Web Ecol., 13, 79–84, 2013
www.web-ecol.net/13/79/2013/
doi:10.5194/we-13-79-2013

Open Access Web Ecology

Experiments with *Lymantria dispar* larvae do not support the idea of physiological adjustment to host individuals by insect herbivores

H. Ruhnke[1,4], D. Matthies[2], and R. Brandl[3]

[1]UFZ – Helmholtz Centre for Environmental Research, Department of Community Ecology, Halle, Germany
[2]Plant Ecology, Department of Biology, Philipps-University Marburg, Marburg, Germany
[3]Animal Ecology, Department of Biology, Philipps-University Marburg, Marburg, Germany
[4]private address: Wiesenstraße 26, 16321 Bernau, Germany

Correspondence to: H. Ruhnke (h.ruhnke@web.de)

Received: 14 May 2013 – Revised: 30 August 2013 – Accepted: 4 September 2013 – Published: 24 September 2013

Abstract. All organisms have to cope with spatial and temporal heterogeneity of the environment. At short temporal and small spatial scales, organisms may respond by behavioural or physiological mechanisms. To test for physiological adjustments to variation in host quality among tree individuals within a host species, we performed a transfer experiment in a climate chamber using larvae of the polyphagous gypsy moth (*Lymantria dispar*). We reared larvae for two weeks on leaves of one of three *Quercus robur* individuals. We found differences in the growth rate of larvae across the host individuals, which indicate that the oak individuals differed in their quality. Furthermore, families of larvae varied in their growth rate and there was variation among the families of gypsy moth larvae in response to leaves from the different oak individuals. After two weeks we offered larvae either leaves of the same or a different individual of the three oaks. We found no effect of transferring larvae to a different tree individual. The results thus do not support the idea of physiological adjustment of a generalist insect herbivore to variation in leaf quality among host individuals.

1 Introduction

All organisms have to cope with spatial and temporal heterogeneity of the environment (e.g. Hutchings et al., 2000). At small spatial and short temporal scales behavioural and temporal adjustments allows organisms to react to environmental heterogeneity (e.g. Karasov et al., 2011), whereas at large scales evolutionary adaptations track environmental changes. At intermediate scales reversible or irreversible phenotypic plasticity (Piersma and Gils, 2011) as well as epigenetics (e.g. Bräutigam et al., 2013) provide possibilities to react to environmental heterogeneity. Almost all environmental characteristics show at least some heterogeneity in space or time. For herbivorous insects, for example, the quality of diet shows considerable heterogeneity across all temporal and spatial scales (e.g. Scriber, 2002; Glynn et al., 2004; Osier and Lindroth, 2004; and Ruhnke et al., 2009).

This heterogeneity may be disadvantageous for insects because not all host species or host individuals may have a chemical composition which match the needs of the herbivore and/or can be handled efficiently by the physiological machinery of the insect. Compared to the lifespan of the host (e.g. trees), the lifespan of herbivorous insects is often much shorter. Therefore, evolutionary responses may occur even to a single host individual. The adaptive deme formation hypothesis suggests that short-lived insects respond to resource heterogeneity among long-lived hosts by evolving genetically distinct lineages adapted to particular host individuals (Edmunds and Alstad, 1978). A prerequisite for the evolution of adaptive demes is a certain predictability of leaf quality from year to year. However, the leaf quality of host individuals is not always consistent across years (Laitinen et al., 2000; Covelo and Gallardo, 2001). Therefore, explicit tests of the hypothesis have given conflicting results (e.g. Cobb and Whitham, 1998; and Ruhnke et al., 2006).

Published by Copernicus Publications on behalf of the European Ecological Federation (EEF).

However, resource heterogeneity may also be advantageous for herbivores that can switch among host species or individuals, because the different diets may complement each other to satisfy the needs of the herbivore (e.g. Mody et al., 2007; and Unsicker et al., 2008).

Irrespective of whether resource heterogeneity has positive or negative effects on herbivorous insects, individuals need to react to this variation if they feed for a longer time span on a host individual. One possibility is phenotypic plasticity in morphological, physiological and behavioural traits (e.g. Leclaire and Brandl, 1994), which itself has a genetic basis (Scheiner, 1993; Windig, 1994). For example, in herbivorous insects, the exposure of larvae to specific stimuli in the diet can alter the responsiveness of taste cells (Jermy et al., 1968; Glendinning et al., 1999), which may lead to changes in behaviour (Blaney et al., 1986). Furthermore, the composition and concentration of enzymes in the midgut change with the diet (Mainguet et al., 2000). In oligophagous and polyphagous herbivores, feeding on a particular host species can induce a strong preference for that plant species (Jermy et al., 1968), a process fairly well documented for Lepidoptera (Bernays and Weiss, 1996). Such observations suggest that herbivore individuals adjust their physiological machinery to handle efficiently the diet provided by the host.

In this paper, results of a transfer experiment that tested the effect of dietary experience during larval development on feeding efficiency are presented. In the experiment, we reared groups of larvae of the polyphagous gypsy moth (*Lymantria dispar*) on three individual oak trees (*Quercus robur*). After two weeks we offered either leaves of the same individual or of one of the two other oak individuals. If larvae had adjusted their physiological machinery to the rearing tree individual, we should find stronger growth of larvae which received leaves of the rearing tree individual compared to larvae that received leaves from one of the other two tree individuals.

2 Methods

The study was carried out with leaves from oaks growing in the "Leipzig flood-plain forest" (city of Leipzig, Saxony, Germany). The dominant tree species in the study area are ash (*Fraxinus excelsior*), oak (*Quercus robur*), sycamore (*Acer pseudoplatanus*), lime (*Tilia cordata*), and hornbeam (*Carpinus betulus*). In 2001 a project was launched to investigate the canopy of this forest by installing a canopy crane (Morawetz and Horchler, 2002, 2004; Unterseher et al., 2007). From a gondola attached to the crane it was possible to work in the tree canopy within an area of 1.6 ha. For the experiment, three individuals of the common oak (*Quercus robur* L., Fagaceae) were selected in 2003. Two of the oaks were neighbours and the third grew approx. 130 m from the two others.

The insect species used in the experiment was the gypsy moth (*Lymantria dispar* (L.), Lepidoptera: Lymantriidae), whose caterpillars are leaf feeders (Rossiter, 1987). *L. dispar* uses numerous tree species as hosts. Nevertheless, *L. dispar* prefers oak species (Alalouni et al., 2013). The C : N ratio of sun leaves collected in 2003 in the canopy varied among the three selected oak individuals (for more information see Ruhnke et al., 2009; mean and standard error: Oak 1: 18.4 ± 1.00; Oak 2: 19.4 ± 0.50; Oak 3: 20.4 ± 0.58). Furthermore, preliminary work indicated that there were significant differences among the three selected oak individuals in the amount of leaf tissue consumed by caterpillars of *Lymantria*, as well as in the relative growth rates of caterpillars feeding on these oak individuals (data not shown; for results with *Spodoptera littoralis* on the same individuals see Ruhnke et al., 2009). This information suggested that leaves of the three selected oak individuals differed in their quality as food for herbivores.

Eleven full-sib families of gypsy moth larvae were selected in early June 2003 (Doane, 1968). The egg batches originated from a laboratory stock maintained on an artificial diet based on wheatgerm over several generations. Due to the large number of individuals used for the experiments, it was not possible to treat all larvae on the same day. Hence, the hatching time of larvae was staggered and the experiments were carried out over a period of 10 days between 27 June and 7 July 2003. Newly hatched larvae from each family were split into three groups of approximately equal numbers (Oak 1: 322; Oak 2: 465, Oak 3: 374 larvae). Each group was then reared on leaves of one of the three oak individuals (rearing tree) in a climate chamber at 22 °C. Every two days we provided larvae with fresh leaves from their rearing tree. Leaves were collected in the upper, sunny parts of the canopy, and we selected only leaves with no signs of herbivory.

After two weeks of rearing, a reciprocal transfer experiment was performed with third instar larvae. Each group of larvae of a particular rearing tree was subdivided into three subgroups as similar in size as possible, resulting in nine subgroups. Each larva was weighed and placed individually into a Petri dish on moist filter paper and received oak leaves ad libitum of a test tree individual. We used the same three oak individuals used as rearing trees also as test trees. The first subgroup of larvae received leaves of its rearing tree; i.e. test tree and rearing tree individual were identical (control subgroup), the other two subgroups received leaves of one of the two other tree individuals (novel tree subgroups). Larvae were kept in a climate chamber at 26 °C for 12 h in the light and then for 12 h in the dark. To avoid effects of adjustment to the test tree, tests were terminated after 24 h. Note also that larvae had no experience with oak leaves and therefore, our experiments tested physiological adjustments to the rearing tree during two weeks. The larvae were then killed by freezing, dried at 60 °C to weight constancy and weighed. Final sample sizes were as follows (rearing tree Oak 1 – test tree Oak 1: 118; Oak 1 – Oak 2: 108; Oak 1 – Oak 3: 96; Oak 2 –

Table 1. The effects of moth family, rearing tree individual and test tree individual on the final dry mass of the larvae of *Lymantria dispar*. Results of a general linear model with (log) initial larval mass as a covariate. Larvae were reared on leaves of one of three oak individuals and their growth was then tested feeding them leaves from the same or one of the other two trees (test trees). We partitioned the interaction rearing tree × test tree into a contrast that tests the effect of transfer to leaves from a novel tree vs. the effect of transfer to leaves from the same tree (control) and a rest.

Source of variation	Error term	d.f.	MS	*F*
Initial larval mass	Residual	1	4.517	54.01^{***}
Family	Residual	10	0.406	4.86^{***}
Rearing tree	Fam. × Rear. tree	2	1.554	5.47^{*}
Test tree	Fam. × Test tree	2	0.186	3.67^{*}
Family × Rearing tree	Residual	20	0.284	3.40^{***}
Family × Test tree	Residual	20	0.051	0.60
Rearing tree × Test tree	Fam. × R. t. × T. t.	4	0.124	1.98
Control vs. Novel tree	Fam. × R. t. × T. t.	1	0.176	2.81
Rest	Fam. × R. t. × T. t.	3	0.106	1.70
Family × Rearing tree × Test tree	Residual	40	0.063	0.75
Residual		1061	0.084	

* $P < 0.05$, *** $P < 0.001$.

Oak 1: 153; Oak 2 – Oak 2: 165; Oak 2 – Oak 3: 147; Oak 3 – Oak 1: 119; Oak 3 – Oak 2: 125; Oak 3 – Oak 3: 130). Initial larval fresh mass was converted into dry mass using a linear regression equation obtained by measuring the fresh mass and dry mass of 77 additional larvae ($r = 0.76$, $P < 0.001$; dry mass [mg] = 0.1446 times fresh mass [mg] + 0.3457).

Prior to the analysis, initial and final larval dry masses were log-transformed. The effects of family, rearing tree, and test tree on final larval dry mass were analysed by a three-way ANCOVA (analysis of covariance; GLM Procedure of SAS, Version 8.02) with initial larval dry mass as covariate. By using type I sums of squares, confounding effects of initial larval dry mass were removed from the analysis and thus in effect the relative growth rate of the larvae was evaluated (Raubenheimer and Simpson, 1992; Horton and Redak, 1993). In the statistical model "family" was considered as a random factor and "rearing tree" and "test tree" as fixed factors. The interaction rearing tree × test tree was partitioned into a contrast between the subgroups control and novel tree and a rest to test whether the growth of larvae that were feeding on leaves from novel trees was slower than that of larvae feeding on leaves from the tree they had been reared on (see Table 1).

3 Results

Adjusted final dry mass of the larvae varied among the families of gypsy moth (least square means: 3.07–3.69 mg, Table 1) indicating genetic variation among families in larval growth. Furthermore, the response of the larvae to the three host individuals differed among the 11 families (significant interaction family × rearing tree, Fig. 1a), and depended on the oak individual used as rearing tree (Table 1; Fig. 1a). These differences were small, but consistent. Therefore, leaves of the three oak individuals differed in their quality as food for the larvae and these differences continued to influence the growth rate of the larvae during the one-day experiment with the test tree.

The identity of the test tree from which the leaves for the transfer experiment were taken affected the final dry mass of the larvae (Fig. 1b). This indicates that the individual trees also differed in the quality of their leaves as food for the gypsy moths during the test phase of the experiment. However, there was no significant interaction between the effects of the rearing and the test tree. In particular, larvae did not grow faster on leaves from the tree they had been feeding on for the preceding two weeks than on leaves from a new tree (contrast control vs. novel tree in Table 1). Thus, there was no evidence for a physiological adjustment of the larvae to the leaves of the individual trees.

4 Discussion

The quality of leaf tissue of trees shows seasonal changes and varies across years (e.g. Kause et al., 1999; and Ruusila et al., 2005). For instance, Ruusila et al. (2005) reported rapid seasonal changes in the quality of leaf tissue in birch individuals as food for the larvae of the geometrid moth *Epirrita autumnalis*. We also found considerable variation in leaf quality across years in several tree species occurring in flood-plain forests, where we sampled the oaks for the present experiments (Ruhnke et al., 2009). Thus, plants represent moving targets for herbivores (Hartley and Jones, 1997). Such temporal variation in leaf quality may have two important effects. First, this variation may prevent the evolution of a genetic response. Second, non-predictable temporal variation and other idiosyncratic variations of host quality may lead to the evolution of phenotypic plasticity in behavioural and

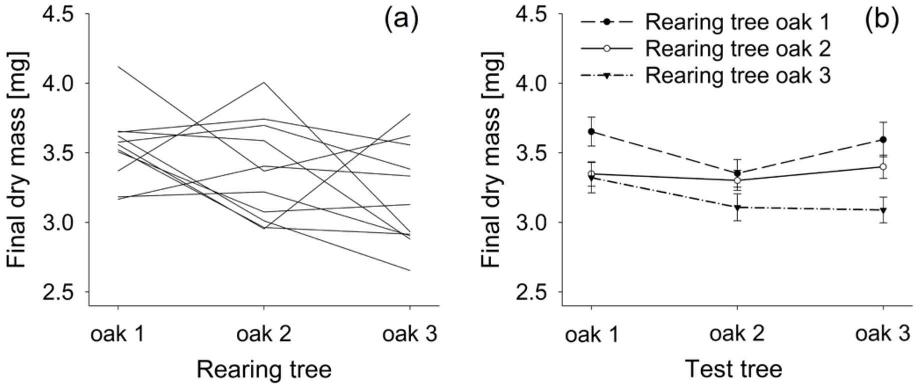

Figure 1. **(a)** Response of different families of *Lymantria dispar* to the three rearing trees. **(b)** Effect of the rearing tree and the test tree on the final dry mass of larvae of *Lymantria dispar* (±1 standard error; for sample size see Methods section). Test trees are the leaves of tree individuals that larvae received after the transfer. We used the same three trees as rearing and test tree. A test tree could be the same as the rearing tree (control) or one of the two other trees (novel trees).

physiological mechanisms to cope with this variation. Our experiments tested for such adjustments to variation in leaf quality between host individuals.

Although we found differences in the C : N ratios among the three tree individuals, these were not responsible for the differences in the quality of the three tree individuals for *L. dispar*, as the growth rate of the insect was not related to leaf C : N ratio (see also Ruhnke et al., 2009). Host quality may be influenced by numerous chemical compounds and interactions between compounds further complicate the interpretation. In contrast, growth rates of insects under constant environmental conditions (e.g. determined by experiments in climate chambers) are a good measure of host quality (e.g. Ruusila et al., 2005). Therefore, our feeding experiments showed clearly that the oak individuals differed in the quality of their leaves as food for the larvae of gypsy moths.

The families of larvae differed in their growth on different oak individuals (significant interaction family × rearing tree), even though the moths had been reared on an artificial diet for generations. Hence, there was genetic variation among families in their reaction to host individuals. However, after the transfer larvae did not grow best when feeding on oak individuals they had been reared on. The experiment thus provides no evidence for physiological adjustment to a particular host individual during larval development in our test organism.

The failure to demonstrate physiological adjustment may have several reasons. First, the time span to measure the growth rate on the test tree may have been too short to find a response. Second, the larvae may have adjusted more important fitness variables than growth (e.g. egg production). Third, we selected a polyphagous species for the experiment. However, monophagous and oligophagous herbivores may respond to variation among host individuals and/or host species differently than polyphagous species (see e.g. Hanson, 1976; and Lajeunesse and Forbes, 2002) However, a test of the adaptive deme formation hypothesis comparing a monophagous and an oligophagous sawfly (Hymenoptera: Tenthredinidae) found no evidence for adaptive demes in both species (Ruhnke et al., 2006).

Our experiment has also some bearing on the possible importance of diet mixing. For polyphagous species like Orthoptera, the positive effects of diet mixing are well known (e.g. Unsicker et al., 2008). Furthermore, Mody et al. (2007) showed recently for the host specialist *Chrysopsyche imparilis*, (Lasiocampidae) occurring in West Africa, that feeding on several host tree individuals of *Combretum fragrans* (Combretaceae) has positive effects on caterpillar performance. However, these and similar experiments were designed to show the effects of diet mixing. Therefore, during the experiments larvae were confronted with leaves from one host species or individual, or leaves from several species or individuals simultaneously, whereas in our experiments we offered the host individuals sequentially. Furthermore, diet mixing is in nature only of relevance if the organisms can move freely between host individuals. As far as we know, larvae of *L. dispar* usually complete their development on a single host individual.

In conclusion, while we found genetic variation in the response to host quality in *L. dispar*, there was no evidence for physiological adjustment to host individuals. It is not clear, however, whether the results of our study represent a general pattern in herbivorous insects or are due to our test organisms or the particular set-up of our experiment.

Acknowledgements. This work was part of the Leipzig Crane Project and the EU funded Network of Excellence, EVOLTREE. Andreas Linde and Dörte Goertz (University of Applied Sciences, Eberswalde, Germany) provided the insects. We thank two anonymous reviewers for their helpful comments on the manuscript.

Edited by: F. Pugnaire
Reviewed by: M. Goßner and one anonymous referee

References

Alalouni, U., Schädler, M., and Brandl, R.: Natural enemies and environmental factors affecting the population dynamics of the gypsy moth, J. Appl. Entomol., in press, doi:10.1111/jen.12072, 2013.

Bernays, E. A. and Weiss, M. R.: Induced food preferences in caterpillars: The need to identify mechanisms, Entomol. Exp. Appl., 78, 1–8, 1996.

Blaney, W. M., Schoonhoven, L. M., and Simmonds, M. S. J.: Sensitivity variations in insect chemoreceptors – a review, Experientia, 42, 13–19, 1986.

Bräutigam, K., Vining, K. J., Lafon-Placette, C., Fossdal, C. G., Mirouze, M., Marcos, J. G., Fluch, S., Fraga, M. F., Guevara, M. A., Abarca, D., Johnsosn, Ø., Maury, S., Strauss, S. H., Campbell, M. M., Rohde, A., Díaz-Sala, C., and Cervera, M.-T.: Epigenetic regulation of adaptive responses of forest tree species to the environment, Ecol. Evol., 3, 399–415, 2013.

Cobb, N. S. and Whitham, T. G.: Prevention of deme formation by the pinyon needle scale: Problems of specializing in a dynamic system, in: Genetic structure and local adaptation in natural insect populations, edited by: Mopper, S. and Strauss, S. Y., Chapman & Hall, New York, 37–63, 1998.

Covelo, F. and Gallardo, A.: Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain, Can. J. Bot., 79, 1262–1269, 2001.

Doane, C. C.: Aspects of mating behavior of the gypsy moth, Ann. Entomol. Soc. Am., 61, 768–773, 1968.

Edmunds, G. F. and Alstad, D. N.: Coevolution in insect herbivores and conifers, Science, 199, 941–945, 1978.

Glendinning, J. I., Ensslen, S., Eisenberg, M. E., and Weiskopf, P.: Diet-induced plasticity in the taste system of an insect: Localization to a single transduction pathway in an identified taste cell, J. Exp. Biol., 202, 2091–2102, 1999.

Glynn, C., Ronnberg-Wastljung, A. C., Julkunen-Tiitto, R., and Weih, M.: Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance, Entomol. Exp. Appl., 113, 1–14, 2004.

Hanson, F. E.: Comparative studies on induction of food choice preferences in lepidopterous larvae, in: The host-plant relation to insect behaviour and reproduction, edited by: Jermy, T., Symposia biologica Hungarica 16, Akademiai Kiado, Budapest, 71–77, 1976.

Hartley, S. E. and Jones, C. G.: Plant chemistry and herbivory, or why the world is green, in: Plant ecology, edited by: Crawley, M. J., 2nd Edn., Blackwell Science, Oxford, 284–324, 1997.

Horton, D. R. and Redak, R. A.: Further comments on analysis of covariance in insect dietary studies, Entomol. Exp. Appl., 69, 263–275, 1993.

Hutchings, M. J., John, E. A., and Stewart, A. J. A. (Eds.): The Ecological consquences of environmental heterogeneity, 40th Symposium of the British Ecological Society, Blackwell Science, Oxford, 2000.

Jermy, T., Hanson, F. E., and Dethier, V. G.: Induction of specific food preference in lepidopterous larvae, Entomol. Exp. Appl., 11, 211–230, 1968.

Karasov, W. H., Martínez del Rio, C., and Caviedes-Vidal, E.: Ecological physiology of diet and digestive systems, Annu. Rev. Physiol., 73, 69–93, 2011.

Kause, A., Ossipov, V., Haukioja, E., Lempa, K., Hanhimaki, S., and Ossipova, S.: Multiplicity of biochemical factors determining quality of growing birch leaves, Oecologia, 120, 102–112, 1999.

Laitinen, M. L., Julkunen-Tiitto, R., and Rousi, M.: Variation in phenolic compounds within a birch (*Betula pendula*) population, J. Chem. Ecol., 26, 1609–1622, 2000.

Lajeunesse, M. J. and Forbes, M. R.: Host range and local parasite adaptation, P. Roy. Soc. Lond. B Bio., 269, 703–710, 2002.

Leclaire, M. and Brandl, R.: Phenotypic plasticity and nutrition in a phytophagous insect – Consequences of colonizing a new host, Oecologia, 100, 379–385, 1994.

Mainguet, A. M., Louveaux, A., El Sayed, G., and Rollin, P.: Ability of a generalist insect, *Schistocerca gregaria*, to overcome thioglucoside defense in desert plants: tolerance or adaptation?, Entomol. Exp. Appl., 94, 309–317, 2000.

Mody, K., Unsicker, S. B., and Linsenmair, K. E. Fitness related diet-mixing y intraspecific host-plant-switching pf specialist insect herbivores, Ecology, 88, 1012–1020, 2007.

Morawetz, W. and Horchler, P. J.: The Leipzig Canopy Crane Project, in: The global canopy handbook. Techniques of access and study in the forest roof, edited by: Mitchell, A. W., Secoy, K., and Jackson, T., Global canopy programme, Oxford, 54–57, 2002.

Morawetz, W. and Horchler, P.J.: The Leipzig canopy crane project (LAK), Germany, in: Studying forest canopies from above: The international canopy crane network, edited by: Basset, Y., Horlyck, V., and Wright, S. J., Smithsonian tropical research institute, Panama, 79–85, 2004.

Osier, T. L. and Lindroth, R. L.: Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance, Oecologia, 139, 55–65, 2004.

Piersma, Th. and van Gils, J. A.: The flexible phenotype: A body-centred integration of ecology, physiology, and behaviour, Oxford University Press, Oxford, 2011.

Raubenheimer, D. and Simpson, S. J.: Analysis of covariance – an alternative to nutritional indexes, Entomol. Exp. Appl., 62, 221–231, 1992.

Rossiter, M. C.: Genetic and phenotypic variation in diet breadth in a generalist herbivore, Evol. Ecol., 1, 272–282, 1987.

Ruhnke, H., Schädler, M., Matthies, D., Klotz, S., and Brandl, R.: Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis, Evol. Ecol. Res., 8, 1039–1048, 2006.

Ruhnke, H., Schädler, M., Klotz, S., Matthies, D., and Brandl, R.: Variability in leaf traits, insect herbivory and herbivore performance within and among individuals of four broad-leaved tree species, Basic Appl. Ecol., 10, 726–736, 2009.

Ruusila, V., Morin, J. P., van Ooik, T., Saloniemi, I., Ossipov, V., and Haukioja, E.: A short-lived herbivore on a long-lived host: tree resistance to herbivory depends on leaf age, Oikos, 108, 99–104, 2005.

Scheiner, S. M.: Genetics and evolution of phenotypic plasticity, Annu. Rev. Ecol. Syst., 24, 35–68, 1993.

Scriber, J. M.: Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits, Entomol. Exp. Appl., 104, 217–235, 2002.

Unsicker, S. B., Oswald, A., Kohler, G., and Weisser, W. W.: Complementarity effects through dietary mixing enhance the performance of a generalist insect herbivore, Oecologia, 156, 313–324, 2008.

Unterseher, M., Morawetz, W., Klotz, S., and Arndt, E.: The canopy of a temperate floodplain forest. Results from five years of research at the Leipzig canopy crane. University Leipzig, Helmholtz Zentrum für Umweltforschung, Stadt Leipzig, 161 pp., 2007.

Windig, J. J.: Reaction norms and the genetic basis of phenotypic plasticity in the wing pattern of the butterfly *Bicyclus anynana*, J. Evol. Biol., 7, 665–695, 1994.