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The consequences of alternating diet on performance and food preferences of a specialist leaf beetle

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ABSTRACT

The food quality of a given host plant tissue will influence the performance and may also affect the preference behavior of herbivorous animals. As nutrient contents and defense metabolite concentrations can vary significantly between different parts of a plant and change over time, herbivores are potentially confronted with diet differing in quality even when feeding on a single plant individual. Here we investigated to what extent feeding exclusively either on young or old, mature leaves of *Brassica rapa* or on a mixed diet of young and old leaves offered in alternating order affects the larval performance, food consumption, and the host preference behavior of adult mustard leaf beetles, *Phaedon cochleariae*. Analyzing different leaf quality traits, we found lower water contents, no changes in C:N ratio but more than threefold higher glucosinolate concentrations in young compared to old leaves. Individuals reared on mixed diet performed as well as animals reared on young leaves. Thus, compared to animals feeding exclusively on highly nutritious young leaves, diet-mixing individuals may balance the lower nutrient intake by a dilution of adverse secondary metabolites. Alternatively, they may integrate over the variation in their food, using a previously assimilated resource for growth at times of scarcity. Animals reared on old leaves grew less and had a prolonged larval developmental time, although they showed increased consumption indicating compensatory feeding. Additionally, we found that experience with a certain diet affected the preference behavior. Whereas individuals reared exclusively on young leaves preferred young over old leaves for feeding and oviposition, we did not find any preferences by animals reared exclusively on old leaves or by females reared on alternating diet. Thus, in contrast to positive feedbacks for animals reared on young leaves, an integrative growth of diet-mixing individuals potentially leads to a lack of feedback during development. Taken together, our results suggest that different diet regimes can lead to comparable performance of mustard leaf beetles but experienced feedbacks may differ and thus convey distinct diet preferences.

1. Introduction

For an herbivorous animal, the quality of a host plant is determined by the balance of nutrients and the amount of secondary metabolites that may act deterrent or toxic. Even within a single plant, the composition and concentration of primary and secondary metabolites can vary significantly between parts or organs and these differences can have profound influences on the performance and preferences of herbivores (Lambdon et al., 2003; Travers-Martin and Müller, 2008). According to the optimal defense hypothesis (ODH), the most valuable parts of a plant should be defended best (McKey, 1974). Since young leaves should contribute most to the future production of photoassimilates, they are highly valuable. Consequently, young leaves of many plant species contain higher levels of soluble proteins and nitrogen (Mattson, 1980; Travers-Martin and Müller, 2008), but at the same time they are better protected than mature leaves by secondary metabolites (Barton and Koricheva, 2010), such as iridoid glycosides (Bowers et al., 1992), alkaloids (van Dam et al., 1994), or, in the case of Brassicaceae, glucosinolates (Fahey et al., 2001; Brown et al., 2003; Reifenrath and Müller, 2007). Herbivores are thus confronted with a heterogeneous nutritional landscape (Behmer, 2009).

Several studies show that diet mixing can be advantageous for both generalist and specialist herbivores (Bernays et al., 1994; Moreau et al., 2003) when feeding on imbalanced food (Raubenheimer and Simpson, 1999). Whereas generalists may profit when feeding on leaves of different plant species varying in quality, specialists may also explore the intraspecific and intraindividual variation in plant metabolite composition (Mody et al., 2007). In both cases, diet mixing may allow regulating a balanced nutrient intake (Pulliam, 1975). Furthermore, herbivores can decrease the intake of

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toxic plant compounds by feeding on various hosts or host tissues and thus reduce toxic effects, as stated by the toxin dilution hypothesis (Freeland and Janzen, 1974). However, there are at least two other important factors influencing the growth of an animal when feeding on alternating diet, namely the frequency of diet alternation and the variability of the food. *Daphnia magna* (Onychura:Daphniidae) feeding on high and low quality food in alternating order show optimal growth when high quality food is available only for half of each day (Sterner and Schwalbach, 2001). This phenomenon was termed integrative growth (Sterner and Schwalbach, 2001; Litchman, 2008) suggesting the ability to carry over assimilated resources for growth from the nutrient-rich to the nutrient-poor environment (Fig. 1). Besides the absolute amounts of nutrients and toxins that are ingested, the variability plays an important role. Insects reared on diet highly variable in nutritional quality can have a reduced performance compared to those reared on food varying only little (Stockhoff, 1993). In contrast to different effects of diet mixing, herbivores feeding solely on low quality diet may countervail negative dietary impacts by compensatory feeding and/or metabolic adaptations in food digestion (Slansky, 1993; Berner et al., 2005; Warbrick-Smith et al., 2006).

Apart from influences on the performance, the experience with a particular food source can influence the food preference behavior of these animals. Individuals of many species were shown to prefer as adults the host they experienced as larvae (Anderson et al., 1995; Zhang and Liu, 2006), particularly when the diet led to an improved performance (Scheirs and de Bruyn, 2002; Egusa et al., 2006; Stamps and Davis, 2006). For individuals having experienced a positive feedback of high quality food on their performance it may thus be beneficial to show an induced stronger preference of this particular diet and reduce time for food exploration (Bernays, 1998; del Campo et al., 2001; Villalba and Provenza, 2009). In contrast, animals reared under poor conditions seem to demonstrate reduced host selectivity (Stamps and Davis, 2006; Davis, 2008). For them it should be advantageous to maintain a higher plasticity instead of developing a host fixation. Little is known how feeding on diet of alternating quality may affect preferences. Such effects may highly depend on the post-ingestive feedbacks during development (Villalba and Provenza, 2009) and thus not be predictable *a priori*.

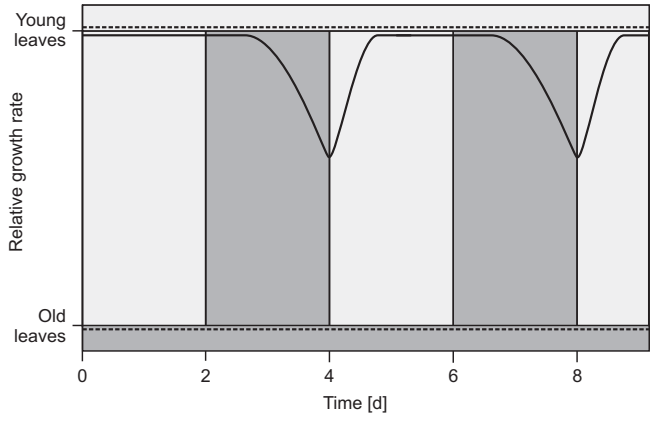

Fig. 1. Hypothetical progress of the relative growth rate of larvae of *Phaedon cochleariae* feeding on alternating diet or exclusively on young or old leaves. The curve indicates an integrative growth by carrying over benefits from one environment to another with a low decrease of growth rate from high to low quality conditions (i.e., young and old leaves). The upper and lower dashed lines show the relative growth rate of larvae exclusively feeding on young and old leaves, respectively. Light grey background: young leaves offered; dark grey background: old leaves offered. (Figure modified after Sterner and Schwalbach, 2001.)

In this study, we investigated the influence of feeding pure *versus* mixed diet on performance and consumption parameters of the specialist herbivore *Phaedon cochleariae* (Coleoptera: Chrysomelidae). Furthermore, we tested to what extent diet experience affects food preferences. Therefore, individuals of *P. cochleariae* were either reared on young or on old (mature) cabbage leaves further manipulated in quality or on both diets offered in alternating order during larval development and adulthood. Several leaf quality traits were measured in the leaves, including nutrient levels as well as glucosinolates as characteristic secondary metabolites of Brassicaceae. It is known that the growth and fertility of *P. cochleariae* are impacted by the age of their host tissues, i.e., the beetles perform better on young leaves than on old leaves when feeding exclusively on the respective diet (Reifenrath and Müller, 2009; Kühnle and Müller, 2011; Tremmel and Müller, 2013). However, feeding highly nutritious but well defended young leaves and nutrient-poor but low defended leaves in alternating order might affect the performance of the beetles in different ways. Lower nutrient intake may be balanced off by a reduced intake of adverse secondary metabolites, i.e., toxin dilution, as glucosinolates and specifically their breakdown products can affect both specialist and generalists negatively (Agrawal and Kurashige, 2003; Gols et al., 2008; Kos et al., 2012). Additionally, if larvae are able to take benefits from one environment into the other, we expected an integration of growth in animals reared on different food in alternating order with a hypothetical progress of the growth rate as shown in Fig. 1, where these animals develop as well as larvae reared on young leaves. The preference behavior of adult *P. cochleariae* was hypothesized to depend on the probability that herbivores gain a positive feedback for a certain diet.

2. Materials and methods

2.1. Plant preparation and insect rearing

Cabbage plants (*Brassica rapa* L. ssp. *pekinensis* var. Michihili, Brassicaceae) and monks cress (*Tropaeolum majus* L., Tropaeolaceae) were grown in a greenhouse at 20 °C and a photoperiod of L16:D8. Seeds were obtained from Kiepenkerl (Bruno Nebelung GmbH, Konken, Germany) and grown in composted soil (plant nursery, Bielefeld University) in pots (diameter: 12 cm). Cabbage plants used for larval rearing and bioassays were 7–10 weeks old and non-flowering. Leaf discs (diameter: 25 mm) of fully developed young inner and older outer leaves of the rosette were used as substrates for insect feeding. Young leaves were treated with 20 µl hexane per disc (referred to as "young leaves" in Section 2.2. analysis of leaf traits and Section 2.3. performance experiments hereafter). Discs from old leaves were treated each with 20 µl of a hexane extract of *T. majus* ("old leaves") to lower the food quality of old cabbage leaves and thus increase the difference between young and old leaf quality in a mechanistic way, as the extract of this plant species was shown to reduce feeding of *P. cochleariae* in a 20 h choice test (Kühnle and Müller, 2009). The extract concentration corresponded to the amount of a *T. majus* leaf disc equivalent to the size of a *B. rapa* leaf disc. To prepare the extract, about 3–4 g of leaves and stems of several eight weeks old, flowering *T. majus* plants were harvested, frozen in liquid nitrogen, lyophilized, and ground in 50 ml of hexane. The resulting crude extract was filtered and concentrated under an air-stream. The solvents were allowed to evaporate before using the plants for chemical analyses or offering the leaf discs to the insects.

Adults of *P. cochleariae* (F.) (Coleoptera: Chrysomelidae) were collected in the field near Berlin, Würzburg and Pevestorf (Germany). They were crossed and reared in a climate chamber (20 °C, 70% r.h., L16:D8) for several generations on *B. rapa* leaves,