 1998; WallisDeVries and Bobbink, 2017) enable nettles to increasingly spread into farmland, in which they might form large mono-specific stands (Taylor, 2009; Müllerová et al., 2014). Nettles benefit from fertilizer quantities of up to 600 kg N ha^{-1} yr^{-1}, which cause several changes in their tissue chemistry (Grevsen et al., 2008; Rutto et al., 2012). It is unknown whether these host-plant quality changes are still within the tolerance range of both *Aglais* species or may lead to unexpected negative responses.

Herbivorous insects and plants substantially differ in their stoichiometry with about ten-fold lower C:N ratios in insects than in plants (Mattson, 1980). Therefore, the host-plant quality and its nitrogen concentration determine the performance of herbivorous insects (Mattson, 1980; White, 1993). Accordingly, the nitrogen-limitation hypothesis proposed by White (1993) predicts that higher nitrogen contents in the diet support the performance of herbivorous insects. Several empirical studies (e.g., Slansky and Feeny, 1977; Tabashnik, 1982; Chen et al., 2008) confirm this hypothesis for Lepidoptera. Larvae developing on high-quality host plants have higher metabolism efficiencies, enabling them to increase their growth rate and to shorten their development time despite a lower food intake (Slansky and Feeny, 1977; Morehouse and Rutowski, 2010). Furthermore, several studies observed heavier pupae and larger forewings in the adult stage when individuals had fed on diets with high nitrogen contents (Myers and Post, 1981; Mevi-Schütz and Erhardt, 2003). All these trait shifts imply fitness advantages for the individuals lowering their probability to die before reproduction and enhance their fecundity (Loader and Damman, 1991; Awmack and Leather, 2002). The pupal time of the individuals though received less attention or remained constant (Mevi-Schütz and Erhardt, 2003). Too low nitrogen concentrations in the host plants instead induce not only fitness losses but can also lead to the death of the larvae (Myers and Post, 1981; Ravenscroft, 1994).

Within the nitrogen-limitation hypothesis, both sexes are subject to different constraints, and therefore, to maximize their fitness, develop sex-specific responses to different food qualities (Nylin and Gotthard, 1998; Quezada-García et al., 2014). In most Lepidoptera species females represent the larger sex with longer development times and higher food intakes, rendering them more vulnerable to unsuitable diet (Quezada-García et al., 2014; Szekely et al., 2014). Hence, a generation feeding on an unsuitable diet is characterized by a male-biased sex ratio (Quezada-García et al., 2014). Furthermore, the fecundity of females depends mainly on their body size, which is assumed to be the main driver in sexual selection (Nylin and Gotthard, 1998). In contrast, according to the theory of protandry, males optimize their fitness through shorter development times to increase the number of matings with the later hatching females (Wiklund and Fagerström, 1977; Nylin and Gotthard, 1998).

Recently, the well-accepted general validity of the nitrogen-limitation hypothesis was questioned by empirical findings of hump-shaped or negative relationships between the performance of Lepidoptera species and the nitrogen content in their diet (Fischer and Fiedler, 2000a; Sarfraz et al., 2009; Han et al., 2014). However, considering the overwhelming number of studies supporting the nitrogen-limitation hypothesis, these negative evidences should be considered anecdotal (Waring and Cobb, 1992; Throop and Lerdau, 2004).

The present study aims at overcoming the lack of knowledge about the response of *A. io* and *A. urticae* to nettles growing in the intensive farmland and explicitly tests their assumed pre-adaptation to nettles receiving fertilizer quantities commonly used in modern-day agriculture. To our knowledge, this study represents one of the first fertilization experiments with non-pest butterflies considering agriculturally used fertilizer quantities (but see Prudic et al., 2005). According to the assumed pre-adaptation of both *Aglais* species and the nitrogen-limitation hypothesis we predict the following reactions: Individuals developing on fertilized nettles have (i) a higher survival rate; (ii) a shorter larval period; (iii) no change in the duration of the pupal period; (iv) heavier pupae and (v) longer forewings compared to individuals feeding on unfertilized plants. All these responses of the butterflies are expected to depend on the fertilizer supply to the host-plants resulting in a greater increase of the trait attribute in individuals feeding on more fertilized plants. Concerning the sex-specific responses to food quality we expect females to be more vulnerable to unsuitable diet. Besides the standardized fertilization procedure, we controlled the host-plant quality by the measurement of the nitrogen concentration and C:N ratio, predicting higher nitrogen concentrations and lower C:N ratios in fertilized nettles and in a dose dependent manner.

2. Materials and methods

2.1. Study species

Both studied butterfly species, *Aglais io* (Linnaeus, 1758) (peacock) and *Aglais urticae* (Linnaeus, 1758) (small tortoiseshell), have many similarities in their biology and ecology. The distribution of the highly mobile and common species ranges from Europe to the temperate zone of Asia (Bräu et al., 2013). They occur in a wide variety of habitats including urban, rural, agricultural, and forest landscapes (Ebert and Rennwald, 1991; Bräu et al., 2013). Usually, these habitats are characterized by high densities of flowering plants as nectar resources and stands of the main host plant, *Urtica dioica* L. (stinging nettle). Females of both species deposit their eggs as batches on the host plant, and larvae feed gregariously (Bräu et al., 2013). However, oviposition site preferences clearly differ among the two species. Females of *A. io* prefer semi-shaded to sunny *U. dioica* plants growing on sites with high air humidity (Bräu et al., 2013). In contrast, females of *A. urticae* lay their eggs on fully sunny host-plant individuals (Bräu et al., 2013). Both species hibernate as an adult. *Aglais io* is univoltine or partially bivoltine and the larvae appear in May to June and July to August (Ebert and Rennwald, 1991; Bräu et al., 2013). In contrast, *A. urticae* has two or even three generations per year with larvae occurring from May to June and July to August (Ebert and Rennwald, 1991; Bräu et al., 2013). Although *A. urticae* populations have highly fluctuated in recent years (Bräu et al., 2013), it is considered not threatened in Europe just like *A. io* (van Swaay et al., 2010).

The main host plant of the two *Aglais* species, *U. dioica*, is widespread throughout the temperate zone of the Palearctic and naturalized in other temperate regions (e.g., North America) (Grime et al., 2007; Verheyen et al., 2012). It is recorded from many terrestrial habitats and particularly abundant on fertile soils on the banks of rivers, streams and ditches, unmanaged road verges, and hedgerows (Grime et al., 2007; Taylor, 2009). The distribution and abundance of *U. dioica* has strongly increased in western and central Europe due to agricultural fertilization and airborne nitrogen deposition (Ellenberg and Leuschner, 2010;

Verheyen et al., 2012).

2.2. Host-plant treatments

The application of fertilizer in the host-plant treatments was based on quantities commonly used in agriculture, ranging from zero to over 500 kg N ha^{-1} yr^{-1} in different grasslands across Europe (Herzog et al., 2006; Kleijn et al., 2009). Therefore, the experiment with both *Aglais* species comprised three host-plant treatments including one control group without fertilization and two fertilization treatments with an input of 150 kg N ha^{-1} yr^{-1} (hereafter referred to as N150) or 300 kg N ha^{-1} yr^{-1} (N300). To ensure a fast and sufficient nitrogen uptake by the plants, the fertilization took place with the common fertilizer ammonium nitrate sulfate containing 26% ammonium nitrate (Karmoker et al., 1991; Salvagiotti et al., 2009). For the application, this fertilizer was dissolved in water.

In autumn of the previous year, seedlings of *U. dioica* were dug out in the agricultural landscape near Dresden and potted in garden soil. In spring, these plants were transplanted to bigger pots with new garden soil and randomly distributed to the treatments. The potted nettles grew under field conditions but received water in ample supply, evenly distributed to all treatments. A subsequent rearrangement of the pots took place every week. The whole fertilizer amount, calculated on the basis of the pot areas, was divided into different portions during the larval period of the study species. When the neonates hatched, the plants got 5% of the fertilizer amount. A few days passed until the transfer of the larvae to the treatments when the plants received the first of five further servings, which contained altogether 95% (5 × 19%) of the whole amount. After the larvae had finished three-quarters of their development, the nettles got the last 19% serving.

Ten random samples of leaf cuttings of *U. dioica* of each treatment were gathered five days after the plants received the last fertilizer serving. The leaf cuttings were dried, ground to a homogeneous powder and analyzed with the C/N Analyzer vario EL III (company Elementar) to determine the nitrogen (N%) and carbon (C%) concentration, as well as the C:N ratio in reference to the dry weight.

2.3. Rearing experiment

In order to obtain larvae of *A. io* for the fertilization experiment, nine females were caught in May 2015 in the agricultural landscape near Dresden, Germany. The females originated from arable fields, urban gardens, and hedgerows. Larvae of *A. urticae* were collected in April 2015 from different larvae webs across ditches in Dresden, Germany. These larvae were reared with unfertilized nettles until adult emergence. For mating, individuals of both sexes were kept in small groups in cages with sugar saturated flowers, cotton balls, and fresh leaf cuttings of nettles. After the first egg batches had occurred, females were separated singly. The rearing of this first generation of *A. urticae* took place under field conditions except in the case of rain. For the fertilization experiments, we used the offspring of 14 reared females of *A. urticae*.

The females of both *Aglais* species were kept individually in oviposition cages (30 cm × 30 cm × 30 cm). These cages contained cuttings of unfertilized nettles, cotton balls, and flowers prepared with sugar solution and stood outside except in the case of rain. Every three days, a control of all cages took place to collect and separate the egg batches female-specific in ventilated plastic boxes (10 cm × 10 cm × 6 cm). The boxes with the eggs and neonates were controlled daily to ensure a sufficient humidity and an ample supply of fresh leaves of unfertilized nettles. After the larvae had reached a length of 10 mm, they were randomly distributed to the three host-plant treatments following the approach of a split-brood design. Due to this procedure, the neonates initially benefited from the suitable microclimate within the communal larvae webs, and the experiment did not contain biased results because of their randomly occurring food refusal. The synchronous development in *A. io* allowed the distribution of the larvae from all female lineages on the same day. In *A. urticae*, the variable growth of the individuals resulted in a staggered distribution of the larvae from different female lineages to the host-plant treatments after the fertilization procedure, which was considered in the statistical analysis. Depending on the size of the egg batches, a maximum of 30 randomly chosen larvae of each female (maximum per treatment: 10 larvae) took part in the experiment, resulting in a total number of 270 *A. io* individuals and 375 *A. urticae* individuals. For reasons of feasibility, larvae of the same female and treatment were reared in small groups in ventilated plastic boxes (16 cm × 14 cm × 9.5 cm). All rearing boxes contained moistened filter paper at the bottom to ensure a sufficient humidity and fresh randomly chosen leaf cuttings of nettles of the specific treatment in ample supply. Depending on the food and humidity requirements of the larvae, the boxes were controlled and rearranged daily. Pupae were separated in additional boxes for daily inspection of adult eclosion and weighed one day after pupation as soon as the puparium was cured. The rearing conditions of the larvae met the diurnal and seasonal variation of temperature.

The survival rate for the offspring of each female corresponded to the proportion of individuals that became an adult and capable of movement compared with the number of larvae, which was distributed to the treatments. Furthermore, for each surviving larvae, the following traits (further referred to as individual-measured traits) were recorded: duration of the larval and pupal period, pupal weight, forewing length, and sex. After the experiments surviving individuals were released in their habitats.

2.4. Statistical analysis

Differences in the nitrogen concentration and C:N ratio of *U. dioica* plants between the three treatments were analyzed using linear models (LM). In order to test the influence of the treatment on the survival rate and sex ratio of each *Aglais* species, we fitted binomial generalized mixed-effect models (GLMM) with Laplace approximation for each species and parameter with the package lme4 (Bates et al., 2014). To account for the genetic similarity of larvae from one female, female identity was implemented as a random factor. In *A. urticae*, we considered the distribution date of the larvae to the treatments as an additional categorical random factor as well as an individual observer-level to correct for over-dispersion (Bolker et al., 2009) leading to crossing random effects in the models. A Wald χ^2 test on the full model tested the significance of the treatment as fixed factor in each GLMM (Bolker et al., 2009). The treatment effect on the individual-measured traits of both *Aglais* species was analyzed with sex-specific linear mixed-effect models (LMM) (package lme4, Bates et al., 2014) separately for each trait with a restricted maximum likelihood estimation (REML) to handle the species-specific structure of random factors (see above). We used a *F* test on the full model to test the significance of the treatment. The calculation of the degrees of freedom and appropriate *P* values were done using the Kenward-Roger approximation (packages lmertest (Kuznetsova et al., 2014), pbkrtest (Halekoh and Højsgaard, 2014)). Multiple studies prefer this method compared to other approximations (Schaalje et al., 2002; Spilke et al., 2005). In the present analyses, the results of the Kenward-Roger and Satterthwaite approximation did not differ significantly.

The determination of marginal R^2 (R_m^2) defined as the variance explained by the fixed factors, and conditional R^2 (R_c^2) summing up the variance explained by the fixed and random factors (Nakagawa and Schielzeth, 2013) took place with the package MuMIn (Barton, 2015). However, it should be noted that models with individual observer-levels have misleadingly high R_c^2 because the observer effect represents a nuisance parameter with low biological importance (Harrison, 2014). To test the differences in the response variables between the three treatments in each model, Bonferroni *t* tests as post-hoc tests were used to prevent type I error in pairwise comparisons (package lsmeans

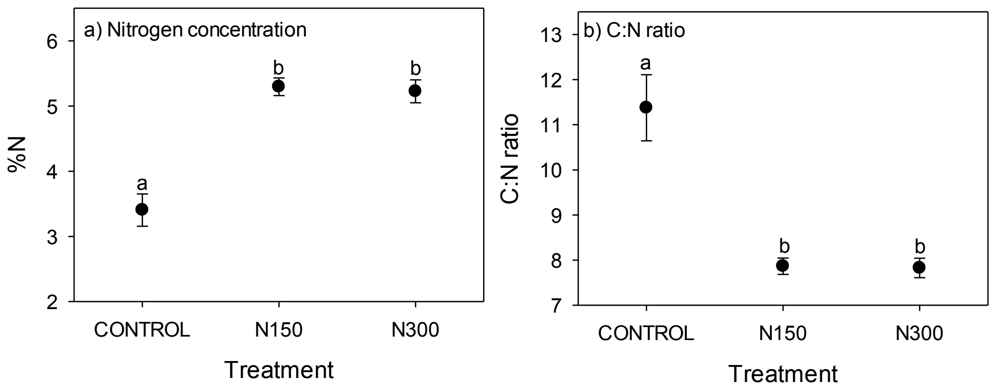

Fig. 1. Nitrogen concentration and C:N ratio of *Urtica dioica* depending on the treatment. Data are expressed as mean (± SE). LM was used to analyse the treatment effect. Different letters above error bars indicate significant differences between treatments (Bonferroni *t* tests as post-hoc test, $P < 0.05$). CONTROL = control group; N150 and N300, respectively = fertilization treatment with 150 and 300 kg N ha^{-1} yr^{-1}, respectively. Statistics: Nitrogen concentration (a) $F_{2, 27} = 31.17$, $P < 0.001$, $R^2 = 0.68$; C:N ratio (b) $F_{2, 27} = 20.24$, $P < 0.001$, $R^2 = 0.57$.

(Lenth and Herve, 2015)). All statistical analyses were conducted using R 3.0.3 (R Core Team, 2014).

3. Results

Fertilization of *Urtica dioica* had strong effects on host-plant quality. The nitrogen concentration nearly doubled in both fertilization treatments compared to the control group, resulting in a corresponding decrease in the C:N ratio in both fertilization treatments (Fig. 1).

In both *Aglais* species, host-plant fertilization enhanced the survival of the individuals (Fig. 2). In *A. io* the survival rate in the N150 fertilization treatment was significantly higher than in the control group. Survival in the N300 fertilization treatment, however, did not differ from the two other treatments. In contrast, in *A. urticae*, survival was twice as high in the two fertilization treatments compared to the control group.

Also in the individual-measured traits, the differences were most pronounced between the two fertilization treatments and the control group, but usually not among the fertilization treatments (Fig. 3, Table 1). The trait shifts in *A. io* were less strong than in *A. urticae*. In *A. io*, neither the forewing length nor the pupal weight of females shifted across the three treatments. Pupal weight was only significantly higher in males in the N150 treatment compared to the control group. Nevertheless, host-plant fertilization caused about six days shorter larval and less than one day longer pupal periods in both sexes of *A. io*. In *A. urticae*, host-plant fertilization resulted in both sexes in a nearly one-third shorter development time of larvae and pupae, as well as about 20% increased pupal weights and forewing lengths. Among the fertilization treatments, significant differences were only observed in males. Males of *A. urticae* had heavier pupae and longer forewings in the N300 fertilization than in the N150 fertilization treatment.

Due to the random selection of larvae, the sex ratio should be balanced. Consequently, different sex ratios in the three treatments referred to variable survival probabilities of the sexes in both *Aglais* species. While in the control group 30% of the individuals were males, the ratio shifted to a more balanced sex ratio in both fertilization treatments (Fig. 4). However, the sex ratio significantly differed only between the control group and the N150 fertilization treatment in *A. urticae*.

4. Discussion

Fertilization of *Urtica dioica* had strong positive effects on host-plant quality, as well as on survival and individual-measured traits of both nettle-feeding *Aglais* species. The differences were most pronounced between the two fertilization treatments and the control group, but usually not among the fertilization treatments. However, in *A. io*, the survival rate tended to be highest in the N150 fertilization treatment, significantly differing from the control group but not from the lower survival in the N300 treatment.

4.1. Host-plant quality

The lower C:N ratio of nettles in both fertilization treatments in comparison to the control group is the result of increased nitrogen concentrations within the plants. Several other fertilization experiments with different plant species produced similar findings (e.g., Estiarte et al., 1994; Chen et al., 2008; Han et al., 2014). The lack of difference in the nitrogen concentration among both fertilization treatments results from a non-linear nitrogen uptake from the soil, resulting in a saturation curve between nitrogen concentration in the nettles and in the soil (Melzer et al., 1984). Despite the significant fertilizer-induced changes, the nitrogen concentration of nettles in all three treatments ranged within the values of this species under natural conditions (Serruys and van Dyck, 2014; Merckx et al., 2015). Since almost all nettles had a nitrogen concentration above 2.5%, few, if any, of the individuals were subject to nitrogen limitation in the present experiment (cf. Müllerová et al., 2014). Under natural conditions, nettles reach even higher nitrogen concentrations up to 7.4% (Serruys and van Dyck, 2014), which were not observed in the present experiment.

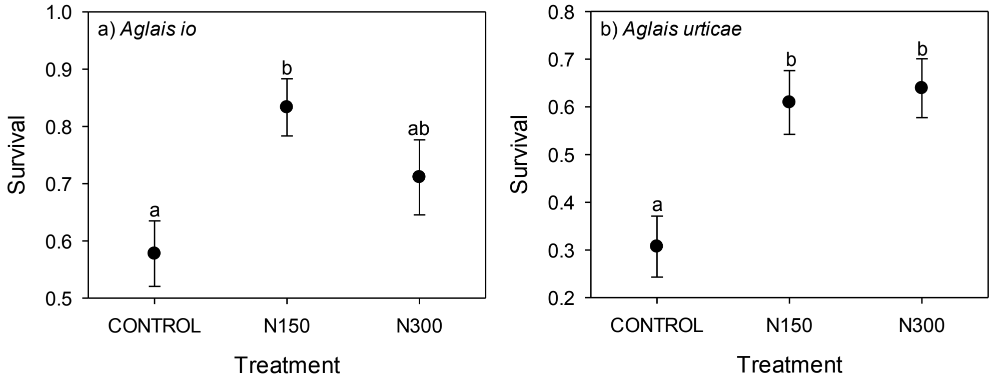

Fig. 2. Survival rate of *Aglais io* and *Aglais urticae* depending on the treatment. Data are expressed as mean (± SE). GLMM were used to analyse the treatment effect. Different letters above error bars indicate significant differences between treatments (Bonferroni *t* tests as post-hoc test, $P < 0.05$). CONTROL = control group; N150 and N300, respectively = fertilization treatment with 150 and 300 kg N ha^{-1} yr^{-1}, respectively. Statistics: *A. io* (a) $\chi^2_2 = 13.87$, $P < 0.001$, $R^2_m = 0.08$, $R^2_c = 0.12$; *A. urticae* (b) $\chi^2_2 = 21.50$, $P < 0.001$, $R^2_m = 0.12$, $R^2_c = 0.17$. *Aglais io*: 9 female lineages, 85 ♂♂, 106 ♀♀; *A. urticae*: 14 female lineages, 105 ♂♂, 88 ♀♀.

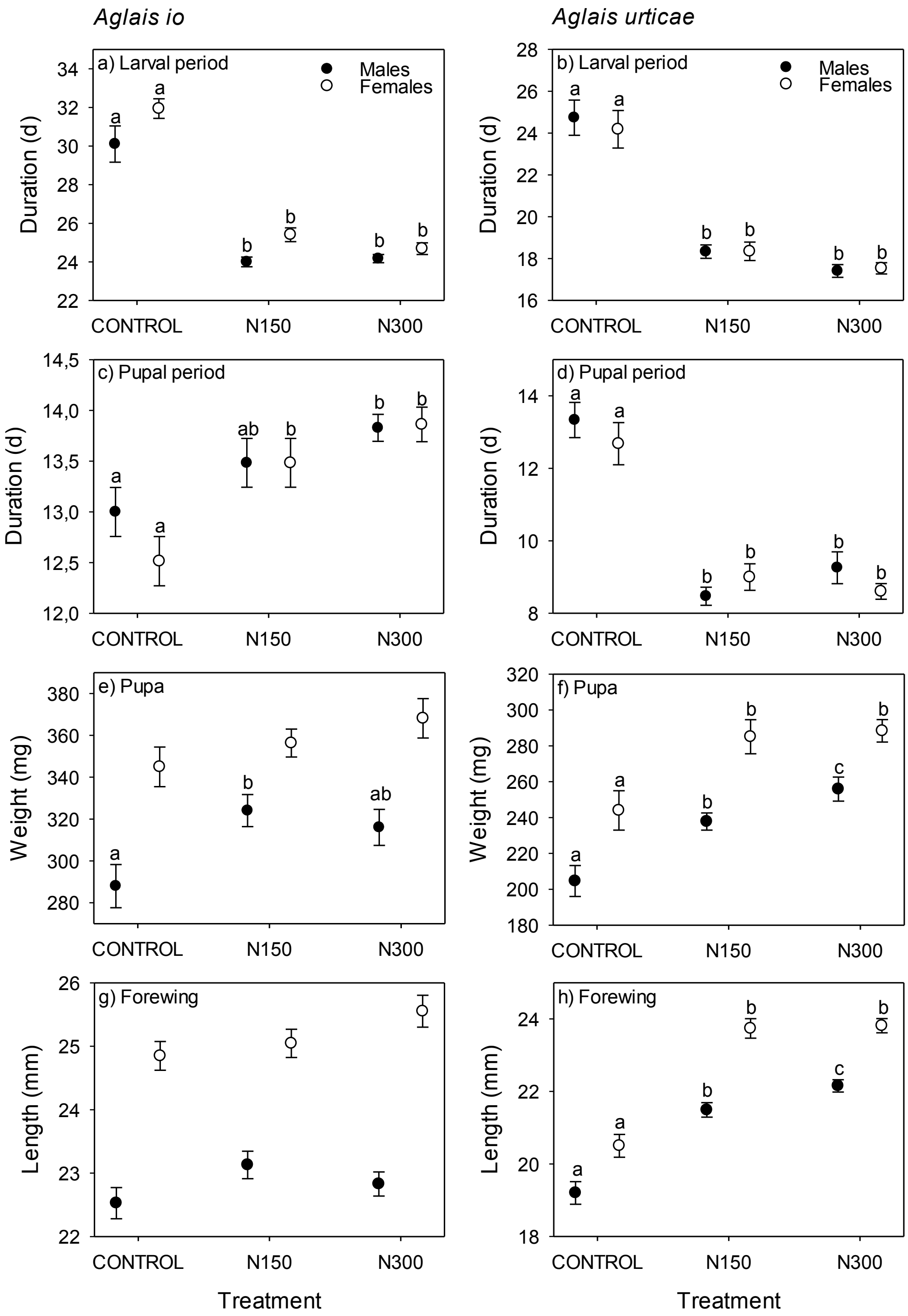

Fig. 3. Larval period duration, pupal period duration, pupal weight and forewing length of both sexes of *Aglais io* (a, c, e, g) and *Aglais urticae* (b, d, f, h) depending on the treatment. Data are expressed as mean (± SE). Sex-specific LMM were used to analyse the treatment effect (see Table 1). Different letters above error bars indicate significant differences between the treatments within a sex (Bonferroni *t* tests as post-hoc test, $P < 0.05$). CONTROL = control group; N150 and N300, respectively = fertilization treatment with 150 and 300 kg N ha^{-1} yr^{-1}, respectively.

Hence, under natural conditions, both *Aglais* species have to cope with similar, if not higher, nitrogen concentrations in nettles.

4.2. General positive response of both Aglais species to fertilized nettles

The positive response of *A. io* and *A. urticae* to host-plant fertilization corresponds with the predictions of the nitrogen-limitation hypothesis (White, 1993). Due to the higher plant quality of fertilized nettles, the individuals had higher survival rates, shorter larval periods and, with the exception of *A. io* females, greater pupal masses and in *A. urticae* longer forewings. All these trait shifts rest upon a higher metabolism efficiency of the larvae feeding on fertilized plants (Slansky and Feeny, 1977; Mattson, 1980; Estiarte et al., 1994). These beneficial responses are known from fertilization experiments with other Lepidoptera species (e.g., Myers and Post, 1981; Mevi-Schütz and Erhardt, 2003) and have been observed in both *Aglais* species feeding on regrown compared to old nettle leaves (Pullin, 1986, 1987). However, the changes in the pupal period of both *Aglais* species with an increase in *A. io* and a decrease in *A. urticae* are not in line with the findings of previous studies with a constant pupal time across host-plant treatments (Mevi-Schütz and Erhardt, 2003). This inter-specific difference does not correlate with other individual-measured traits, which have the same tendency in both species. Thus, most likely unknown physiological mechanisms induce the increasing or decreasing pupal time.

Besides the obvious advantage of a higher survival rate, also all other trait shifts imply not only fitness advantages for the individual but also support the growth rate of the population. Based on the same

Table 1
Results of sex-specific LMM testing the effect of the treatment ($df = 2$) on duration of larval and pupal period, pupal weight, and forewing length in both *Aglais* species (*err.df* error degrees of freedom). *Aglais io*: 9 female lineages, 85 ♂♂, 106 ♀♀; *A. urticae*: 14 female lineages, 105 ♂♂, 88 ♀♀. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Trait	Males				Females			
	err.df	F	P	R^2_m/R^2_c	err.df	F	P	R^2_m/R^2_c
a) *Aglais io*								
Duration larval period	77.0	64.46	***	0.56/0.65	96.0	167.90	***	0.63/0.81
Duration pupal period	79.9	3.48	*	0.08/0.09	100.9	10.37	***	0.17/0.17
Pupal weight	78.2	4.29	*	0.09/0.19	97.4	1.94	0.150	
Forewing length	80.1	1.66	0.197		99.8	1.98	0.144	
b) *Aglais urticae*								
Duration larval period	90.4	101.31	***	0.41/0.81	79.8	75.33	***	0.55/0.74
Duration pupal period	93.3	25.17	***	0.33/0.39	84.3	30.86	***	0.44/0.46
Pupal weight	98.3	11.08	***	0.16/0.36	84.3	5.90	**	0.13/0.21
Forewing length	97.8	32.04	***	0.35/0.52	84.5	43.88	***	0.52/0.56

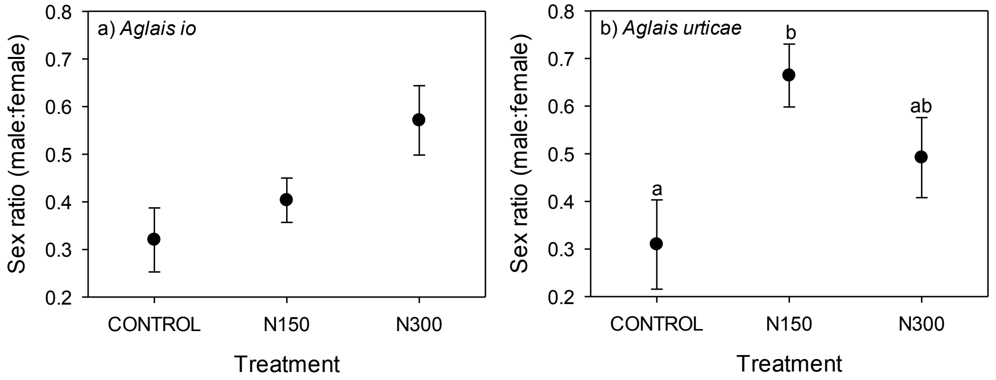

Fig. 4. Sex ratio of *Aglais io* and *Aglais urticae* depending on the treatment. Data are expressed as mean (± SE). GLMM were used to analyse the treatment effect. Different letters above error bars indicate significant differences between treatments (Bonferroni *t* tests as post-hoc test, $P < 0.05$). CONTROL = control group; N150 and N300, respectively = fertilization treatment with 150 and 300 kg N ha^{-1} yr^{-1}, respectively. Statistics: *A. io* (a) $\chi^2_2 = 4.28$, $P = 0.12$; *A. urticae* (b) $\chi^2_2 = 7.02$, $P = 0.030$, $R^2_m = 0.08$, $R^2_c = 0.22$). *Aglais io*: 9 female lineages, 85 ♂♂, 106 ♀♀; *A. urticae*: 14 female lineages, 105 ♂♂, 88 ♀♀.

number of larvae in the rearing boxes and a similar regular food supply, visual observation clearly suggested that larvae of both *Aglais* species in the control group substantially fed more plant biomass than larvae in the fertilization treatments. A shorter larval period, potentially combined with a lower food intake of the individuals feeding on fertilized nettles, reduces, according to the slow-growth-high-mortality hypothesis, their exposure to predators or parasites and thus their risk to die before reproduction (Loader and Damman, 1991). Additionally, the population benefits from a shorter development time of the individuals due to the potential establishment of a further generation within the same season (Pullin, 1987; Hunter and McNeil, 1997). Heavier pupae and longer forewings also suggest a higher fitness of the individuals developing on fertilized nettles. Both traits are suitable indicators for the reproductive potential of the adults and influence their life span and dispersal ability (Slansky, 1993; Nylin and Gotthard, 1998; Awmack and Leather, 2002; Colasurdo et al., 2009). In contrast, despite the observed compensatory feeding behavior, the larvae of the control group needed a prolonged development time to reach a certain threshold mass for pupation, and in *A. urticae*, smaller adults hatched (Slansky, 1993; Taylor, 1984). These detrimental trait shifts, as well as the lower survival rate, indicate that the individuals suffer from the higher processing efforts to digest large amounts of less suitable diet, making it impossible to reach the same fitness as the individuals developing on fertilized nettles (Slansky, 1993; Morehouse and Rutowski, 2010).

Due to a similar host-plant quality of nettles in both fertilization treatments, neither *Aglais* species showed a further response to the N300 compared to the N150 fertilization treatment. However, one surprising tendency is, besides the continuous increase of the pupal weight and forewing length in *A. urticae* males (see below), that the survival rate of *A. io* in the N300 fertilization treatment ranged between the N150 treatment and control group. Similar, but significant, hump-shaped relationships between survival and nitrogen supply of the diet have been observed in a few Lepidoptera species (Sarfraz et al., 2009; Han et al., 2014), indicating a limited applicability of the nitrogen-limitation hypothesis. However, whether this slight non-significant decline in the survival rate of *A. io* refers to the vulnerability of this species to higher nitrogen contents in nettles would require further research.

4.3. Sex-specific differences in the response of both Aglais species to fertilized nettles

The sex-specific differences with a female-biased sex ratio in the control group, as well as the more pronounced significant shifts in the post-pupation traits in males compared to females, in both *Aglais* species, is opposite to that observed in other studies (Fischer and Fiedler, 2000b; Mevi-Schütz and Erhardt, 2003; Quezada-García et al., 2014). In monophagous butterflies, such as both *Aglais* species, the correlation between body size and fecundity is stronger in females (Karlsson et al., 1997; Fischer and Fiedler, 2000b). For this reason, the missing shift in the pupal weight of females in *A. io*, despite an appropriate response in males, suggests that males have an unexpectedly stronger benefit from the fertilized plants. This is also true for *A. urticae*, with a continuous increase in the pupal weight and forewing length in males across the three treatments, whereas the response of females was restricted to the N150 fertilization treatment. A similar hump-shaped relationship in the pupal weight occurred in females of *Coenonympha pamphilus*, despite a continuous increase in males (Soontiëns and Bink, 1997). This sex-specific difference may result from the larger fat storage of females as an energy resource for egg-production (Soontiëns and Bink, 1997). Therefore, the development of females depends more strongly on the carbohydrate concentration of the host plant, which does not necessarily increase with the nitrogen content (Soontiëns and Bink, 1997; Quezada-García et al., 2014). However, these more specific requirements of females do not explain the female-biased sex ratio of *A. urticae* in the control treatment, since this treatment offers based on the overall mortality the most unsuitable diet. Even *A. io* showed a similar tendency in the sex ratio. Generally, females are considered as the more susceptible sex to less suitable diets (Quezada-García et al., 2014). Hence, it is remarkable and, in contrast to previous studies (Quezada-García et al., 2014), that the less suitable diet in the control group supports a stronger survival of females than of males. Our data of the nitrogen concentration and C:N ratio in nettles did not explain these sex-specific differences. However, in fertilized nettles, not only the nitrogen content but also other nutrients, flavenoids, and phenolic acids change (Grevsen et al., 2008; Müllerová et al., 2014). Thus, a more extensive physiological investigation on both sides, the butterflies and the host plant, is necessary to uncover the sex-specific responses.

5. Conclusions

In conclusion, all the beneficial responses of both *Aglais* species due to nettles receiving nitrogen inputs of about 150 kg N ha^{-1} yr^{-1}, and, partly also 300 kg N ha^{-1} yr^{-1}, suggest that the positive abundance trends of both butterflies (Öckinger et al., 2006; Nilsson et al., 2008) are not only based on the expansion of nettles (Ellenberg and Leuschner, 2010; Verheyen et al., 2012) but also upon changes in their quality due to the eutrophication of the landscape during recent decades (Ellenberg and Leuschner, 2010; WallisDeVries and Bobbink, 2017). The experiment verified that the commonly assumed pre-adaptation of both *Aglais* species to higher nitrogen contents in their diet also applies to nettles receiving fertilizer quantities commonly used in agriculture. Hence, both species seem to be able to successfully deal with the anthropogenically induced quality changes in their host plants.

Author contributions

All authors designed the experiments. S. Kurze conducted the experiments, analyzed the data and wrote the article. T. Fartmann and T. Heinken made substantial contributions to the manuscript, revising and commenting on subsequent drafts.

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