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

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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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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;