HELMHOLTZ
CENTRE FOR
ENVIRONMENTAL
RESEARCH – UFZ

Effects of resource heterogeneity in trees upon insect herbivory

Dissertation

zur Erlangung des Doktorgrades
der Naturwissenschaften
(Dr. rer. nat.)

dem Fachbereich Biologie
der Philipps-Universität Marburg
vorgelegt von

Haike Ruhnke
aus Wismar

Marburg/Lahn, 2007

Vom Fachbereich Biologie
der Philipps-Universität Marburg
als Dissertation angenommen am 10. Mai 2007

Erstgutachter: Prof. Dr. Roland Brandl
Zweitgutachter: Prof. Dr. Diethart Matthies

Tag der mündlichen Prüfung am 20. Juni 2007

Anschrift der Autorin zur Zeit der Promotion:

Haike Ruhnke
Helmholtz Centre for Environmental Research - UFZ
Department of Community Ecology
Theodor-Lieser-Str. 4
D-06120 Halle
Germany

Tel: +49 345 558 53 02
Fax: +49 345 558 53 29
E-Mail: ruhnke.ufz@web.de

Front page: Larva of the privet sawfly *Macrophya punctumalbum*.

“The great tragedy of Science –
the slaying of a beautiful hypothesis by an ugly fact.”

Thomas H. Huxley

Contents

1. Introduction

1.1. Responses of herbivorous insects to leaf tissue quality

Leaf tissue quality and its effects on herbivorous insects have been topics of interest for several decades. Many plant constituents reduce the usability of plant tissue for phytophagous insects such as tough fibres, high carbohydrate and cellulose content, low nitrogen and water content as well as toxins and digestibility-reducing substances (Hartley & Jones, 1997). Moreover, plants show an extremely high diversity in phytochemicals (Swain, 1977; Nuhn, 1997), which can make especially leaves a poor-quality food for insects. Hence, herbivores have developed various strategies to deal with such substances in leaves. Such strategies encompass feeding and oviposition choice, altering the herbivore's morphology and physiology as well as active manipulation of the host (Karban & Agrawal, 2002). For instance, insects have optimised the size of mouth parts (Caroll & Boyd, 1992) and of the head (Bernays, 1986) to enhance their feeding abilities. Herbivores have developed adaptations to detoxify secondary metabolites of plants, e.g. larvae of the tobacco hornworm *Manduca sexta* induce midgut cytochrome P-450 activity when toxic nicotine of tobacco plants is ingested (Snyder *et al.*, 1993). Further, adaptations of herbivorous insects to novel host species (Lazarevic *et al.*, 2002; Ballabeni *et al.*, 2003), to budburst phenology of hosts (Yukawa, 2000; Tikkanen *et al.*, 2006) and to regional differences of host species composition (Parry & Goyer, 2004) are reported.

Leaf traits differ among plant species and, therefore, the leaf palatability to herbivorous insects (Edwards *et al.*, 1986). Several authors showed that the concentrations of leaf nitrogen and secondary metabolites also vary within species, e.g. among tree individuals (Howard, 1990; Suomela & Ayres, 1994; Laitinen *et al.*, 2000; Osier *et al.*, 2000). Leaf tissue quality varies due to genotype (Glynn *et al.*, 2004) and environmental factors, e.g. soil conditions (Niinemets & Kull, 2003) and light availability (Larsson *et al.*, 1986; Henriksson *et al.*, 2003). These variations in tissue quality affect the feeding behaviour and development of folivorous insects (Ayres *et al.*, 1987; Howard, 1990; Strauss, 1990; Osier & Lindroth, 2001; Fortin & Mauffette, 2002) and may allow for fine-tuned adaptations of insects (Edmunds & Alstad, 1978). Plants may change the chemical composition of leaf tissue following damage by herbivory (induced plant responses, Wratten *et al.*, 1984; Karban & Myers, 1989), thereby influencing subsequent herbivore attack. Effects of previous damage of leaves on herbivores are variable; often the performance of insects declines (Cronin & Abrahamson, 1999) whereas some species prefer damaged leaves (Shibata *et al.*, 2001). Herbivores can change the distribution, structure and dominance hierarchies in plant communities (Louda & Rodman, 1996; Hartley & Jones, 1997). Hence, plant-insect interactions are a complex network of abiotic and biotic traits.

Diverse theories and hypotheses regarding plant-insect interactions have been developed (compilations e. g. in Hartley & Jones, 1997; Price, 1997) often involving mechanisms of adaptation. While some hypotheses are well-investigated (cp. Hartley & Jones, 1997) others have been controversially discussed, e.g. the adaptive deme formation hypothesis (Van Zandt & Mopper, 1998; Cobb & Whitham, 1998). The hypothesis is based on the adaptability of herbivorous insects on a fine-scale and might describe an important process in evolution.

The adaptive deme formation hypothesis assumes that insect populations respond to the resource heterogeneity among individual hosts by forming ecologically and genetically distinct groups – the demes (Edmunds & Alstad, 1978). Gradually over several generations, these demes should become locally adapted to the specific defensive and nutritional characteristics of a host plant individual. Thereby, optimising their performance and gaining an evolutionary advantage. For the formation of adapted demes, the life span of hosts must sustain several generations of the herbivore and host individuals should produce predictable leaf tissue quality over many insect generations. Therefore, adaptive demes are most likely to occur in insects living on clonal growing plants or on trees. Several studies demonstrated genetic variation within and among populations of phytophagous insects (see Mopper, 1996). It may occur even among herbivorous insects occupying different branches of the same tree (Alstad & Corbin, 1990). Tests of the adaptive deme formation hypothesis, however, have not revealed a consistent link between patterns of genetic variation and the formation of locally adapted demes (e.g. Cobb & Whitham, 1998). Nevertheless, Van Zandt & Mopper (1998) suggested that local adaptation is an important phenomenon which occurs in diverse insect systems. In contrast, Cobb & Whitham (1998) doubted that adaptations to individual trees occur at all. In a meta-analysis Van Zandt & Mopper (1998) tested whether the formation of locally adapted demes depends on mobility, feeding mode (endophagous, exophagous) or breeding system (haplodiploid, diploid, parthenogenetic) of the herbivorous insects but they found no significant effects of any of these traits. However, a limited host range has been shown to be an important precondition for the formation of adaptive demes (Lajeunesse & Forbes, 2002).

Adaptations are assumed to evolve over many generations and to be genetically manifested as the adaptive deme formation hypothesis presumes (Edmunds & Alstad, 1978). However, they may also occur over shorter time periods or could be caused by non-genetic effects, e.g. parental effects (Rotem *et al*., 2003). Fine-scale adaptations to host individuals may already take place in an expeditious adaptation during larval development, here called physiological adaptation. Until now, no studies are known on this type of adaptation that is suggested to occur on host individuals within plant species. Reported responses of herbivorous insects to host quality allow the assumption that phytophagous insects are able to adapt physiologically to the specific leaf tissue quality that they encounter during larval development. For instance, feeding on a particular host plant can induce a strong preference for that plant species in larvae of oligophagous and polyphagous herbivores (Jermy *et al*., 1968). It is assumed to be relatively common among lepidopteran larvae (Bernays & Weiss, 1996). The responsiveness of taste cells in caterpillars can be altered by the dietary exposure to specific stimuli (Jermy *et al*., 1968; Schoonhoven, 1969; Glendinning *et al*., 1999) which may lead to changes in behaviour (Blaney *et al*., 1986). Furthermore, induction of specific enzymes in the midgut is reported as early as eight days following exposure to a new host plant species in larvae of the desert locust (Mainguet *et al.*, 2000). The phenotypic capability for adaptation to host individuals may vary genetically among phytophagous individuals and might have an effect on long-term (genetic) adaptabilities, e.g. the formation of locally adapted demes. Investigations of physiological and genetic fine-scale adaptations of herbivores may help to understand processes in evolution.

1.2. Objectives

The primary objective of this thesis is to broaden the understanding of fine-tuned adaptabilities and to test the applicability of the adaptive deme formation hypothesis of Edmunds & Alstad (1978) on populations of herbivorous insects. Therefore, three different aspects of adaptability of phytophagous species, including their preconditions, were investigated: resource heterogeneity for herbivorous insects, physiological adaptability of larvae and the formation of locally adapted demes.

The first part of the thesis (Chapter 3 and 4) analyses the resource heterogeneity for herbivorous insects and its predictability across years. The second part (Chapter 5) probes into the question of whether larvae are able to adapt physiologically to the resource situation on individual hosts that they encounter during their development. The third part (Chapter 6) focuses on the adaptive deme formation hypothesis itself. The hypothesis is tested on populations of two sawfly species.

Resource heterogeneity

Resource heterogeneity for herbivorous insects among host individuals is a prerequisite for fine-scale adaptations. Together with the predictability of host quality over time, it is essential for forming locally adapted demes (Edmunds & Alstad, 1978). Because of their longevity, tree species may represent an excellent system for testing the adaptive deme formation hypothesis. Therefore, the intraspecific variability of leaf tissue quality for herbivorous insects on different tree species was investigated as a first step to test the hypothesis. Leaf traits, the level of leaf damage by herbivory in the field as well as the palatability of leaves and performance of a polyphagous herbivore (*Spodoptera littoralis*; Lepidopera: Noctuidae) in feeding experiments were estimated (first results in Ruhnke *et al.*, 2004). Chapter 3 focuses on results from sycamore (*Acer pseudoplatanus*) in two consecutive years. In Chapter 4 results from ash (*Fraxinus excelsior*), lime (*Tilia cordata*) and common oak (*Quercus robur*) were presented together with the results from sycamore (*Acer pseudoplatanus*). The following questions were aimed to answer: (1) Does the quality of leaf tissue differ among individual trees and between layers within a tree? (2) Are these differences reflected in different levels of herbivore attack in the field? (3) Do the differences in leaf traits cause variation in leaf consumption and growth of polyphagous insect herbivores in the laboratory? (4) Are differences in palatability consistent across two consecutive years?

Physiological adaptability

The ability of phytophagous insects to respond physiologically and behaviourally to the quality of leaf tissue allows the assumption that herbivorous insects may be able to adapt physiologically to the specific leaf tissue quality that they encounter during larval development. This adaptability might affect the formation of locally adapted demes. In

Chapter 5 the hypothesis of physiological adaptation of folivorous insects during larval development was tested together with genetic variation in phenotypic adaptability. Larvae of the polyphagous gypsy moth (*Lymantria dispar*; Lepidoptera: Lymantriidae) were reared on individual oak trees (*Quercus robur*) for about two weeks. After that, reciprocal transfer experiments were performed and the effect of novel host individuals on the relative growth rate of the larvae measured.

Formation of locally adapted demes

Van Zandt & Mopper (1998) suggested that adaptive deme formation is an important evolutionary phenomenon. Tests of the adaptive deme formation hypothesis, however, failed to produce consistent results (Van Zandt & Mopper, 1998). The vast majority of studies used piercing-sucking insects (scale insects, thrips, aphids and phylloxerans) to test the hypothesis. However, leaf constituent levels encountered by herbivorous insects are determined largely by their feeding styles. Therefore, leaf chewers might respond differently to leaf quality than piercing-sucking herbivores (Peeters, 2002).

In this thesis, the adaptive deme formation hypothesis was tested for populations of free-feeding leaf chewers. Reciprocal transfer experiments were performed with larvae of the monophagous black sawfly *Tomostethus nigritus* and the oligophagous privet sawfly *Macrophya punctumalbum* (Hymenoptera: Tenthredinidae) on ash (*Fraxinus excelsior*). These two species differ in biological characteristics, which may affect the formation of locally adapted demes (Chapter 6).

1.3. Study area

The study was performed in a mixed floodplain forest in Central Germany on a small spatial scale. The „Leipzig floodplain forest" (city of Leipzig, Saxony, Germany) covers a total area of circa 1900 ha, mostly consisting of a hard-wood floodplain forest. The actual canopy composition with old oak trees (> 250 years old) and younger trees of this and other species (< 130 years old) was created through selective felling in the past (Morawetz & Horchler, 2004). In spring 2001, a construction crane was installed in the forest. The crane makes it possible to work in the canopy in an area of 1.6 ha. In that area the vegetation is classified as a typical floodplain forest of the upper alluvial zone (Querco-Ulmetum) on nutrient-rich loamy floodplain soils (Morawetz & Horchler, 2004). The forest is rich in plant species and dominated by ash (*Fraxinus excelsior*), sycamore (*Acer pseudoplatanus*), oak (*Quercus robur*), lime (*Tilia cordata*), maple (*Acer platanoides*) and hornbeam (*Carpinus betulus*).

1.4. References

Alstad, D.N. & Corbin, K.W. (1990) Scale insect allozyme differentiation within and between host trees. *Evolutionary Ecology*, **4**, 43-56.

Ayres, M.P., Suomela, J. & MacLean, S.F. Jr. (1987) Growth performance of *Epirrita autumnata* (Lepidoptera: Geometridae) on mountain birch: trees, broods, and tree x brood interactions. *Oecologia*, **74**, 450-457.

Ballabeni, P.; Gotthard, K.; Kayumba, A. & Rahier, M. (2003) Local adaptation and ecological genetics of host-plant specialization in a leaf beetle. *Oikos*, **101**, 70-78.

Bernays, E.A. (1986) Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science*, **231**, 495-497.

Bernays, E.A. & Weiss, M.R. (1996) Induced food preferences in caterpillars: The need to identify mechanisms. *Entomologia Experimentalis et Applicata*, **78**, 1-8.

Blaney, W.M., Schoonhoven, L.M. & Simmonds, M.S.J. (1986) Sensitivity variations in insect chemoreceptors - a review. *Experientia*, **42**, 13-19.

Caroll, C.P. & Boyd, C. (1992) Host race radiation in the soapberry bug: natural history with the history. *Evolution*, **46**, 1052-1069.

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

Cronin, J.T. & Abrahamson, W.G. (1999) Host-plant genotype and of her herbivores influence goldenrod stem galler preference and performance. *Oecologia*, **121**, 392-404.

Edmunds, G.F. & Alstad, D.N. (1978) Coevolution in insect herbivores and conifers. *Science*, **199**, 941-945.

Edwards, P.J., Wratten, S.D. & Greenwood, S. (1986) Palatability of British trees to insects: Constitutive and induced defences. *Oecologia*, **69**, 316-319.

Fortin, M. & Mauffette, Y. (2002) The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. *Canadian Journal of Forest Research*, **32**, 379-389.

Glendinning, J.I., Ensslen, S., Eisenberg, M.E. & Weiskopf, P. (1999) Diet-induced plasticity in the taste system of an insect: Localization to a single transduction pathway in an identified taste cell. *Journal of Experimental Biology*, **202**, 2091-2102.

Glynn, C., Ronnberg-Wastljung, A.C., Julkunen-Tiitto, R., & Weih, M. (2004) Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomologia Experimentalis et Applicata*, **113**, 1-14.

Hartley, S.E. & Jones, C.G. (1997) Plant chemistry and herbivory, or why the world is green. In: Crawley, M.J. (ed.) *Plant ecology*. 2nd ed., pp. 284-324, Blackwell Science, Oxford.

Henriksson, J., Haukioja, E., Ossipov, V., Ossipova, S., Sillanpaa, S., Kapari, L. & Pihlaja, K. (2003) Effects of host shading on consumption and growth of the geometrid Epirrita autumnata: interactive roles of water, primary and secondary compounds. *Oikos*, **103**, 3-16.

Howard, J.J. (1990) Infidelity of leafcutting ants to host plants: resource heterogeneity or defense induction? *Oecologia*, **82**, 394-401.

Jermy, T., Hanson, F.E. & Dethier, V.G. (1968) Induction of specific food preference in lepidopterous larvae. *Entomologia Experimentalis et Applicata*, **11**, 211-230.

Karban, R. & Agrawal, A.A. (2002) Herbivore offense. *Annual Review of Ecology and Systematics*, **33**, 641-664.

Karban, R. & Myers, J.H. (1989) Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, **20**, 331-348.

Laitinen, M.L., Julkunen-Tiitto, R. & Rousi, M. (2000) Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology*, **26**, 1609-1622.

Lajeunesse, M.J. & Forbes, M.R. (2002) Host range and local parasite adaptation. *Proceedings of the Royal Society of London Series B*, **269,** 703-710.

Larsson, S., Wirén, A., Lundgren, L. & Ericsson, T. (1986) Effects of light and nutrient stress on leaf phenolic chemistry in *Salix dasyclados* and susceptibility to *Galerucella lineola* (Coleoptera). *Oikos*, **47**, 205-210.

Lazarevic, J.; Peric-Mataruga, V.; Stojkovic, B. & Tucic, N. (2002) Adaptation of the gypsy moth to an unsuitable host plant. *Entomologia Experimentalis et Applicata*, **102**, 75-86.

Louda, S.M. & Rodman, J.E. (1996) Insect herbivory as a major factor in the shade distribution of a native crucifer (*Cardamine cordifolia* A. Gray, bittercress). *Journal of Ecology*, **84**, 229-237.

Mainguet, A.M., Louveaux, A., El Sayed, G. & Rollin, P. (2000) Ability of a generalist insect, Schistocerca gregaria, to overcome thioglucoside defense in desert plants: tolerance or adaptation? *Entomologia Experimentalis et Applicata*, **94**, 309-317.

Mopper, S. (1996) Adaptive genetic structure in phytophagous insect populations. *Trends in Ecology & Evolution*, **11**, 235-238.

Morawetz, W., Horchler, P.J. (2004) Leipzig Canopy Crane Project (LAK), Germany. In: Basset, Y., Horlyck, V. & Wright, S. J. (ed.): *Studying forest canopies from above: The International Canopy Crane Network*. United Nations Environment Programme, Editorial Panamericana de Colombia, Bogotá.

Niinemets, Ü. & Kull, K. (2003) Leaf structure *vs*. nutrient relationships vary with soil conditions in temperate shrubs and trees. *Acta Oecologica*, **24**, 209-219.

Nuhn, P. (1997) *Naturstoffchemie: mikrobielle, pflanzliche und tierische Naturstoffe*. 3. ed., Stuttgart: S. Hirzel Verlag.

Osier, T.L., Hwang, S.Y. & Lindroth, R.L. (2000) Within- and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. *Biochemical Systematics and Ecology*, **28**, 197-208.

Osier, T.L. & Lindroth, R.L. (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. *Journal of Chemical Ecology*, **27**, 1289-1313.

Parry, D. & Goyer, R.A. (2004) Variation in the suitability of host tree species for geographically discrete populations of forest tent caterpillar. *Environmental Entomology*, **33**, 1477-1487.

Peeters, P.J. (2002) Correlations between leaf constituent levels and the densities of herbivorous insect guilds in an Australian forest. *Austral Ecology*, **27**, 658-671.

Price, P.W. (1997) *Insect Ecology*. 3rd ed. New York: Wiley.

Rotem, K., Agrawal, A.A. & Kott, L. (2003) Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? *Ecological Entomology*, **28**, 211-218.

Ruhnke, H., Brändle, M., Klotz, S., Brandl, R. & Schädler, M. (2004) Heterogeneity of palatability within and between trees. *Mitteilungen der deutschen Gesellschaft für allgemeine und angewandte Entomologie*, **14**, 45-48.

Schoonhoven, L.M. (1969) Sensitivity changes in some insect chemoreceptors and their effect on food selection behavior. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen C*, **72**, 491-498.

Shibata, S., Ishida, T.A., Soeya, F., Morino, N., Yoshida, K., Sato, H. & Kinura, M.T. (2001) Within-tree variation in density and survival of leafminers on oak *Quercus dentata*. *Ecological Research*, **16**, 135-143.

Snyder, M.J., Hsu, E.-L. & Feyereisen, R. (1993) Induction of cytochrome P-450 activities by nicotine in the tobacco hornworm, *Manduca sexta*. *Journal of Chemical Ecology*, **19**, 2903-2916.

Strauss, S.Y. (1990) The role of plant genotype, environment and gender in resistance to a specialist chrysomelid herbivore. *Oecologia*, **84**, 111-116.

Suomela, J. & Ayres, M.P. (1994) Within-tree and among-tree variation in leaf characteristics of mountain birch and its implications for herbivory. *Oikos*, **70**, 212-222.

Swain, T. (1977) Secondary compounds as protective agents. *Annual Review of Plant Physiology*, **28**, 479-501.

Tikkanen, O.P., Woodcock, B., Watt, A. & Lock, K. (2006) Are polyphagous geometrid moths with flightless females adapted to budburst phenology of local host species? *Oikos*, **112**, 83-90.

Van Zandt, P.A. and Mopper, S. (1998) A meta-analysis of adaptive deme formation in phytophagous insect populations. *American Naturalist*, **152**, 595–604.

Wratten, S.D., Edwards, P.J. & Dunn, I. (1984) Wound induced changes in the palatability of *Betula pubescens* and *B. pendula*. *Oecologia*, **61**, 372-375.

Yukawa, J. (2000) Synchronization of gallers with host plant phenology. *Population Ecology*, **42**, 105-113

2. Authors' contribution to the research papers and manuscripts

The following chapters represent a compilation of research papers and manuscripts on resource heterogeneity for and adaptability of herbivorous insects. The authors' contributions to the single chapters are as follows:

Chapter 3

Plant-animal interactions in the canopy: Intraspecific variability in herbivory on sycamore (*Acer pseudoplatanus* L.)

H. Ruhnke, M. Brändle, S. Klotz, R. Brandl & M. Schädler

In: Unterseher, M., Morawetz, W., Klotz, S., & Arndt, E. (eds) *The canopy of a temperate floodplain forest: Results from five years of research at the Leipzig Canopy Crane*. Leipzig: Universitätsverlag Leipzig, pp. 57-63

- Idea in cooperation with Roland Brandl.
- Experimental design together with Roland Brandl.
- Realisation of the experiments.
- Data analysis together with Diethart Matthies.
- Writing. Martin Schädler and Roland Brandl commented on the manuscript.

Chapter 4

Heterogeneity in palatability and utilization of leaves among and within trees of four broad-leaved tree species

H. Ruhnke, M. Schädler, S. Klotz, D. Matthies & R. Brandl

Manuscript intended for submission to *Ecological Entomology*

- Idea in cooperation with Roland Brandl.
- Experimental design together with Roland Brandl.
- Realisation of the experiments.
- Data analysis together with Diethart Matthies.
- Writing. Roland Brandl, Diethart Matthies and Martin Schädler commented on the manuscript.

Chapter 5

Is there an efficient physiological adaptation of a generalist herbivore to individual host plants?

H. Ruhnke, D. Matthies, R. Brandl

Manuscript intended for submission to *Physiological Entomology*

- Idea.
- Experimental design.
- Realisation of the experiments.
- Data analysis together with Diethart Matthies.
- Writing. Roland Brandl and Diethart Matthies commented on the manuscript.

Chapter 6

Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis

H. Ruhnke, M. Schädler, D. Matthies, S. Klotz, R. Brandl

Evolutionary Ecology Research (2006) **8**, 1039-1048

- Idea in cooperation with Roland Brandl.
- Experimental design; supervised by Diethart Matthies
- Realisation of the experiments.
- Data analysis together with Diethart Matthies.
- Writing. Roland Brandl, Diethart Matthies and Martin Schädler commented on the manuscript.

3. Plant-animal interactions in the canopy: Intraspecific variability in herbivory on sycamore (*Acer pseudoplatanus* L.)

Haike Ruhnke, Martin Brändle, Stefan Klotz, Roland Brandl & Martin Schädler

In: Unterseher, M., Morawetz, W., Klotz, S., & Arndt, E. (eds) *The canopy of a temperate floodplain forest: Results from five years of research at the Leipzig Canopy Crane*. Leipzig: Universitätsverlag Leipzig, pp. 57-63

3.1. Abstract

The quality of leaf tissue differs not only among plant species, but may also show considerable variability among and within individuals of a given species. Insect populations may respond to this resource heterogeneity by forming ecologically and genetically distinct groups (adaptive deme formation; ADF, Edmunds & Alstad 1978). To evaluate central assumptions of the ADF-hypothesis, we analysed variability in herbivore attack, palatability and leaf utilization in a feeding experiment as well as leaf traits among and within three sycamore individuals in two consecutive years on the crane plot in Leipzig. Herbivore attack in the field, palatability and leaf utilization by a generalist herbivore as well as leaf traits differed among the investigated individuals of sycamore. Only in 2003 did herbivore attack differ significantly between the upper and the lower tree layer. In the feeding experiments, larvae of *Spodoptera littoralis* showed differences between tree layers only in terms of conversion efficiency in the first year of our study. All measured variables point to considerable heterogeneity of leaf quality among the investigated individuals, with only little variation within trees. Palatability, the relative growth rate and the conversion efficiency of the larvae of *S. littoralis* correlated negatively with the carbon/nitrogen-ratio of the leaf tissue. Herbivore attack in the field, however, was not related to palatability, relative growth rate and conversion efficiency of *S. littoralis* or to the measured leaf traits. Due to confounding environmental effects, levels of herbivory do not always follow the patterns of palatability and leaf traits in space and time. Overall, our analyses support two basic assumptions of the ADF-hypothesis. Firstly, there is considerable variability of quality among individual trees and, secondly, the variability is to some extent predictable across time.

3.2. Introduction

Leaves of trees may differ in palatability to insect herbivores. Several authors showed that concentration of leaf nitrogen and secondary compounds may vary among individual trees within species (Howard 1990; Suomela & Ayres 1994; Laitinen *et al.* 2000; Osier *et al.* 2000b) as well as between sun and shade leaves within individuals (Hollinger 1989; Dudt & Shure 1994). These variations may affect attack, feeding behaviour and development of associated insect herbivores (Ayres *et al.* 1987; Howard 1990; Strauss 1990; Osier & Lindroth 2001; Fortin & Mauffette 2002). Ultimately, differences in the quality of leaves may translate into genetic differentiation between populations of phytophagous insects living on different individuals. The adaptive deme formation hypothesis (hereafter called ADF hypothesis) predicts the evolution of distinct groups (demes) within species of herbivorous insects in response to differences of resource quality among individuals. Demes are adapted to a particular individual (Edmunds & Alstad 1978). Although some studies have demonstrated genetic variation among populations of phytophagous insects, the underlying mechanisms are still poorly understood (Mopper 1996). In our study, we investigated the intraspecific variability of herbivore attack, of palatability and leaf utilization as well as of leaf traits among and within individuals of sycamore (*Acer pseudoplatanus* L.) in two consecutive years. We approached the following questions: (1) Is there any difference in herbivore attack, palatability and leaf utilization as well as of leaf traits among and within individual trees? (2) If such differences exist, is there any correlation between herbivore attack in the field and palatability as well as of leaf traits? (3) Do the differences among individual trees show a consistent pattern across the two consecutive years?

3.3. Materials and methods

We estimated herbivore attack in the field. Palatability and related variables of leaves were measured with laboratory experiments using a polyphagous moth. Furthermore, we measured two leaf traits known to be important for herbivores. For our work we sampled three mature sycamore trees (*Acer pseudoplatanus* L.) in two consecutive years. The maximum distance between individual trees was about 90 m. Within trees we distinguished between an upper (sun leaves) and a lower layer (shade leaves).

Herbivore attack in the field

At the end of growing season in August 2002 and 2003, we quantified the levels of herbivory by estimating the amount of removed leaf material. From each tree individual we selected four branches in each layer. We collected 25 leaves from each branch (200 leaves per tree). Consumed leaf area [mm2] was estimated after digitising and measuring leaf area (SigmaScanPro5). The specific weight of leaf material differed between the two layers. Therefore, we corrected the consumed leaf area of the upper layer by tree-specific correction factors. These correction factors were the ratio of leaf fresh mass to dry mass of 20 samples from each tree layer collected in July of 2002 and 2003.

Feeding experiments

For our feeding experiments we used larvae of the African cotton leafworm (*Spodoptera littoralis* (Boisduval), Lepidoptera: Noctuidae) a polyphagous herbivore. Larvae originated from a laboratory stock and were reared on artificial diet. The experiments were carried out in July 2002 and July 2003, when the foliage was fully mature. In every experiment, we used different branches within each tree layer to avoid possible confounding effects of induced plant defence. The branches selected for the feeding experiments were near to those selected to estimate herbivore attack in the field. We collected leaves from short shoots of each branch. Leaves were sprayed with deionised water to keep them turgescent and leaves were stored at 4 °C. The experiments started a few hours after sampling. From six leaves of each branch we punched leaf discs (diameter

23 mm). Discs were weighed and placed individually in Petri dishes lined with moist filter paper. For each Petri dish we used one larva of *S. littoralis* (third instar). Prior to the experiments, larvae were weighed. The Petri dishes were placed in a climate chamber (26 °C and 12 h light) for 24 h. At the end of the experiment larvae were killed by freezing. Dead larvae and remaining leaf material were dried at 60 °C to weight constancy. Initial larval mass was converted to dry mass using a linear regression equation for each year (30 larvae in each year). The initial fresh mass of leaf discs was converted to dry mass by using the mean water content of leaves for each selected branch (see below). Leaf consumption was expressed as mg consumed leaf dry mass in 24 h.

Osier *et al.* (2000a) showed that the growth of caterpillars reared in bags on trees was highly correlated to the growth of larvae reared in the laboratory on leaves of the same tree. Hence, we expect that the feeding experiments in the lab reflect leaf quality in the field (see also Kleiner 1991). Lab experiments have the advantage that all experiments are performed under identical climatic conditions. In the field microclimatic differences between trees and layers may influence the results.

Leaf traits

Leaf water, nitrogen and carbon contents are known to be closely related to functional leaf traits and to palatability (Schädler *et al.* 2003). Therefore, we measured these traits for the foliage of each branch used during the feeding experiments and to the same time as in the bioassays. Water content was determined by the ratio of leaf dry mass to fresh mass of five leaf discs per branch. To estimate the carbon/nitrogen-ratio a sample of leaves from every branch was vacuum-dried for 48 h, milled with a high-speed rotor mill and analysed for carbon and nitrogen with an element analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany).

Data analyses

For the analysis of consumed leaf area in the field we averaged the 25 individual values of each branch. These means were square-root transformed to normalize the distribution of residuals. The effects of year, tree individual and tree layer were tested using a three-way ANOVA (Proc GLM [Version 8.02]; SAS Institute).

In the analyses of the feeding experiments, for every trait we used the average across the six values of each branch. The effects of year, tree individual and tree layer on consumed leaf material, final larval dry mass, and increase of larval mass during the experiment were analysed using an ANCOVA (see Raubenheimer & Simpson 1992, Horton & Redak 1993). For the analysis of consumed leaf material and final larval dry mass, initial larval dry mass was used as a covariate. By using type I sums of squares, we analysed the effects after removing confounding effects of initial larval dry mass from the analysis. Thereby, we standardized leaf consumption to herbivore mass, and adjusted means are a measure of palatability. Similarly, adjusted means of final larval dry mass measure relative growth rates of larvae; for this analysis we log-transformed initial and final larval dry mass. For the analysis of increase in mass, the consumed leaf material was used as covariate. Thereby, we estimated an equivalent to the efficiency of conversion of ingested food into body substance (see Waldbauer 1968).

Effects of year, tree individual and tree layer on leaf C/N-ratio and water content were analysed using a three-way ANOVA. The relationships between variables measured during the feeding experiments (adjusted means from the ANCOVA for every layer of each tree individual), the herbivore attack in the field, and leaf traits were tested by a Spearman's rank correlation using the mean of every trait per layer, tree and year.

3.4. Results

Herbivore attack in the field

In the field, the consumed leaf area differed significantly between the two consecutive years (Table 1). We observed a significant interaction between year and tree individual indicating different levels of herbivory on the individuals between years (Fig. 1a). Separate statistical analyses for the two years showed, however, significant differences among individual trees only in 2003 (Fig. 1a, ANOVA results not shown). Herbivore attack was larger in the upper tree layer in the second year only (significant year x tree layer interaction, Table 1, Fig. 1b). Furthermore, the effect of tree layer varied among individual trees (significant tree individual x tree layer interaction, Table 1).

Feeding experiments

Palatability, relative growth rate, and conversion efficiency of *Spodoptera littoralis* differed significantly between the two years (Table 2). All traits differed among individual trees (Fig. 2). We found no general difference between the upper and lower tree layer for all three variables. Overall, we found few significant interactions between factors. For the conversion efficiency we found a significant interaction between year and tree layer; in 2002 we found higher conversion efficiency for the lower layer (Fig. 3). For the growth rate we found a three-way interaction.

Table 1. Results of the ANOVA of effects of year, tree individual, and tree layer on the consumed leaf area by herbivores (herbivore attack). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; df: degrees of freedom, MS: mean square.

Source	**df**	**F values**
Year	1	15.37 ***
Tree individual	2	1.56
Tree layer	1	15.08 ***
Year x tree individual	2	3.73 *
Year x tree layer	1	25.10 ***
Tree individual x tree layer	2	4.82 *
Year x tree individual x tree layer	2	1.89
Residual	36	[MS = 8.26]

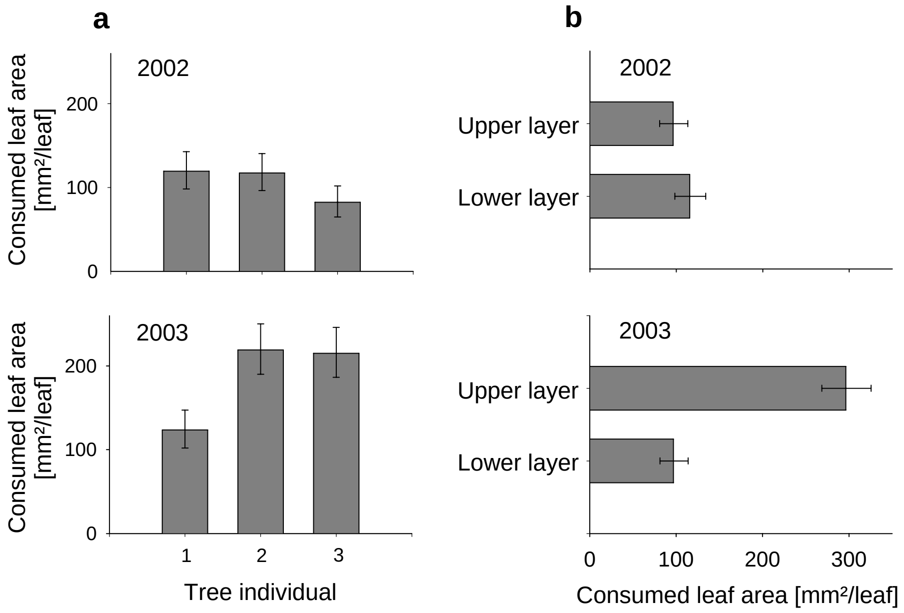


Fig. 1. Herbivore attack measured by the consumed leaf area on sycamore by herbivores in the field. The graphs present means of tree individuals (a) and tree layer (b) for each year (back-transformed means ± 1 SE).

Leaf traits

The C/N-ratio of leaves differed between the two years as well as among individual trees (Table 3). We found a significant interaction of year and tree individual as well as a significant three-way interaction (Table 3). The water content of leaf tissue was similar in the two years but differed among individual trees and between tree layers. Generally, water content was significantly lower in leaves of the upper tree layer. However, the strength of this effect differed between years and individual trees (significant year x tree layer and tree individual x tree layer interaction, Table 3). Palatability ($P = 0.052$), relative growth rate ($P = 0.019$), and the conversion efficiency ($P = 0.003$) of larvae of *S. littoralis* were all negatively correlated to the C/N-ratio of the leaf tissue, although we could only use the mean of the trait per layer and tree and year. In contrast, we could not detect correlations with the water content of leaves (all $P > 0.3$). We found no relationship between herbivore attack in the field and variables measured during the feeding experiments as well as with the measured leaf traits (all $P > 0.3$).

3.5. Discussion

One may answer the three questions posed in the introduction as following: (1) We found significant differences among individual trees in herbivore attack in the field, variables derived from feeding experiments as well as measured leaf traits. (2) We found no correlation between herbivore attack in the field and variables derived from the feeding experiments or measured leaf traits. However, variables derived from the feeding experiments and leaf traits were correlated (3). Although we found significant interactions

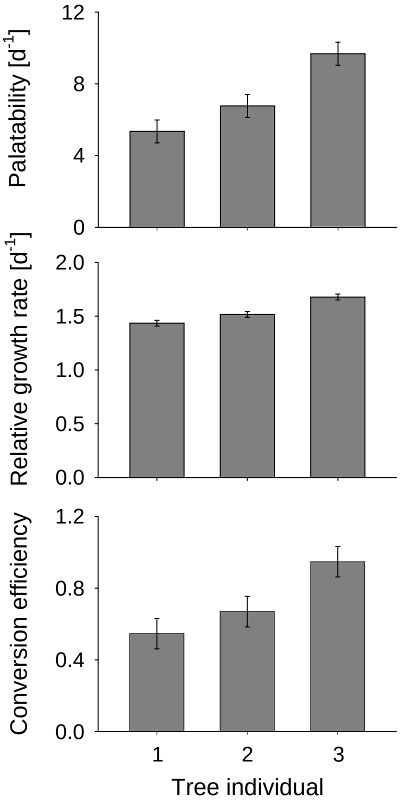


Fig. 2. Effects of tree individual on palatability, relative growth rate, and conversion efficiency of larvae of *S. littoralis* on sycamore (adjusted means of both years ± 1 SE).

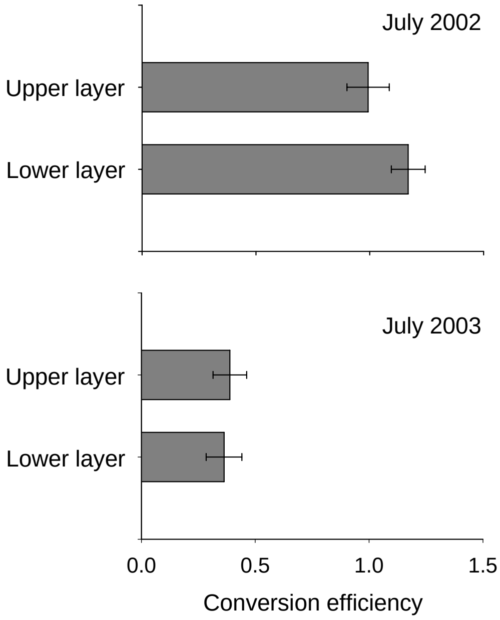


Fig. 3. Effects of tree layer on the conversion efficiency of larvae of *S. littoralis* for 2002 and 2003 (adjusted means ± 1 SE).

for the leaf traits, the results from the feeding experiments provided a consistent ranking of tree individuals across the two years.

A number of studies have shown that the content of certain compounds differs among tree individuals (Howard 1990; Suomela & Ayres 1994; Laitinen *et al.* 2000; Osier *et al.* 2000b). Further, their utilization by herbivores varied among host-plant individuals (Ayres *et al.* 1987; Howard 1990; Strauss 1990; Osier & Lindroth 2001). We found significant differences in herbivore attack, palatability and related variables as well as simple leaf traits among three sycamore individuals. Note that these individuals grow close to each other. However, the variation in herbivore attack in the field was not correlated to the results of the feeding experiments and measured leaf traits. This lack of correlation between field patterns and lab experiments has been reported by a few other authors (Rowe & Potter 1996; Van Nouhuys *et al.* 2003). Rowe & Potter noticed ". . . there is no a priori expectation for whether leaves in the upper or lower canopy will be preferred on the basis of foliar chemistry". This points to fundamental differences between patterns of herbivory in the field and lab studies. In the field the variation in herbivore pressure within

Table 2. Results of an ANCOVA of effects of year, tree individual, and tree layer on palatability, growth rate and conversion efficiency of the larvae of *S. littoralis*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; df: degrees of freedom, MS: mean square.

Source	df	F values		
		Palatability	Growth rate	Conversion efficiency
Covariate	1	52.16 ***	204.23 ***	424.69 ***
Year	1	6.65 *	76.03 ***	52.07 ***
Tree individual	2	23.94 ***	41.98 ***	15.55 ***
Tree layer	1	3.02	0.93	3.48
Year x tree individual	2	0.62	1.19	1.35
Year x tree layer	1	0.88	0.17	6.59 *
Tree individual x tree layer	2	0.76	1.65	1.19
Year x tree individual x tree layer	2	2.58	3.48 *	0.98
Residual	35	[MS = 3.27]	[MS < 0.01]	[MS = 0.06]

and among trees is not only influenced by leaf quality but by a plethora of factors such as microclimate (Stamp & Bowers 1990), predation, parasites (Stamp & Bowers 1988) or migration (Batzer *et al.* 1995; Magalhães *et al.* 2002).

Leaf traits like water content, C/N-ratio, or concentration of secondary compounds influence leaf palatability (Mattson 1980; Scriber & Slansky 1981; Hartley & Jones 1997; Schädler *et al.* 2003). Nevertheless, several studies found that palatability is sometimes not affected by those traits (Dudt & Shure 1994; Rowe & Potter 1996; Osier & Lindroth 2001; Shibata *et al.* 2001). Furthermore, the details of the relationship may depend on the specific plant-insect species combination considered (Howard 1990; Hemming & Lindroth 1995), and the relationship may change with environmental factors (Jansen & Stamp 1997). In our study, palatability, relative growth rate and the conversion efficiency measured with the larvae of *S. littoralis* showed the expected negative relationship to the C/N-ratio of the leaf tissue.

We found significant differences in herbivore attack as well as water content of leaves between tree layers. However, we were not able to demonstrate general differences in palatability, growth rate and conversion efficiency between layers. Fortin & Mauffette (2002) found that leaves from the upper layer of sugar maple were more palatable to larvae of a generalist moth, with positive effects on pupal mass and number of eggs. In addition, these leaves were preferred in feeding tests. Only our results from the second year support these findings. In general, little information is available on the variability of herbivore attack as well as leaf palatability within individual trees (Howard 1990; Rowe & Potter 1996; Kause *et al.* 1999; Fortin & Mauffette 2002). Some authors suggested that differences in leaf quality within plants are directly or indirectly related to the effects of solar irradiation (references in Fortin & Mauffette 2002). However, many published experiments used sun and shade leaves from different plants respectively. Our finding of considerable differences among individuals suggests that results in the literature are confounded by those differences.

Table 3. Results of an ANOVA of effects of year, tree individual, and tree layer on the carbon/nitrogen-ratio and water content of leaves. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; df: degrees of freedom, MS: mean square.

Source	df	F values	
		C/N-ratio	Water content
Year	1	235.26 ***	1.07
Tree individual	2	17.00 ***	19.83 ***
Tree layer	1	2.86	411.99 ***
Year x tree individual	2	6.97 **	1.72
Year x tree layer	1	0.50	19.85 ***
Tree individual x tree layer	2	0.95	3.93 *
Year x tree individual x tree layer	2	8.38 **	1.00
Residual	36	[MS = 0.908]	[MS = 0.001]

The ADF-hypothesis suggests that monophagous herbivorous insects may form distinct adaptive groups in response to resource heterogeneity among host individuals. However, the formation of demes requires temporal predictability of host plant quality for the insects. Mopper *et al.* (2000) showed for a leafminer that ten generations may be necessary to form demes. At least across such temporal scales host individuals should have predictable properties to which the insect can respond. Leaf traits, however, show considerable seasonality (McKinnon *et al.* 1998; Osier *et al.* 2000b; Riipi *et al.* 2004) and leaf traits differ between years (Laitinen *et al.* 2000; Covelo & Gallaro 2001). Thus, insect herbivores have to deal with considerable variability in leaf quality within (Kause *et al.* 1999; Haukioja *et al.* 2002) and between years (McPheron *et al.* 1988; Cronin *et al.* 2001). Cronin *et al.* (2001) even showed fluctuations in host-plant preferences and performance between successive years. Although in our study we found significant interactions between tree individuals and year in their effect on leaf traits, few interaction terms were significant for variables measured during the feeding experiments. The relative ranking among individuals was the same in the two years (see also Riipi *et al.* 2004). Leaves of some tree individuals may be in general a better food source than leaves of other individuals. Hence, our study suggests that resource heterogeneity is predictable between years. But remember that about ten or even more generations are required to form demes. Hence more long-term studies are badly needed to draw safe conclusions (see also Cronin *et al.* 2001).

Acknowledgements

We thank Diethart Matthies (University of Marburg, Germany) and Daniel Prati (UFZ Halle, Germany) for statistical advice. We are grateful to our colleagues of the Department of Community Ecology at the UFZ (Halle, Germany). Thanks to Robert Paxton (University of Belfast, United Kingdom) for helpful comments and to Peter Proksch and Gero Eck (University of Düsseldorf, Germany) for providing *Spodoptera littoralis*.

3.6. References

Ayres, M.P., Suomela, J. & MacLean, S.F.Jr. (1987) Growth performance of *Epirrita autumnata* (Lepidoptera: Geometridae) on mountain birch: trees, broods, and tree x brood interactions. *Oecologia* 74: 450–457.

Batzer, H.O., Martin, M.P., Mattson, W.J. & Miller, W.E. (1995) The forest tent caterpillar in aspen stands: distribution and density estimation of four life stages in four vegetation strata. *Forest Science* 41: 99–121.

Covelo, F. & Gallardo, A. (2001) Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain. *Canadian Journal of Botany* 79: 1262–1269.

Cronin, J.T., Abrahamson, W.G. & Craig, T.P. (2001) Temporal variation in herbivore host-plant preference and performance: constraints on host-plant adaptation. *Oikos* 93: 312–320.

Dudt, J.F. & Shure, D.J. (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology* 75: 86–98.

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

Fortin, M. & Mauffette, Y. (2002) The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. *Canadian Journal of Forest Research* 32: 379–389.

Hartley, S.E. & Jones, C.G. (1997) Plant chemistry and herbivory, or why the world is green. In: Crawley, M. J. (Ed.) Plant ecology 2*nd* edition. 284–324. Oxford: Blackwell Science.

Haukioja, E., Ossipov, V. & Lempa, K. (2002) Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata* 104: 125–136.

Hemming, J.D.C. & Lindroth, R.L. (1995) Intraspecific variation in aspen phytochemistry – Effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* 103: 79–88.

Hollinger, D.Y. (1989) Canopy organization and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. *Functional Ecology* 3: 53–62.

Horton, D.R. & Redak, R.A. (1993) Further comments on analysis of covariance in insect dietary studies. *Entomologia Experimentalis et Applicata* 69: 263–275.

Howard, J.J. (1990) Infidelity of leafcutting ants to host plants: resource heterogeneity or defense induction? *Oecologia* 82: 394–401.

Jansen, M.P.T & Stamp, N.E. (1997) Effects of light availability on host plant chemistry and the consequences for behavior and growth of an insect herbivore. *Entomologia Experimentalis et Applicata* 82: 319–333.

Kleiner, K.W. (1991) Stability of phenolic and protein measures in excised oak foliage. *Journal of Chemical Ecology* 17: 1243–1251.

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

Laitinen, M.L., Julkunen-Tiitto, R. & Rousi, M. (2000) Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology* 26: 1609–1622.

Magalhães, S., Janssen, A., Hanna, R. & Sabelis, M.W. (2002) Flexible antipredator behaviour in herbivorous mites through vertical migration in a plant. *Oecologia* 132: 143–149.

Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* 11: 119– 161.

McKinnon, M.L., Quiring, D.T. & Bauce, E. (1998) Influence of resource availability on growth and foliar chemistry within and among young white spruce trees. *Ecoscience* 5: 295–305.

McPheron, B.A., Smith, D.C. & Berlocher, S.H. (1988) Microgeographic genetic-variation in the apple maggot *Rhagoletis pomonella*. *Genetics* 119: 445–451.

Mopper, S., Stiling, P., Landau, K., Simberloff, D. & van Zandt, P. (2000) Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology* 81: 1577–1587.

Osier, T.L. & Lindroth, R.L. (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. *Journal of Chemical Ecology* 27: 1289–1313.

Osier, T.L., Hwang, S.Y. & Lindroth, R.L. (2000a) Effects of phytochemical variation in quaking aspen *Populus tremuloides*clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. *Ecological Entomology* 25: 197–207.

Osier, T.L., Hwang, S.Y. & Lindroth, R.L. (2000b) Withinand between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. *Biochemical Systematics and Ecology* 28: 197–208.

Raubenheimer, D. & Simpson, S.J. (1992) Analysis of covariance: an alternative to nutritional indices. *Entomologia Experimentalis et Applicata* 62: 221–231.

Riipi, M., Haukioja, E., Lempa, K., Ossipov, V., Ossipova, S. & Pihlaja, K. (2004) Ranking of individual mountain birch trees in terms of leaf chemistry: seasonal and annual variation. *Chemoecology* 14: 31–43.

Rowe, W.J. & Potter, D.A. (1996) Vertical stratification of feeding by Japanese beetles within linden tree canopies: selective foraging or height per se? *Oecologia* 108: 459–466.

Schädler, M., Jung, G., Auge, H. & Brandl, R. (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos* 103: 121–132.

Scriber, J.M. & Slansky, F. (1981) The nutritional ecology of immature insects. *Annual Review of Entomology* 26: 183-211.

Shibata, S., Ishida, T.A., Soeya, F., Morino, N., Yoshida, K., Sato, H. & Kinura, M.T. (2001) Within-tree variation in density and survival of leafminers on oak *Quercus dentata*. *Ecological Research* 16: 135-143.

Stamp, N.E. & Bowers, M.D. (1988) Direct and indirect effects of predatory wasps (*Polistes* sp.: Vespidae) on gregarious caterpillars (*Hemileuca lucina*: Saturniidae). *Oecologia* 75: 619–624.

Stamp, N.E. & Bowers, M.D. (1990) Variation in food quality and temperature constrain foraging of gregarious caterpillars. *Ecology* 71: 1031–1039.

Strauss, S.Y. (1990) The role of plant genotype, environment and gender in resistance to a specialist chrysomelid herbivore. *Oecologia* 84: 111–116.

Suomela, J. & Ayres, M.P. (1994) Within-tree and amongtree variation in leaf characteristics of mountain birch and its implications for herbivory. *Oikos* 70: 212–222.

Van Nouhuys, S., Singer, M.C. & Nieminen, M. (2003) Spatial and temporal patterns of caterpillar performance and the suitability of two host plant species. *Ecological Entomology* 28: 193–202

Waldbauer, G.P. (1968) The consumption and utilization of food by insects. *Advances in Insect Physiology* 5: 229–288.

4. Heterogeneity in palatability and utilization of leaves among and within trees of four broad-leaved tree species

Haike Ruhnke, Martin Schädler, Stefan Klotz & Roland Brandl

Manuscript intended for submission to Ecological Entomology

4.1. Abstract

1. Leaves may show intraspecific variability in quality and quantity of certain compounds and, therefore, in their palatability to insect herbivores. As predicted by the adaptive deme formation hypothesis, this may result in fine-scale adaptations of herbivorous insects to host plant individuals.

2. Intraspecific resource heterogeneity and its temporal predictability were tested for leaves of ash, lime, common oak, and sycamore on a small spatial scale. The herbivore attack in the field was estimated as well as leaf traits (contents of carbon, nitrogen and water) and the utilization of leaves by the polyphagous caterpillars of *Spodoptera littoralis* in feeding experiments.

3. Leaf tissue quality varied among and within tree individuals of all four plant species. The level of herbivore attack in the field differed among the tree individuals on lime, oak and sycamore, but not on ash. Within tree individuals, the consumed leaf area in the field varied between the upper and lower layer on all four species. The bioassays in the laboratory revealed differences of herbivore performance among and within tree individuals on all tree species at least for one measured trait. However, the resource heterogeneitywas not always related to the measured leaf traits.

4. The intraspecific pattern of usability of leaf tissue for herbivorous insects changed across years in lime and oak. Therefore, trees of both species might present moving targets for herbivores and hamper fine-scale adaptations. Ash and sycamore individuals may be more suitable hosts for the formation of adapted demes of herbivores. In these species, the pattern of insect performance among tree individuals remained constant across two investigated years.

Keywords: *Acer pseudoplatanus*, *Fraxinus excelsior*, herbivory, intraspecific variation, palatability, *Quercus robur*, resource heterogeneity, *Spodoptera littoralis*, *Tilia cordata*

4.2. Introduction

Leaf traits such as thickness, water content, C/N-ratio and content of secondary compounds may differ both among individuals of a given tree species (Howard, 1990; Suomela & Ayres, 1994; Laitinen *et al*., 2000; Osier *et al*. 2000b) and within an individual tree, e.g. between leaves exposed to the sun and leaves in the shade (Hollinger, 1989; Dudt & Shure, 1994; Henriksson *et al*., 2003). These differences may affect the feeding behaviour and development of insect herbivores (Ayres *et al*., 1987; Howard, 1990; Strauss, 1990; Osier & Lindroth, 2001; Fortin & Mauffette, 2002). It has been suggested that differences in leaf quality among plant individuals lead to the evolution of genetically distinct groups (demes) of herbivorous insects on individual plants within host species (adaptive deme formation hypothesis, Edmunds & Alstad, 1978). The hypothesis of the formation of adaptive demes has been discussed controversially (e.g. Van Zandt and Mopper, 1998; Cobb & Whitham, 1998) and the results of experimental tests have been equivocal. While some studies have demonstrated adaptations to individual hosts, the results of other studies have not supported the hypothesis (see Ruhnke *et al*., 2006).

An important prerequisite for the evolution of adaptive demes is that the differences in leaf quality among individual host plants remain the same for several generations of insects. Because of their longevity, trees therefore represent excellent systems for testing the hypothesis of the formation of adaptive demes. Resource heterogeneity for herbivorous insects has been investigated in several species of trees, including birch (*Betula pubescens*, Ayres *et al*., 1987; Suomela & Nilson, 1994), aspen (*Populus tremuloides*, Hemming & Lindroth, 1995; Osier & Lindroth, 2004), cottonwood (*Populus trichocarpa* x *P. deltoides*, James & Newcombe, 2000), various oak (*Quercus*) species (e.g. Eliason & Potter, 2001; Roslin *et al*., 2006), maple (*Acer* spp., Marquis, 1988; Reynolds & Crossley, 1997; Fortin & Mauffette, 2002), willow (*Salix viminalis* x *S. dasyclados*, Glynn *et al*., 2004) and lime (*Tilia cordata*, Rowe & Potter, 1996). However, little is known about the spatial and temporal variability of the palatability of tree leaves. Moreover, very few studies have related levels of herbivore attack in the field to the palatability and usability of the leaves for insects in laboratory feeding tests, i.e. without the confounding effects of different environmental conditions (Rowe & Potter, 1996).

In the work reported here, intraspecific variability in the quality of leaf tissue for herbivorous insects was studied in ash (*Fraxinus excelsior*), lime (*Tilia cordata*), common oak (*Quercus robur*), and sycamore (*Acer pseudoplatanus*). The study was performed in a mixed flood-plain forest on a small spatial scale. The variation among individuals in leaf damage by herbivores was studied in the field and the utilization of leaves by a polyphagous herbivore in feeding experiments in the laboratory. The following questions were aimed to answer: (1) Does the quality of leaf tissue differ among individual trees and between layers within a tree? (2) Are these differences reflected in different levels of herbivore attack in the field? (3) Do the differences in leaf traits cause variation in leaf consumption and growth of polyphagous insect herbivores in the laboratory? (4) Are differences in palatability consistent across two consecutive years?

By examining central assumptions of the adaptive deme formation hypothesis, the intraspecific resource heterogeneity and its temporal predictability, our results may present a first step to test the applicability of the hypothesis to these model systems.

4.3. Methods

The study area and tree species

The study was performed in Central Germany in the „Leipzig floodplain forest” (city of Leipzig, Saxony, Germany) that covers a total area of circa 1900 ha. Selective felling in the past has led to the actual composition of the canopy that consists of old oak trees (> 250 years old) and younger trees of this and other species (< 130 years old, Morawetz & Horchler, 2004). In spring 2001 a construction crane was set up in the forest, which makes it possible to work in a 1.6 ha area of the canopy. In the crane plot, the vegetation is classified as a typical floodplain forest of the upper alluvial zone (Querco-Ulmetum) on nutrient-rich loamy flood-plain soils (Morawetz & Horchler, 2004). It is rich in species and dominated by ash, sycamore, oak, lime, maple and hornbeam. In this study, four of the dominate tree species were used: Ash (*Fraxinus excelsior*), lime (*Tilia cordata*), common oak (*Quercus robur*), and sycamore (*Acer pseudoplatanus*).

Levels of herbivore attack in the field

At the end of growing season in two consecutive years, in August 2002 and 2003, the levels of herbivory in the field were quantified. Three mature individuals of each tree species that were growing less than 150 m from each other were selected. Within the trees an upper layer consisting of sun leaves and a lower layer of shade leaves were distinguished. In each layer of each tree 25 leaves from each of four branches were collected (200 leaves per tree). Total leaf area was measured after scanning (Sigma Scan Pro 5) and the leaf area consumed by herbivores estimated. Because the specific weight of leaf material differed between the two sampled layers, the consumed leaf area in the upper tree layer was corrected by tree-specific factors. The factors were derived from 20 leaves collected per tree individual and layer in July 2002 and July 2003.

Feeding experiments

The utilization of leaves by an herbivorous insect was examined in laboratory experiments using the same tree individuals and layers as in the study of herbivore attack in the field. However, to avoid potential effects of induced plant defence, four different branches were used. Six leaves of short shoots were sampled per branch. Palatability and usability of leaf tissue were measured in feeding experiments in the laboratory using larvae of the African cotton leafworm (*Spodoptera littoralis* (BOISDUVAL); Lepidoptera: Noctuidae), a polyphagous herbivore. Larvae used in the feeding trials originated from a laboratory breeding on an artificial diet (bean based) to avoid adaptations of the insects to specific trees. To control for any potential differences among larvae originating from different egg batches, individuals from each egg batch were dispersed across experimental treatments. The bioassays were carried out in July 2002 and July 2003. To that time, the foliage was fully mature and in both years of comparable age. After sampling, the leaves were immediately sprayed with deionised water to keep them turgescent, put in plastic bags, and transported in a cooled box to a refrigerator (4 °C). The feeding experiments started a few hours after sampling. One disc of 23 mm diameter per leaf was punched, weighed and placed individually into a Petri dish together with a piece of moistened filter paper. Early third instar larvae of *S. littoralis* were weighed and one larva was added to each Petri dish. The Petri dishes were placed in a climate chamber at 26 °C with 12 h of light. After 24 h the larvae were killed by freezing. The larvae and the remaining leaf material were dried at 60 °C to weight constancy. Initial larval mass in the feeding experiments was converted into dry mass by using a calibration curve obtained from 60 further larvae. Initial fresh mass of leaf discs in the feeding trial was converted into dry mass using the mean water content of leaves for every branch (see below). Leaf consumption of larvae was expressed as mg consumed leaf dry mass in 24 h.

Osier *et al.* (2000a) demonstrated that the growth of caterpillars reared in bags on trees was highly correlated with the growth of larvae reared in the laboratory on leaves of the same tree. Further, foliage kept turgescent under insect-rearing temperature or refrigerated was shown to have stable protein and phenolic compound content for up to 48 h following field sampling (Kleiner, 1991). Hence, our results should represent the field conditions without the confounding effects of microclimatic differences.

Leaf traits

Leaf traits like water, nitrogen and carbon content are known to be related to leaf palatability (Schädler *et al.*, 2003). Thus, these traits were assessed for the foliage of every branch that was used in the feeding trials and at the same time as the bioassays were performed.

Leaf water content was determined by measuring the fresh mass and dry mass of five reference disks for each branch. For the assessment of leaf carbon and nitrogen, a subsample of leaves from every branch was vacuum-dried for 48 h, milled and kept at -20 °C until C and N concentrations were measured using an element analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany).

Data analyses

The analyses were carried out separately for every tree species. For the analyses of herbivore attack in the field, the 25 individual values per branch of consumed leaf area were averaged. To normalize the distribution of residuals, these means were square-root transformed. The effects of year, tree individual and tree layer were tested with a three-way ANOVA (Proc GLM [Version 9.1]; SAS Institute).

For the analyses of the feeding experiments, the six values obtained for each branch were averaged and the effects of year, tree individual and tree layer on leaf consumption and larval performance were analysed by ANCOVA. For the analyses of consumed leaf material and final larval dry mass, initial larval dry mass was used as a covariate. Thus, possibly confounding effects of initial dry mass were removed from the analysis of the effects of the factors of interest and analyses of relative consumption rate and relative growth rate of larvae were obtained (Raubenheimer & Simpson, 1992; Horton & Redak, 1993). Values of initial and final larval dry mass were $\log_e$-transformed in the analyses for the relative growth rate. For the analyses of biomass gain of larvae, consumed leaf material was used as covariate, giving an estimate of the efficiency of conversion of ingested food into body substance (ECI) used by Waldbauer (1968).

Effects of year, tree individual and tree layer on leaf C/N-ratio and water content of the leaves were analysed using three-way ANOVAs with arcsin-square root transformed values of the water content. The relationships between the utilization of leaf material in the bioassays (adjusted means from the ANCOVA for every layer of each individual tree), herbivore attack in the field, leaf C/N-ratio, and leaf water content were analysed by Spearman rank correlations (software “R”, version 2.0.0) of mean values of every trait per tree layer, tree individual and experiment.

Table 1. ANOVA results of effects of year, tree individual, and tree layer on the carbon/nitrogen-ratio and water content of the leaves of four tree species. Levels of significance: $^{(*)}$ $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. MS = Mean square.

	Source	d.f.	F values			
			Ash	Lime	Sycamore	Oak
C/N-ratio	Year	1	92.17 ***	12.04 **	235.26 ***	106.33 ***
	Tree individual	2	7.12 **	11.67 ***	17.00 ***	6.43 **
	Tree layer	1	66.22 ***	21.11 ***	2.86 $^{(*)}$	0.88
	Year x tree individual	2	0.61	9.40 ***	6.97 **	1.88
	Year x tree layer	1	3.70 $^{(*)}$	23.82 ***	0.50	4.97 *
	Tree individual x tree layer	2	9.70 ***	10.60 ***	0.95	1.81
	Year x tree individual x tree layer	2	1.07	0.41	8.38 **	0.39
	Residual	36	[MS = 1.002]	[MS = 0.716]	[MS = 0.908]	[MS = 1.506]
Water content	Year	1	23.87 ***	36.90 ***	1.07	2.04
	Tree individual	2	8.20 **	2.00	19.83 ***	2.62 $^{(*)}$
	Tree layer	1	160.96 ***	589.87 ***	411.99 ***	85.12 ***
	Year x tree individual	2	0.21	2.24	1.72	2.95 $^{(*)}$
	Year x tree layer	1	5.48 *	2.15	19.85 ***	0.19
	Tree individual x tree layer	2	12.99 ***	25.07 ***	3.93 *	0.34
	Year x tree individual x tree layer	2	1.73	1.19	1.00	0.40
	Residual	36	[MS < 0.001]	[MS < 0.001]	[MS < 0.001]	[MS < 0.001]

4.4. Results

Leaf traits

The C/N-ratio of the tree leaves differed among the individual trees in all studied species (mean per individual across the two years: ash 14.1 to 15.4, lime 13.5 to 14.8, oak 16.2 to 17.7, sycamore 16.5 to 18.2, Table 1). Within trees, it varied in ash and lime between the two tree layers, but there was no consistent pattern among the individual trees (significant interactions tree individual x tree layer in both species, Table 1). Across the two years, the level of the C/N-ratio of the leaf tissue varied significantly in all studied tree species. In lime and sycamore, also the pattern of the C/N-ratio among the tree individuals changed across the two years. Further, the pattern of the C/N-ratio within trees between the two layers changed in lime and in oak.

The water content of the leaf tissue differed significantly among individual trees in ash and sycamore (mean per individual across the two years: ash 63.6 % to 65.4 %, sycamore: 63.2 % to 65.9 %, Table 1). In all studied species it was higher in shade leaves (mean lower layer: ash 66.7 %, lime 68.0 %, oak 57.6 %, sycamore 68.1 %) than in sun leaves (mean upper layer: ash 61.8 %, lime 59.3 %, oak 53.4 %, sycamore 60.9 %). The strength of this effect, however, varied among the tree individuals (ash, lime and sycamore) and across years (ash, sycamore).

Table 2. Results of the ANOVA of effects of year, tree individual, and tree layer on the amount of leaf material of four tree species consumed by herbivores in the field. Levels of significance: $^{(*)}$ $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. MS = Mean square.

Source	d.f.	F values			
		Ash	Lime	Sycamore	Oak
Year	1	5.19 *	4.51 *	15.37 ***	22.12 ***
Tree individual	2	0.84	17.65 ***	1.56	8.52 ***
Tree layer	1	5.31 *	137.63 ***	15.08 ***	1.01
Year x tree individual	2	0.98	0.17	3.73 *	3.53 *
Year x tree layer	1	0.53	7.90 **	25.10 ***	10.92 **
Tree individual x tree layer	2	0.52	2.90 $^{(*)}$	4.82 *	2.33
Year x tree individual x tree layer	2	1.15	7.58 **	1.89	0.40
Residual	36	[MS = 4.18]	[MS = 6.44]	[MS = 8.26]	[MS = 6.48]

Levels of herbivore attack in the field

Less than five percent of the total foliage was consumed by herbivores on each of the four tree species. The herbivore attack in the field differed significantly among the individual trees of lime, sycamore and oak, but not in ash (Fig. 1a, Table 2). Within trees, the leaf area consumed differed between the two tree layers in all four tree species (Fig. 1b). There was, however, no consistent effect of layer on herbivore attack on different tree species: In ash, the upper layer of the trees suffered more leaf damage than the lower layer. In contrast, in lime the lower tree layer suffered more leaf damage than the upper layer; though, the effect size differed across years and marginally among tree individuals (Table 2). In sycamore and oak, there was no consistent pattern between the tree layers present. Across the two years, the level of the consumed leaf area varied significantly in all studied tree species (mean 2002/2003: ash 56/38 mm^2/leaf, lime 144/109 mm^2/leaf, sycamore 106/183 mm^2/leaf, oak 127/61 mm^2/leaf). In oak and sycamore, the pattern of the herbivore attack among trees changed across years with different ranking of the tree individuals (Fig. 1a). Further, the pattern of the consumed leaf area between the two tree layers varied in lime, oak and sycamore across the two years (Fig. 1b, Table 2).

Feeding experiments

In the bioassays with *S. littoralis*, the relative consumption rate of the larvae differed among the tree individuals in ash, sycamore and oak (Fig. 2, Table 3), but showed similar patterns in both years. Within trees, it varied between the two tree layers in lime, oak and marginally also in sycamore. The larvae consumed more leaf material from the upper layer in lime and oak (Fig. 2). In sycamore, however, there was no consistent pattern present.

The relative growth rate of the larvae differed among the individuals in all four investigated tree species (Fig. 2, Table 3). Though, the pattern among individuals of oak changed across years. The relative growth rate differed also within tree individuals

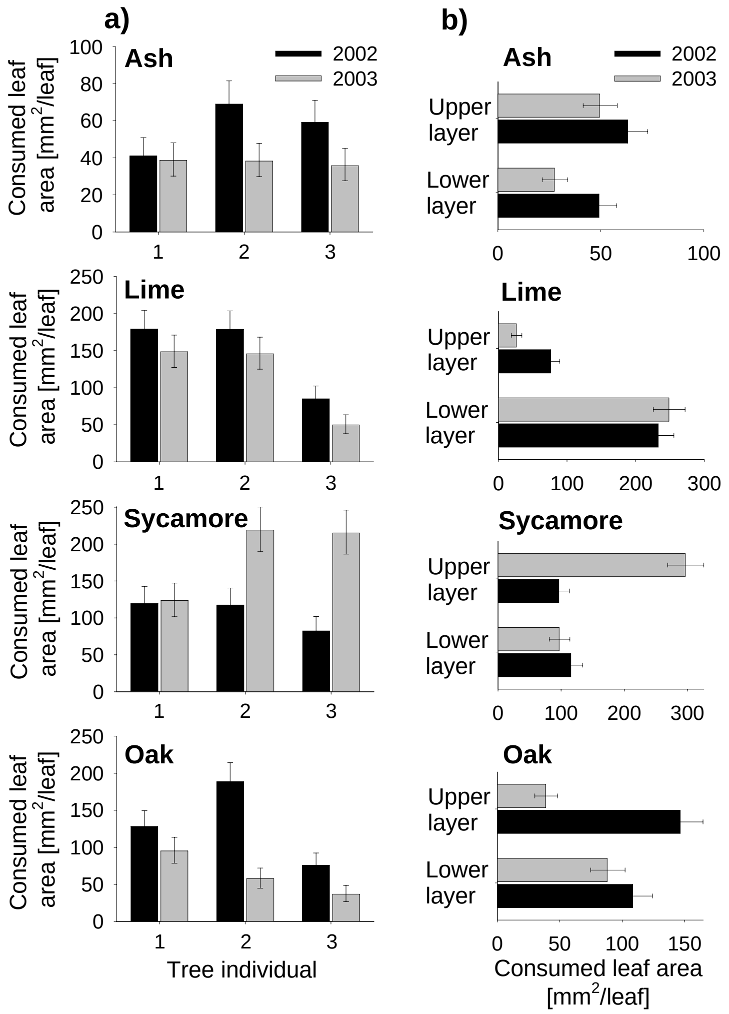


Fig. 1. The consumed leaf area by herbivores in the field. Effects of tree individual (a) and tree layer (b) in ash, lime, sycamore and oak (back-transformed least squares means from ANOVAs ± 1 back-transformed SE).

between the two layers in lime and oak, but showed no consistent pattern across years (Fig. 2, Table 3). Nevertheless, in lime the variation of palatability and insect performance showed much clearer effects within trees than among tree individuals (see mean squares in Table 3).

The efficiency of conversion of ingested food into body substance of the larvae differed among individuals in lime, oak and sycamore (mean per individual across the two years: lime 0.698 to 0.867, sycamore 0.546 to 0.947, oak –0.008 to –0.243, Table 3), but not in ash (mean 0.786). Across years, the pattern among the tree individuals changed just in oak significantly. Within trees, the conversion efficiency of the larvae varied between the tree layers in all four tree species, but it did not show a consistent pattern among the tree individuals (ash, oak) and across the two years (lime, sycamore, oak) (Table 3).

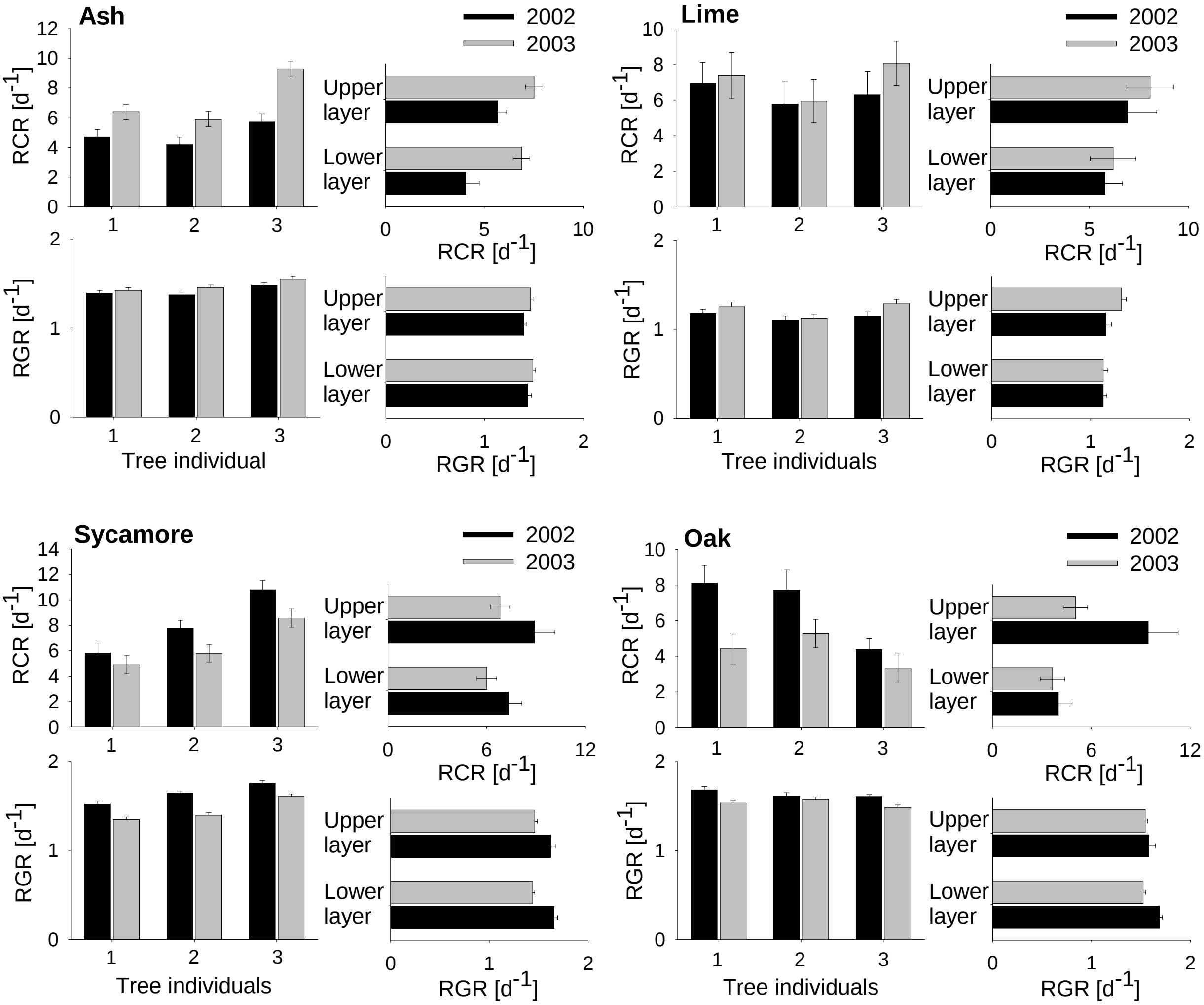


Fig. 2. Relative consumption rate (RCR) and relative growth rate (RGR) of larvae of *S. littoralis* feeding on ash, lime, sycamore or oak measured across 24 hours (least squares means from ANCOVAs ± 1 SE).

Correlations between leaf traits, herbivore attack and bioassays

The analyses did not reveal any consistent pattern of correlations across measured parameters or species. In oak, the herbivore attack in the field was negatively related to the C/N-ratio (Spearman's rank correlation: $r^2 = 0.41$; $P = 0.028$). In lime, the herbivore attack was positively related to the water content of the leaves ($r^2 = 0.54$; $P = 0.009$) as well as negatively to the measured parameters of performance of *S. littoralis* in the bioassays ($r^2 = 0.45$ to 0.69; all $P < 0.03$). In contrast, there was no correlation found for the herbivore attack in the field with leaf traits or results of the bioassays in ash and sycamore.

For the results of the bioassays with larvae of *S. littoralis* on ash, there were no relations found to the leaf traits or herbivore attack in the field. In lime, the relative consumption rate of the larvae was negatively related to the water content of the leaves ($r^2 = 0.28$; $P = 0.084$), but there were again no correlations with the C/N-ratio and also no

Table 3. ANCOVA results of the effects of year, tree individual, and tree layer on relative consumption rate, relative growth rate and conversion efficiency of the larvae of *S. littoralis*. Levels of significance: $^{(*)}$ $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. MS = Mean square.

	Source	d.f.	F values			
			Ash	Lime	Sycamore	Oak
Relative consumption rate	Covariate	1	35.93 ***	38.23 ***	52.16 ***	68.16 ***
	Year	1	17.01 ***	1.35	6.65 *	0.02
	Tree individual	2	15.37 ***	1.95	23.94 ***	3.61 *
	Tree layer	1	2.27	4.98 *	3.02 $^{(*)}$	5.42 *
	Year x tree individual	2	2.10	0.58	0.62	0.75
	Year x tree layer	1	0.39	0.37	0.88	2.21
	Tree individual x tree layer	2	1.33	1.02	0.76	0.66
	Year x tree individual x tree layer	2	0.58	< 0.01	2.58 $^{(*)}$	1.52
	Residual	35	[MS = 1.836]	[MS = 4.843]	[MS = 3.272]	[MS = 3.100]
Relative growth rate	Covariate	1	155.17 ***	271.25 ***	204.23 ***	1001.76 ***
	Year	1	7.60 **	1.06	76.03 ***	47.72 ***
	Tree individual	2	9.24 ***	8.62 ***	41.98 ***	9.51 ***
	Tree layer	1	1.38	22.91 ***	0.93	0.12
	Year x tree individual	2	0.44	1.90	1.19	3.61 *
	Year x tree layer	1	0.19	8.53 **	0.17	4.67 *
	Tree individual x tree layer	2	1.57	0.73	1.65	3.25 $^{(*)}$
	Year x tree individual x tree layer	2	0.04	0.69	3.48 *	0.28
	Residual	35	[MS = 0.006]	[MS = 0.007]	[MS = 0.006]	[MS = 0.003]
Conversion efficiency	Covariate	1	101.66 ***	396.93 ***	424.69 ***	20.95 ***
	Year	1	0.16	11.40 **	52.07 ***	32.85 ***
	Tree individual	2	1.97	6.77 **	15.55 ***	6.33 **
	Tree layer	1	5.58 *	11.85 **	3.48 $^{(*)}$	6.33 *
	Year x tree individual	2	0.77	0.42	1.35	4.62 *
	Year x tree layer	1	< 0.01	16.07 ***	6.59 *	13.23 ***
	Tree individual x tree layer	2	2.94 $^{(*)}$	0.13	1.19	3.30 *
	Year x tree individual x tree layer	2	0.37	1.81	0.98	0.26
	Residual	35	[MS = 0.066]	[MS = 0.016]	[MS = 0.058]	[MS = 0.070]

relations between relative growth rate or conversion efficiency and leaf traits. In sycamore, the relative consumption rate ($r^2 = 0.34$; $P = 0.052$), the relative growth rate ($r^2 = 0.46$; $P = 0.019$) and the conversion efficiency ($r^2 = 0.65$; $P = 0.003$) of larvae of *S. littoralis* were negatively correlated with the C/N-ratio of the leaf tissue, but not with the water content. In oak, the relative consumption rate of the larvae was negatively related ($r^2 = 0.41$; $P = 0.028$) and the relative growth rate ($r^2 = 0.26$; $P = 0.091$) and the conversion efficiency ($r^2 = 0.33$; $P = 0.054$) marginally positive related to the water content of the leaves. In addition, the relative growth rate of larvae was marginally negative related to the C/N-ratio of leaf tissue in oak ($r^2 = 0.31$; $P = 0.067$).

4.5. Discussion

Intraspecific variability in the quality of leaf tissue may affect herbivorous insects considerably (Edmunds & Alstad, 1978). In this study, results of the variability of leaf tissue quality, level of herbivory in the field and insect performance in bioassays are reported from ash, lime, common oak and sycamore across two consecutive years.

1) Leaf traits and their effects on herbivore attack and insect performance

The carbon, nitrogen and water content of leaf tissue are considered as important determinants of leaf palatability (Mattson, 1980; Scriber & Slansky, 1981; Hartley & Jones, 1997; Schädler *et al.*, 2003). Plant palatability is assumed to increase with increasing water content and decreasing C/N-ratio of the leaves (Schädler *et al.*, 2003). The variability of these traits may therefore be predicative for herbivore activity and distribution. In this study, the C/N-ratio differed among the individuals of the investigated tree species and, in addition, the water content of leaves in ash, sycamore and marginally in oak. Across the tree species, only water content showed a predictable pattern of intra-individual variability with a higher content in the leaves of the lower layer. The variability of the C/N-ratio among and within individuals showed an ambiguous pattern which does not allow for any generalisations. Therefore, the analyses of leaf traits may suggest a variability of leaf consumption and herbivory among and within tree individuals, although there is no clear indication toward any general patterns of herbivory.

Relationships between leaf chemical parameters and level of herbivore damage (Haukioja *et al.*, 1985) or insect performance (Osier *et al.* 2000a; Fortin & Mauffette, 2002) are well documented. In our study, however, the level of herbivore attack in the field as well as the insect performance in feeding experiments was only rarely related with the measured leaf traits. A lack of correlation was also documented in several other studies (Dudt & Shure, 1994; Rowe & Potter, 1996; Shibata *et al.*, 2001; Cippolini *et al.*, 2002). Possibly, the measurement of the content of specific phytochemicals is a better predictor of leaf palatability than the C/N-ratio or water content. However, several authors have emphasised that the quality of leaves for herbivorous insects must depend on many variables and even show synergistic effects of all the individual compounds (references in Loeffler, 1993). Further, the relationship between leaf traits and palatability may depend on the specific plant-insect association (Howard, 1990; Hemming & Lindroth, 1995), and may even change with environmental factors, e. g. light availability (Jansen & Stamp, 1997). Therefore, it seems that it is often hardly possible to derive general statements of palatability to herbivores from leaf traits.

2) Herbivore attack in the field

Leaves of different tree individuals were differently damaged by herbivores in three of the four tree species investigated (lime, sycamore and oak). There was no evidence for a general effect of tree layer on herbivore attack. Even if there were some considerable differences of consumed leaf area between tree layers, the magnitude and the direction of this effect varied among tree species, tree individuals and across years.

There are no similar studies known on ash (*Fraxinus*) to the best of our knowledge. Studies on oak (*Quercus*: Eliason & Potter, 2001; Roslin *et al*., 2006), maple (*Acer*: Marquis, 1988; Reynolds & Crossley, 1997) and lime (*Tilia cordata*: Rowe & Potter, 1996) revealed comparable variations in herbivory as mentioned here. Although in some studies the effect of tree layers differed, e.g. on red maple (*Acer rubrum*), percentage leaf area removed was significantly greater in the lower tree layer compared with the upper tree layer (Reynolds & Crossley, 1997) whereas there was no consistent pattern in this study on the related species *A. pseudoplatanus* (sycamore). In lime (*T. cordata*), Rowe & Potter (1996) showed that Japanese beetles (*Popillia japonica*) caused more damage in the upper canopy than in the lower canopy. In the study reported here, however, herbivore attack in the field was strikingly more pronounced in the lower tree layer than in the upper layer of lime trees. It is not clear which factors the different patterns between tree layers in both studies caused.

3) Insect performance in feeding experiments

The bioassays in the laboratory revealed differences of leaf palatability and herbivore performance among and within tree individuals in all four tree species. Though, there was no consistent pattern among the investigated tree species. For example, between the two tree layers the relative growth rate of the larvae varied just in lime and oak. It was higher on leaves from the upper layer in lime, but higher on leaves from the lower layer in oak.

In contrast to the results reported here, Rowe & Potter (1996) found no effect of tree layer in laboratory choice tests with the Japanese beetles (*Popillia japonica*) on lime (*T. cordata*). Fortin & Mauffette (2002) showed in bioassays with the forest tent caterpillar on sugar maple (*Acer saccharum*) increased performance of larvae on leaves from the upper canopy of trees. In this study, however, larvae of *S. littoralis* did not show a clear effect within sycamore trees.

Only for lime, the results of the feeding experiments were related to the level of herbivore attack in the field. In contrast to expectations, they were negatively correlated. Thus, the insect performance in bioassays showed opposite patterns to herbivore attack in the field. In ash, sycamore and oak there was no correlation between the level of herbivore attack in the field and insect performance in feeding experiments with *S. littoralis*. Rowe & Potter (1996) reported a lack of correlation for experiments with a beetle species on lime between results from the field and from the laboratory. A number of factors can alter the expected pattern of herbivory in the field such as microclimate variations (Stamp & Bowers, 1990), predation, parasites (Stamp & Bowers, 1988; Dicke & Grostal, 2001) and migration of the herbivores (Batzer *et al*., 1995; Magalhães *et al*., 2002). Therefore, results from feeding trials in the laboratory might reflect the actual palatability of leaf tissue more precisely than herbivore attack in the field. Moreover, the consumed leaf area in the field is usually caused by a multitude of herbivorous species, whereas in bioassays the palatability and usability of leaf tissue is commonly tested with singly selected herbivore species. However, different herbivorous insects respond differently toward leaf tissue quality (Hemming & Lindroth, 1995; Cippolini *et al*., 2002; Schädler *et al*., 2005; own unpublished data). This variation of response among insect species may also contribute to the often missing correlation between herbivore attack in the field and results with given herbivore species.

4) Temporal predictability of resource heterogeneity and implications for the formation of adaptive demes

Water content and C/N-ratio of leaves did not remain constant across two consecutive years. Though, the water content showed a predictable pattern of intra-individual variability with a higher content in the leaves of the lower layer. This pattern of variability remained constant across tree individuals and years. Senn *et al.* (1992) found that in mountain birch (*Betula pubescens* ssp. *tortuosa*) the water content of leaves of individuals correlated positively between two successive years. This was also the case in ash and sycamore in the study reported here. In contrast, the C/N-ratio of the leaf tissue showed no consistent patterns across years in this study. Osier *et al.* (2000b) showed for quaking aspen (*Populus tremuloides*), however, that concentrations of leaf nitrogen and phytochemicals were highly correlated between two consecutive years. It seems that, unlike the here measured leaf traits, phytochemicals are often correlated between years, suggesting that the phytochemical profiles are specific for individuals (Haukioja *et al.*, 1985; Laitinen *et al.*, 2000; Riipi *et al.*, 2004). This might not be true for all phytochemicals and for proteins and sugars (Riipi *et al.*, 2004). Hence, the palatability of plants in a population and their ranking is not necessarily constant over time.

Levels of herbivore attack in the field and the performance of larvae of *S. littoralis* grown on leaves varied across the two investigated years. Though, the performance of *S. littoralis* among tree individuals of ash and sycamore remained constant and might therefore be predictable. Likewise, the relative ranking of individual trees of mountain birch in terms of larval growth rates of herbivores remained relative constant between two years, in spite of overall differences (Senn *et al.*, 1992; Hanimäki *et al.*, 1995). The host-plant preference of herbivores, however, may be modified between years (Cronin *et al.*, 2001).

Resource heterogeneity among host individuals is an essential precondition for the adaptive deme formation hypothesis of Edmunds & Alstad (1978). Edmunds & Alstad (1978) considered a tree individual as a homogene resource for insects. However, this may often not be the case. Different palatability and usability of leaves within tree individuals may influence the possibility to form locally adapted demes by reducing the amount of available leaf tissue of similar quality. This might not be crucial if the variability within hosts is smaller than among plants, so that tree individuals remain distinct from each other. Roslin *et al.* (2006) showed for an oak–moth system, however, that variation within trees can be significant larger than among them. Together with changes of leaf palatability within and across years it might hamper fine-scale adaptations on certain host species. In contrast, Suomela & Nilson (1994) found that most variation in larval growth of the fifth instar of *Epirrita autumnata* was explained by trees and only a minor part through within-tree variation. Though, the pattern differed for the fourth instar of the same species. There, the variation among ramets was higher than among trees. In the study reported here, the resource heterogeneity for herbivorous insects showed usually clearer effects among than within tree individuals. In lime, however, the variation of usability of leaf tissue showed much clearer effects within trees than among tree individuals. Besides the pronounced variation between tree layers, the pattern of insect performance within trees varied across the two years. This might hamper the opportunity for insects to adapt on a fine-scale. Resource heterogeneity has to be consistent over several years for forming locally adapted demes. At least ten generations may be necessary for forming these demes (Mopper *et al.*, 2000). Results of two consecutive years were considered in this study, which might represent rather a minimum of variation and just an indication of long-term variability. But even then, the pattern of usability of leaf tissue for herbivorous insects changed across years in lime and oak. Therefore, trees of lime and common oak might present moving targets for herbivores and rule out fine-scale adaptations, although resource heterogeneity for herbivorous insects is sufficiently existent in both host species. Ash and sycamore individuals may be more suitable hosts for forming adapted demes of herbivores. In these species, the pattern of insect performance among tree individuals remained constant across two investigated years.

Conclusions

Leaf tissue quality, level of herbivory in the field, as well as leaf palatability and insect performance varied in ash, lime, oak and sycamore. Though, the pattern of herbivory and insect performance was not always related with the measured leaf traits. Resource heterogeneity among tree individuals may be common. Their temporal predictability, however, is not always given and might differ among plant species. The feasibility of herbivorous insects to adapt to individual hosts might therefore vary depending on the specific plant-insect system, e.g. ash and sycamore individuals may be more suitable hosts for local adaptation than lime or oak.

Acknowledgements

This study was done within the *Leipzig Canopy Crane Project.* We thank our colleagues of the Department of Community Ecology at the UFZ (Halle, Germany) for assistance, especially Daniel Prati, Hannelore Jany, Antje Thondorf and Ines Stolpe. We are grateful to Peter Proksch and Gero Eck (University of Düsseldorf, Germany) for providing *Spodoptera littoralis*. Thanks to Peter Horchler and Martin Unterseher, the coordinators for the *Leipzig Canopy Crane Project* (Leipzig, Germany).

4.6. References

Ayres, M.P., Suomela, J. & MacLean, S.F. Jr. (1987) Growth performance of *Epirrita autumnata* (Lepidoptera: Geometridae) on mountain birch: trees, broods, and tree x brood interactions. *Oecologia*, **74**, 450-457.

Batzer, H.O., Martin, M.P., Mattson, W.J. & Miller, W. E. (1995) The forest tent caterpillar in aspen stands: distribution and density estimation of four life stages in four vegetation strata. *Forest Science* **41**, 99-121.

Cipollini, M.L., Paulk, E. & Cipollini, D.F. (2002) Effect of nitrogen and water treatment on leaf chemistry in horsenettle (*Solanum carolinense*), and relationship to herbivory by flea beetles (*Epitrix* spp.) and tobacco hornworm (*Manduca sexta*). *Journal of Chemical Ecology*, **28**, 2377-2398.

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

Cronin, J.T., Abrahamson, W.G. & Craig, T.P. (2001) Temporal variation in herbivore host-plant preference and performance: constraints on host-plant adaptation. *Oikos*, **93**, 312–320.

Dicke, M. & Grostal, P. (2001) Chemical detection of natural enemies by arthropods: An ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1-23.

Dudt, J.F. & Shure, D.J. (1994) The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, **75**, 86-98.

Edmunds, G.F. & Alstad, D.N. (1978) Coevolution in insect herbivores and conifers. *Science*, **199**, 941-945.

Eliason, E.A. & Potter, D.A. (2001) Spatial distribution and parasitism of leaf galls induced by *Callirhytis cornigera* (Hymenoptera: Cynipidae) on pin oak. *Environmental Entomology*, **30**, 280-287.

Fortin, M. & Mauffette, Y. (2002) The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. *Canadian Journal of Forest Research*, **32**, 379-389.

Glynn, C., Ronnberg-Wastljung, A.C., Julkunen-Tiitto, R. & Weih, M. (2004) Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomologia Experimentalis et Applicata*, **113**, 1-14.

Hanimäki, S., Senn, J., Haukioja, E. (1995) The convergence in growth of foliage-chewing insect species on individual mountain birch trees. *Journal of Animal Ecology*, **64**, 543-552.

Haukioja, E., Niemelä, P. & Sirén, S. (1985) Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the mountain birch *Betula pubescens* ssp *tortuosa*. *Oecologia*, **65**, 214-222.

Hartley, S.E. & Jones, C.G. (1997) Plant chemistry and herbivory, or why the world is green. In: Crawley, M.J. (ed.) *Plant ecology*. 2nd ed., pp. 284-324, Blackwell Science, Oxford.

Hemming, J.D.C. & Lindroth, R.L. (1995) Intraspecific variation in aspen phytochemistry - Effects on performance of gypsy moths and forest tent caterpillars. *Oecologia*, **103**, 79-88.

Henriksson, J., Haukioja, E., Ossipov, V., Ossipova, S., Sillanpaa, S., Kapari, L. & Pihlaja, K. (2003) Effects of host shading on consumption and growth of the geometrid Epirrita autumnata: interactive roles of water, primary and secondary compounds. *Oikos*, **103**, 3-16.

Hollinger, D.Y. (1989) Canopy organization and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. *Functuonal Ecology*, **3**, 53-62.

Horton, D.R. & Redak, R.A. (1993) Further comments on analysis of covariance in insect dietary studies. *Entomologia Experimentalis et Applicata*, **69**, 263-275.

Howard, J.J. (1990) Infidelity of leafcutting ants to host plants: resource heterogeneity or defense induction? *Oecologia*, **82**, 394-401.

James, R.R. & Newcombe, G. (2000) Defoliation patterns and genetics of insect resistance in cottonwoods. *Canadian Journal of Forest Research*, **30**, 85-90.

Jansen, M.P.T & Stamp, N.E. (1997) Effects of light availability on host plant chemistry and the consequences for behavior and growth of an insect herbivore. *Entomologia Experimentalis et Applicata*, **82**, 319-333.

Kleiner, K.W. (1991) Stability of phenolic and protein measures in excised oak foliage. *Journal of Chemical Ecology*, **17**, 1243-1251.

Laitinen, M.L., Julkunen-Tiitto, R. & Rousi, M. (2000) Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology*, **26**, 1609-1622.

Loeffler, C.C. (1993) Host plant and habitat effects on behavior, survival, and growth of early instar *Dichomeris leuconotella* (Lepidoptera: Glechiidae), leaf-folders on goldenrods. *Journal of Research on the Lepidoptera*, **32**, 53-74.

Magalhães, S., Janssen, A., Hanna, R. & Sabelis, M.W. (2002) Flexible antipredator behaviour in herbivorous mites through vertical migration in a plant. *Oecologia*, **132**, 143-149.

Marquis, R.J. (1988) Intra-crown variation in leaf herbivory and seed production in striped maple, *Acer pensylvanicum* L. (Aceraceae). *Oecologia*, **77**, 51-55.

Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119-161.

Mopper, S., Stiling, P., Landau, K., Simberloff, D. & van Zandt, P. (2000) Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology*, **81**, 1577-1587.

Morawetz, W., Horchler, P.J. (2004) Leipzig Canopy Crane Project (LAK), Germany. In: Basset, Y., Horlyck, V. & Wright, S. J. (ed.): *Studying forest canopies from above: The International Canopy Crane Network.* United Nations Environment Programme, Editorial Panamericana de Colombia, Bogotá.

Osier, T.L., Hwang, S.Y. & Lindroth, R.L. (2000a) Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. *Ecological Entomology*, **25**, 197-207.

Osier, T.L., Hwang, S.Y. & Lindroth, R.L. (2000b) Within- and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. *Biochemical Systematics and Ecology*, **28**, 197-208.

Osier, T.L. & Lindroth, R.L. (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. *Journal of Chemical Ecology*, **27**, 1289-1313.

Osier, T.L. & Lindroth, R.L. (2004) 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.

Raubenheimer, D. & Simpson, S.J. (1992) Analysis of covariance - an alternative to nutritional indexes. *Entomologia Experimentalis et Applicata*, **62**, 221-231.

Reynolds, B.C. & Crossley, D.A. (1997) Spatial variation in herbivory by forest canopy arthropods along an elevation gradient. *Environmental Entomology*, **26**, 1232-1239.

Riipi, M., Haukioja, E., Lempa, K., Ossipov, V., Ossipova, S. & Pihlaja, K. (2004) Ranking of individual mountain birch trees in terms of leaf chemistry: seasonal and annual variation. *Chemoecology*, **14**, 31-43.

Roslin, T., Gripenberg, S., Salminen, J.P., Karonen, M., O'Hara, R.B., Pihlaja, K. & Pulkkinen, P. (2006) Seeing the trees for the leaves - oaks as mosaics for a host-specific moth. *Oikos*, **113**, 106-120.

Rowe, W.J. & Potter, D.A. (1996) Vertical stratification of feeding by Japanese beetles within linden tree canopies: selective foraging or height per se? *Oecologia*, **108**, 459-466.

Ruhnke, H., Schädler, M., Matthies, D., Klotz, S. & R. Brandl (2006) Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis. *Evolutionary Ecology Research*, **8**, 1039-1048.

Schädler, M., Jung, G., Auge, H. & Brandl, R. (2003) Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos*, **103**, 121-132.

Schädler, M., Roeder, M., Brandl, R. & Matthies, D. (2005) Is palatability of a root hemiparasite influenced by its host species? *Oecologia*, **146**, 227-233.

Shibata, S., Ishida, T.A., Soeya, F., Morino, N., Yoshida, K., Sato, H. & Kinura, M.T. (2001) Within-tree variation in density and survival of leafminers on oak *Quercus dentata*. *Ecological Research*, **16**, 135-143.

Scriber, J.M. & Slansky, F. (1981) The Nutritional Ecology of Immature Insects. *Annual Review of Entomology*, **26**, 183-211.

Senn, J., Hanhimäki, S., Haukioja, E. (1992) Among-tree variation in leaf phenology and morphology and its correlation with insect performance in the mountain birch. *Oikos*, **63**, 215-222.

Stamp, N.E. & Bowers, M.D. (1988) Direct and indirect effects of predatory wasps (*Polistes* sp.: Vespidae) on gregarious caterpillars (*Hemileuca lucina*: Saturniidae). *Oecologia*, **75**, 619-624.

Stamp, N.E. & Bowers, M.D. (1990) Variation in food quality and temperature constrain foraging of gregarious caterpillars. *Ecology*, **71**, 1031-1039.

Strauss, S.Y. (1990) The role of plant genotype, environment and gender in resistance to a specialist chrysomelid herbivore. *Oecologia*, **84**, 111-116.

Suomela, J. & Ayres, M.P. (1994) Within-tree and among-tree variation in leaf characteristics of mountain birch and its implications for herbivory. *Oikos*, **70**, 212-222.

Suomela, J. & Nilson, A. (1994) Within-tree and among-tree variation in growth of *Epirrita autumnata* on mountain birch leaves. *Ecological Entomology*, **19**, 45-56.

Van Zandt, P.A. and Mopper, S. (1998) A meta-analysis of adaptive deme formation in phytophagous insect populations. *American Naturalist*, **152**, 595–604.

Waldbauer, G.P. (1968) The consumption and utilization of food by insects. *Advances in insect physiology*, **5**, 229-288.

5. Is there an efficient physiological adaptation of a generalist herbivore to individual host plants?

Haike Ruhnke, Diethart Matthies & Roland Brandl

Manuscript intended for submission to Physiological Entomology

5.1. Abstract

Adaptations are usually assumed to evolve over many generations. Induction of food preferences, conditioning as well as food imprinting are, however, already documented for herbivorous insects and occur during the developmental period of the larvae. Hence, it might be possible that herbivores are able to adapt physiologically to the different palatability of individual plants within one generation. To our knowledge no previous study has examined the physiological adaptability of herbivorous insects within host species. This ability may also have an effect on forming locally adapted demes (ADF hypothesis). To test for physiological adaptation as well as genetic variation in physiological adaptability, reciprocal transfer experiments with the polyphagous gypsy moth (*Lymantria dispar*) were performed on oak (*Quercus robur*). The results indicate that variation in growth on different oak individuals exists among the families of gypsy moth. Even though the parents were reared on artificial diet for generations, the genetic variation among the families is an important factor for the utilization of different oak individuals. However, there was no significant effect of transfer among individual oaks present on the relative growth rate of the larvae. Therefore, the results do not support the idea of physiological adaptation of a generalist herbivore to host individuals. Further investigations are required to draw a general conclusion of physiological adaptabilities of herbivorous insects.

Keywords: herbivory, intraspecific variation, *Lymantria dispar*, physiological adaptation, plant-insect interactions, relative growth rate.

5.2. Introduction

Adaptations on evolutionary time scales can be defined as conformity of the organism and the environment (Pianka, 2000). Adaptations may be due to differences in the genetic programme but also due to plasticity in physiological and behavioural traits (e.g. Leclaire & Brandl, 1994). Phenotypic plasticity itself has a genetic basis (Scheiner, 1993; Windig, 1994; Mori *et al.*, 2005). In herbivorous insects, adaptations range from adaptations to host species characterized by certain defence strategies (e.g. Scriber, 2002) down to special fine-tuned adaptations to individual host plants (adaptive deme formation; Edmunds & Alstad, 1978).

A precondition for fine-tuned adaptations is the ability of the herbivorous insects to distinguish among host individuals and to respond accordingly to the specific defensive and nutritional characteristics of the host individual. This is well documented for some host-herbivore-systems (e.g. Glynn *et al.*, 2004; Osier & Lindroth, 2004; Ruhnke *et al.*, in press). The adaptive deme formation hypothesis states that insect populations respond to the resource heterogeneity among host individuals by evolving genetically distinct lineages adapted to one particular host individual - the locally adapted demes (Edmunds & Alstad, 1978). However, tests of the hypothesis were inconclusive (e.g. Cobb & Whitham, 1998; Ruhnke *et al.*, 2006). A reason could be that leaf quality of host individuals is not always consistent across years (Laitinen *et al.*, 2000; Covelo & Gallardo, 2001). This challenge might be evaded by efficient and expeditious adaptations of the insects within one generation, in the study reported here called physiological adaptation. These physiological adaptations should not depend on heritability across generations of insects and should also be possible if the environment changes across years.

The exposure of larvae to specific stimuli of the diet can alter the responsiveness of taste cells (Jermy *et al.*, 1968; Schoonhoven, 1969; Glendinning *et al.*, 1999), which may lead to changes in behaviour (Blaney *et al.*, 1986). Even the composition and concentration of enzymes in the midgut change with the diet (Mainguet *et al.*, 2000). In oliphagous and polyphagous herbivores feeding on a particular host plant can induce a strong preference for that plant species (Jermy *et al.*, 1968). Among lepidopterans induced, and therefore phenotypic, preference is assumed to be common (Bernays & Weiss, 1996). Clearly, insects have sufficient plasticity to respond to the resource quality of hosts. Nevertheless, we are not aware of any study which addressed the issues of phenotypic response of herbivorous insects to variations in resource quality among host individuals. Therefore, transfer experiments with the polyphagous gypsy moth (*Lymantria dispar*) were performed on oak individuals (*Quercus robur*) to test for physiological adaptation as well as genetic variation in phenotypic plasticity.

5.3. Methods

The study was carried out in the ‘Leipzig flood-plain forest’ (city of Leipzig, Saxony, Germany) where a canopy crane was installed in 2001. The crane allowed to work with a gondola in the top of the trees and to investigate the canopy within an area of 1.6 ha. Around the crane, ash (*Fraxinus excelsior*), oak (*Quercus robur*), sycamore (*Acer pseudoplatanus*), lime (*Tilia cordata*), and hornbeam (*Carpinus betulus*) dominate the tree layer.

To test physiological adaptation as well as genetic variation in phenotypic plasticity, reciprocal transfer experiments with the phytophagous gypsy moth (*Lymantria dispar* (L.); Lepidoptera: Lymantriidae) were performed in 2003. Although a generalist (Rossiter, 1987), the larvae prefer oak leaves. For the experiment, three individuals of the common oak (*Quercus robur* L.) were selected for a full factorial experimental design. Two of the oaks were neighbours and the third grew circa 130 m away. Preliminary and unpublished experiments showed that the amount of consumed leaf tissue by as well as the relative growth rate of caterpillars of the gypsy moth differed among these three individuals.

For the experiments, 11 families of full sibs (Doane, 1968) of gypsy moth larvae were used. The egg batches originated from a laboratory stock held on artificial diet (wheat-germ based). Newly hatched larvae of each family were split into three groups of about equal numbers. Each group was then reared on leaves of a different oak individual (rearing tree) in a climate chamber at 22 °C. Every two days, freshly collected leaves were

Table 1. Results of an ANCOVA of the effects of family, rearing tree, novel tree, and transfer on the relative growth rate of the larvae of *Lymantria dispar* (effects after incorporating the covariate). We partitioned the sum of squares of the interaction rearing tree x novel tree into one part which tests effect of transfer to leaves from a new tree *vs*. leaves from the same tree and a part which summarizes all other effects (rest). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Term no.	Source of variation	d.f.	MS	Error term	*F*
1	Initial larval mass	1	4.517	9	54.01 ***
2	Family	10	0.406	9	4.86 ***
3	Rearing tree	2	1.554	5	5.47 *
4	Novel tree	2	0.186	6	3.67 *
5	Family x rearing tree	20	0.284	9	3.40 ***
6	Family x novel tree	20	0.051	9	0.60
7	Rearing tree x novel tree	4	0.124	8	1.98
	Transfer	1	0.176	8	2.81
	Rest	3	0.106	8	1.70
8	Family x rearing tree x novel tree	40	0.063	9	0.75
9	Residual	1061	0.084		

provided. After about two weeks, reciprocal transfer experiments were performed with a total of 1161 third instar larvae. Each group of larvae was subdivided into three subgroups of equal numbers of larvae as far as possible (resulting in nine subgroups containing 95 to 164 larvae). Each larva was weighed and placed individually into a Petri dish on moist filter paper. The first subgroup received leaves of their rearing tree *ad libitum* (control), the other two subgroups received leaves of the two other tree individuals (novel trees). Larvae were kept in a climate chamber at 26 °C and 12:12 h light:darkness for 24 h. Then, larvae were killed by freezing, dried at 60 °C to weight constancy and weighed again. Because of the large number of larvae, it was not possible to perform all experiments in one day. Hence, the hatching time of larvae was staggered and the experiments were carried out over a period of 10 days between the 27th of June and the 7th of July 2003.

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 $\log_e$-transformed. The effects of family, rearing tree, and novel tree on final larval dry mass were analysed by a three-way ANCOVA (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, the relative growth rate of the larvae was evaluated (Raubenheimer & Simpson, 1992; Horton & Redak, 1993). In the statistical model ‘family’ was considered as a random factor and ‘rearing tree’ and ‘novel tree’ as fixed factors. Furthermore, the interaction rearing tree x novel tree was partitioned into a

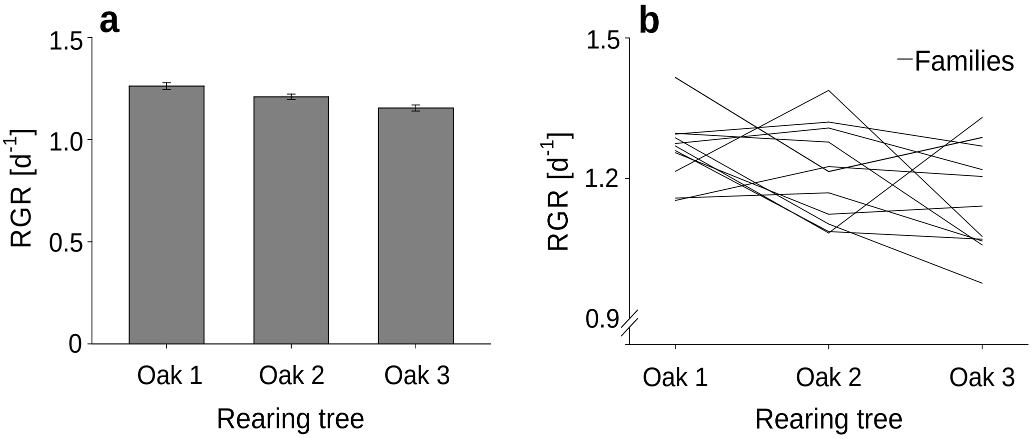


Fig. 1. (a) Mean effect of the rearing tree on the relative growth rate of larvae of *Lymantria dispar*. (b) Response of the growth rate of larvae from different families to the three rearing trees. We give relative growth rates (RGR) measured across 24 hours from an ANCOVA (least squares means ± 1 SE).

contrast transfer *versus* no transfer 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 (error terms see Table 1).

5.4. Results

The relative growth rate of the larvae varied among families of gypsy moth (least squares means: 1.12 to 1.31/day, Table 1). This indicates genetic variation among families in traits influencing growth of larvae. Moreover, the relative growth rate of the larvae varied depending on the oak individual on which they had been reared for the first two weeks (Fig. 1a). These differences were small, but consistent. This shows that the leaves of the three oak individuals differed in their quality as food for the larvae and that these differences continued to influence the growth rate of the larvae during the one-day experiment. These influences differed among the 11 families (significant interaction family x rearing tree, Fig. 1b).

The identity of the host tree (novel tree), from which the leaves for the transfer experiment were taken also affected the relative growth rate of the larvae. Again this indicates that the individual trees differed in the quality of their leaves for gypsy moth. However, there was no significant interaction between the effects of the rearing tree and that of the novel tree. In particular, during the experiment larvae did not grew faster on leaves from the tree they had been feeding on for the preceding two weeks than on leaves from a new tree (transfer contrast in Table 1). Thus, there was no evidence for physiological adaptation of the larvae to the leaves of individual trees.

5.5. Discussion

The results of the reciprocal transfer experiments show that the oak individuals differ in the quality of their leaves for the larvae of gypsy moth. Further, phenotypic variation in growth on different oak individuals exists among the families of larvae (significant interaction family x rearing tree), even though the parents were reared on artificial diet for generations. Hence, there was genetic variation among families in their reaction to host individuals. The transfer, however, did not show an effect on the relative growth rate of the larvae of gypsy moth. Therefore, the described experiment provides no evidence for efficient physiological adaptation to a particular host individual during early larval development.

Induction of food preferences of larvae of herbivorous insects is a well known phenomenon (e.g. Bernays & Weiss, 1996). Larvae of insects can be conditioned to a certain host species (Schoonhoven, 1967), i.e. larvae show an increased response to a specific host. Even food imprinting is, as a special type of learning, documented (Szentesi & Jermy, 1990) where larvae after a period of experience rather starve to death than feed on a different host plant. Further, phenotypic plasticity in the reaction to environmental factors is known from several species (e.g. Peppe & Lomonaco, 2003; Ayrinhac *et al.*, 2004). Together, these findings suggest that there should be ample opportunities of physiological adaptation toward specific defensive and nutritional characteristics of a particular host individual. However, although the larvae showed phenotypic plasticity, the experiments failed to demonstrate physiological adaptation to a specific individual host.

It might be that monophagous and oligophagous herbivores respond to host individuals in a different way than polyphagous species do. Lajeunesse & Forbes (2002) found that parasites, including herbivores, with a broad host range are less likely to show adaptations than parasites with a narrow host range. In contrast, Hanson (1976) found that polyphagous herbivores are more often conditioned to certain host species than species with a more restrictive diet. However, Hanson (1976) regarded that correlation as spurious rather than causal. In tests of the adaptive deme formation hypothesis, using a monophagous and an oligophagous sawfly (Hymenoptera: Tenthredinidae), Ruhnke *et al.* (2006) found no evidence for adaptation as well. The design of those tests did not allow distinguishing between genetic, parental, and physiological effects.

The quality of leaf tissue varies in time and may undergo seasonal changes (e.g. Kause *et al.*, 1999; Ruusila *et al.*, 2005). Thus, plants represent moving targets (Hartley & Jones, 1997). Ruusila *et al.* (2005) found that temporal changes in the quality of leaf tissue of birch explained more of the variance in leaf consumption and growth of *Epirrita* larvae than did the identity of the host tree. Such variation may have two effects. First, temporal variation may prevent the evolution of genetic adaptation. Secondly, temporal variation may lead to a general plasticity to cope with this variation if the variation occurs within the time scale of the development of the insect. It is suggested that within the reaction norm of the family larvae in changing environments respond directly to quality of leaf tissue without fine-tuned adaptations. This might enable herbivorous insects to cope with changing conditions.

Physiological adaptation during larval development has received little attention by entomologists. As far as we know this is the first study of physiological adaptability of an herbivorous insect within a host species. The results do not support the idea of physiological adaptation of a generalist herbivore to host individuals. Therefore, it is suggested that insect populations respond to variation in host quality across individuals and across time directly without physiological adaptations and by differences in the reaction norm of insect families. It is not clear, however, whether the results of the present study represent a general pattern in herbivorous insects. Generalisations have to await the results of further studies.

Acknowledgements

This study was done within the *Leipzig Crane Project* and the EU funded *Network of Excellence* EVOLTREE. We thank our colleagues of the Department of Community Ecology at the UFZ (Halle, Germany) for assistance. We are grateful to Andreas Linde and Dörte Goertz (University of Applied Sciences Eberswalde, Germany) for providing the insects. Thanks to Peter Horchler and Martin Unterseher, the coordinators for the *Leipzig Crane Project* (Leipzig, Germany).

5.6. References

Ayrinhac, A., Debat, V., Gibert, P., Kister, A.G., Legout, H., Moreteau, B., Vergilino, R. & David, J.R. (2004) Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Functional Ecology*, **18**, 700-706.

Bernays, E.A. & Weiss, M.R. (1996) Induced food preferences in caterpillars: The need to identify mechanisms. *Entomologia Experimentalis et Applicata*, **78**, 1-8.

Blaney, W.M., Schoonhoven, L.M. & Simmonds, M.S.J. (1986) Sensitivity variations in insect chemoreceptors - a review. *Experientia*, **42**, 13-19.

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

Covelo, F. & Gallardo, A. (2001) Temporal variation in total leaf phenolics concentration of *Quercus robur* in forested and harvested stands in northwestern Spain. *Canadian Journal of Botany*, **79**, 1262-1269.

Doane, C.C. (1968) Aspects of mating behavior of the gypsy moth. *Annals of the Entomological Society of America*, **61**, 768-773.

Edmunds, G.F. & Alstad, D.N. (1978) Coevolution in insect herbivores and conifers. *Science*, **199**, 941-945.

Glendinning, J.I., Ensslen, S., Eisenberg, M.E. & Weiskopf, P. (1999) Diet-induced plasticity in the taste system of an insect: Localization to a single transduction pathway in an identified taste cell. *Journal of Experimental Biology,* **202**, 2091-2102.

Glynn, C., Ronnberg-Wastljung, A.C., Julkunen-Tiitto, R. & Weih, M. (2004) Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomologia Experimentalis et Applicata*, **113**, 1-14.

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

Hartley, S.E. & Jones, C.G. (1997) Plant chemistry and herbivory, or why the world is green. In: Crawley, M.J. (ed.) *Plant ecology*. 2nd ed., pp. 284-324, Blackwell Science, Oxford.

Horton, D.R. & Redak, R.A. (1993) Further comments on analysis of covariance in insect dietary studies. *Entomologia Experimentalis et Applicata,* **69**, 263-275.

Jermy, T., Hanson, F.E. & Dethier, V.G. (1968) Induction of specific food preference in lepidopterous larvae. *Entomologia Experimentalis et Applicata*, **11**, 211-230.

Kause, A., Ossipov, V., Haukioja, E., Lempa, K., Hanhimaki, S. & Ossipova, S. (1999) Multiplicity of biochemical factors determining quality of growing birch leaves. *Oecologia,* **120**, 102-112.

Laitinen, M.L., Julkunen-Tiitto, R. & Rousi, M. (2000) Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology,* **26**, 1609-1622.

Lajeunesse, M.J. & Forbes, M.R. (2002) Host range and local parasite adaptation. *Proceedings of the Royal Society of London Series B - Biological Sciences*, **269**, 703-710.

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

Mainguet, A.M., Louveaux, A., El Sayed, G. & Rollin, P. (2000) Ability of a generalist insect, Schistocerca gregaria, to overcome thioglucoside defense in desert plants: tolerance or adaptation? *Entomologia Experimentalis et Applicata*, **94**, 309-317.

Mori, T., Hiraka, I., Kurata, Y., Kawachi, H., Kishida, O. & Nishimura, K. (2005) Genetic basis of phenotypic plasticity for predator-induced morphological defenses in anuran tadpole, *Rana pirica*, using cDNA subtraction and microarray analysis. *Biochemical and Biophysical Research Communications,* **330**, 1138-1145.

Osier, T.L. & Lindroth, R.L. (2004) 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.

Peppe, F.B. & Lomonaco, C. (2003) Phenotypic plasticity of *Myzus persicae* (Hemiptera : Aphididae) raised on *Brassica oleracea L. var. acephala* (kale) and *Raphanus sativus* L. (radish). *Genetics and Molecular Biology*, **26**, 189-194.

Pianka, E.R. (2000) *Evolutionary ecology*. 6th ed., Addison Wesley Longman, San Francisco.

Raubenheimer, D. & Simpson, S.J. (1992) Analysis of covariance - an alternative to nutritional indexes. *Entomologia Experimentalis et Applicata*, **62**, 221-231.

Rossiter, M. C. (1987) Genetic and phenotypic variation in diet breadth in a generalist herbivore. *Evolutionary Ecology*, **1**, 272-282.

Ruhnke, H., Brändle, M., Klotz, S., Brandl, R. & Schädler, M. (in press) Plant-animal interactions in the canopy: Intraspecific variability in herbivory on sycamore (*Acer pseudoplatanus* L.). In: Morawetz, W., Arndt, E., Klotz, S. & Unterseher, M. (eds) *The canopy of a temperate floodplain forest: Results from 5 years of research at the Leipzig Canopy Crane*. (in press).

Ruhnke, H., Schädler, M., Matthies, D., Klotz, S. & Brandl, R. (2006) Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis. *Evolutionary Ecology Research*, **8**, 1039-1048.

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

Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, **24**, 35-68.

Schoonhoven, L.M. (1967) Loss of hostplant specificity by Manduca sexta after rearing on an artificial diet. *Entomologia Experimentalis et Applicata*, **10**, 270-272.

Schoonhoven, L.M. (1969) Sensitivity changes in some insect chemoreceptors and their effect on food selection behavior. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen C*, **72**, 491-498.

Scriber, J.M. (2002) Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits. *Entomologia Experimentalis et Applicata*, **104**, 217-235.

Szentesi, A. & Jermy, T. (1990) The role of experience in host plant choice by phytophagous insects. In: Bernays, E.A. (ed.) *Insect-plant interactions*. pp. 39-74, CRC Press, Boca Raton, Florida.

Windig, J.J. (1994) Reaction norms and the genetic basis of phenotypic plasticity in the wing pattern of the butterfly *Bicyclus anynana*. *Journal of Evolutionary Biology*, **7**, 665-695.

6. Are sawflies adapted to individual host trees? A test of the adaptive deme formation hypothesis

Haike Ruhnke, Martin Schädler, Diethart Matthies, Stefan Klotz & Roland Brandl

Evolutionary Ecology Research (2006) **8**, 1039-1048

6.1. Abstract

Question: Are populations of two sawfly species adapted to individual host trees?
Hypothesis: The adaptive deme formation hypothesis suggests that selection may lead to populations (demes) adapted to host individuals.
Organisms: Larvae of the black sawfly *Tomostethus nigritus* and the privet sawfly *Macrophya punctumalbum* (Hymenoptera: Tenthredinidae) living on ash (*Fraxinus excelsior*).
Methods: We reciprocally transferred larvae of the two sawfly species to leaves of different ash individuals of a floodplain forest in Central Germany. After 24 h, we assessed the relative growth rate of the larvae.
Conclusions: There was no effect of the transfer between host individuals on the relative growth rates of sawfly larvae. Based on our results, together with published literature, we conclude that under certain conditions local adaptations may fine-tune herbivore populations to individual hosts. However, the formation of adaptive demes does not appear to be a general phenomenon in herbivorous insects.

Keywords: adaptive deme formation, feeding trial, herbivory, intraspecific variation, local adaptation, relative growth rate, *Macrophya punctumalbum*, *Tomostethus nigritus*.

6.2. Introduction

Several studies have demonstrated genetic variation within and among populations of phytophagous insects (Mopper, 1996). Genetic structure may occur even among herbivorous insects occupying different branches of the same tree (Alstad and Corbin, 1990). The mechanisms behind this structure, however, are poorly understood. One possible mechanism is the adaptation of insects to host individuals.

The quality of leaf tissue differs not only among plant species, but varies also among plant individuals within a species (Howard, 1990; Laitinen *et al.*, 2000; Osier *et al.*, 2000b). This variation in the quality of plant tissue may influence feeding behaviour and development of insect herbivores (Ayres *et al.*, 1987; Howard, 1990; Fortin and Mauffette, 2002; Glynn and Herms, 2004). Based on transfer experiments with a herbivorous insect species, Edmunds and Alstad (1978) formulated the adaptive deme formation hypothesis. According to this hypothesis, selection forms populations (demes) of herbivorous insects adapted to the specific defensive and nutritional characteristics of a host plant individual. Of course, such selection can only work within a framework of preconditions, set by the host as well as the herbivore.

First, the life span of a host individual must sustain several generations of the herbivore. Therefore, adaptive demes are most likely to occur in insects living on trees. Another important precondition is predictability of host quality. At least the ranking of the quality of leaf tissue across individuals should remain constant over time. One important attribute of the insect's biology, which might influence the formation of adaptive demes, is mobility. Gene flow between insect populations on individual trees counteracts local adaptations. The particular defence system of a host individual may result in physiological trade-offs in the insect, which may lead to disadvantages on other host individuals. In turn, this will select for a reduced gene flow between insect populations on different host individuals, which would reinforce the formation of genetically distinct groups [adaptive demes (Edmunds and Alstad, 1978)]. However, low gene flow among populations may also preclude adaptation to host plants by limiting genetic variation upon which selection can act (Slatkin, 1987). Besides mobility, the breeding system and the feeding mode of herbivorous insects may affect the formation of adaptive demes. In a meta-analysis, however, van Zandt and Mopper (1998) found no evidence that mobility constrained local adaptation. In addition, they found no convincing effect of the feeding mode (exophagous *vs*. endophagous) or the breeding system on the formation of adaptive demes.

Tests of the adaptive deme formation hypothesis failed to produce consistent results (van Zandt and Mopper, 1998, table 2). Nevertheless, based on their meta-analysis van Zandt and Mopper (1998) suggested that endophagous, parthenogenetic, and diplodiploid herbivorous insects are most likely to form adaptive demes. But different authors have arrived at very different conclusions. Whereas Boecklen and Mopper (1998) suggested that adaptive deme formation is an important evolutionary phenomenon, Cobb and Whitham (1998) doubted that adaptations to individual trees occur at all. We tested the adaptive deme formation hypothesis for populations of the black sawfly *Tomostethus nigritus* and the privet sawfly *Macrophya punctumalbum* in a floodplain forest in Central Germany. These two species differ in biological characteristics, which should affect the formation of locally adapted demes. The parthenogenetic *M. punctumalbum* should show stronger adaptation to host individuals than the haplodiploid and sometimes outbreaking species *T. nigritus* (cf. Price *et al.*, 1990; van Zandt and Mopper, 1998).

6.3. Methods

The study was carried out in the 'Leipzig floodplain forest' (city of Leipzig, Saxony, Germany). In spring 2001, a construction crane was installed in the forest. Equipped with a gondola, the crane allowed us to work in the canopy so that we could investigate mature tree individuals within an area of 1.6 ha. The vegetation around the crane is a typical floodplain forest of the upper alluvial zone on nutrient-rich loamy soils [Querco-Ulmetum (Morawetz and Horchler, 2004)]. The forest is rich in plant species. The dominant tree species are ash (*Fraxinus excelsior*), oak (*Quercus robur*), sycamore (*Acer pseudoplatanus*), lime (*Tilia cordata*), and hornbeam (*Carpinus betulus*).

The experiments were performed with larvae of the black sawfly *Tomostethus nigritus* (F.) (Hymenoptera: Tenthredinidae, Blennocampinae) and the privet sawfly *Macrophya punctumalbum* (L.) (Hymenoptera: Tenthredinidae, Tenthredininae) on ash (*Fraxinus excelsior*). These sawfly species are univoltine. Females of the two species oviposit under the epidermis of leaves. The larvae are, however, solitary and free-feeding leaf chewers.

Tomostethus nigritus appears to be monophagous on ash (Lorenz and Kraus, 1957; Mrkva, 1965) and has a haplodiploid breeding system. One female can lay 80–120 eggs, which are placed singly into young leaves (Mrkva, 1965). Larvae develop fairly fast. Gradations of the species are known to defoliate ash populations (Mrkva, 1965). However, no outbreaks have been observed in the 'Leipzig floodplain forest' during the last 40 years (A. Sickert and L. Fischer, personal communication). *Macrophya punctumalbum* feeds on several species of the family Oleaceae, in particular on *Fraxinus excelsior* and *Ligustrum vulgare* (Hoebeke and Johnson, 1985; Chevin, 1995). Males of *M. punctumalbum* are extremely rare. The species is therefore considered to be parthenogenetic (Hoebeke and Johnson, 1985; Chevin, 1995). The females usually oviposit groups of about 2–8 eggs into fully expanded leaves. Compared with *T. nigritus*, *M. punctumalbum* larvae have a slow growth rate. Outbreaks are not known for *M. punctumalbum*.

We performed reciprocal transfer experiments with larvae of the two species of sawflies collected from individual host trees. The ash trees sampled during our study were mature (height 29–34 m) and showed phenotypic variation in bud burst (personal observations). Furthermore, in a preliminary study we found significant differences in the palatability of leaf tissue among individual ash trees of our study area using a generalist herbivorous insect (unpublished data). We collected eggs and early instars of the sawflies from the canopy of several ash individuals within the crane area. Larvae were reared in the laboratory on leaves collected from the same branch on which we had sampled the particular insect individual. Every 2 days we provided freshly collected leaves. When the larvae had reached the third larval stage, we reciprocally transferred them between leaves of certain pairs of ash trees. Half of the larvae remained as controls on the original trees. We used two pairs of trees and 339 larvae for the experiments with *T. nigritus* and four pairs of trees and 1550 larvae for the experiments with *M. punctumalbum*. The distance between the ash individuals of a pair was about 30 m and 90 m respectively for the trees used in the experiments with *T. nigritus*. For *M. punctumalbum*, the distance between ash individuals of a pair was 10 m (one pair) and about 130 m (the other three pairs).

The experiments with *T. nigritus* were carried out between 24 May and 9 June 2004 in a climate chamber (18 °C and 16 h light), and with *M. punctumalbum* between 15 June and 5 July 2004 (22 °C and 16 h light). Before the experiments, larvae were weighed and placed individually in petri dishes with moist filter paper. Non-transferred larvae were provided *ad libitum* with leaves from the branch on which they had been sampled. Transferred larvae received leaves of the corresponding novel tree individual of the tree pair. After 24 h, the larvae were re-weighed.

Before all analyses, initial and final mass of larvae were log-transformed. The effects of tree pair, natal tree, novel tree, and home versus away on larval mass at the end of the experiment were analysed by analysis of covariance [ANCOVA; Proc GLM (Version 8.02); SAS Institute]. Initial larval mass had a significant effect on the growth of larvae for both sawfly species and was therefore used as the covariate. By using Type I sums of squares, the confounding effects of initial larval mass were removed. Thus, after removing the effects of initial mass, we obtained for our analyses an estimate of the relative growth rate of the larvae (Raubenheimer and Simpson, 1992; Horton and Redak, 1993). In our statistical model, the factor 'tree pair' tested the effect of the specific tree pairs, 'natal tree' the genetic differentiation of the sawfly populations between trees, and ‘novel tree’ the quality of the ash individuals. The factor ‘home versus away’ tested whether larvae were adapted to the tree on which they had been sampled.

We decided to measure the growth of larvae in the laboratory, not in the field, because microclimatic differences between host individuals could have compromised the test whether differences in host quality had an effect on the growth rate of insect larvae. A drawback of such laboratory experiments, however, is that we had to remove leaves from the tree, which could have affected tissue quality. However, Osier *et al.* (2000a) showed that the growth of caterpillars reared in bags on trees was highly correlated with the growth of larvae reared in the laboratory with leaves of the same tree. Furthermore, over 2 days, leaves removed from the tree do not show significant changes in nutritional quality (Kleiner, 1991).

6.4. Results

Larvae of *M. punctumalbum* sampled from different tree individuals had different relative growth rates (ANCOVA; Table 1, Fig. 1a). This indicates genetic differences among populations living on different individuals of host trees. However, we found no adaptation to the leaves of the tree on which larvae were sampled. The transfer of larvae from one ash individual to another had no significant negative effect on their relative growth rate (ANCOVA; Table 1, Fig. 1b). Instead, sawfly larvae on the four tree pairs tended to respond to the transfer differently (ANCOVA, interaction of tree pair and home versus away; Table 1). Growth of the larvae of *M. punctumalbum* on leaves of novel trees was not different from that of larvae on leaves of the trees they originated from.

For *T. nigritus*, the relative growth rate of sawflies from different tree individuals was not significantly different (ANCOVA; Table 1). However, the identity of the novel host tree affected the relative growth rate of the larvae (ANCOVA; Table 1, Fig. 1c). This indicates that the quality of leaves differed between ash individuals. Again, the transfer of

Table 1. Results of two analyses of covariance of the effects of tree pair, natal tree, novel tree, and transfer (home vs. away) on the relative growth rate (effect after incorporating the covariate) of the larvae of *Macrophya punctumalbum* and *Tomostethus nigritus*.

	M. punctumalbum			*T. nigritus*		
Source of variation	*df*	MS	*F*	*df*	MS	*F*
Initial larval mass	1	119.099	5730.95 ***	1	16.995	1396.02 ***
Tree pair	3	0.099	4.78 **	1	0.001	0.09
Natal tree	4	0.528	25.41 ***	2	0.016	1.36
Novel tree	4	0.031	1.51	2	0.100	8.21 ***
Home vs. away	1	< 0.001	0.04	1	< 0.001	0.01
Tree pair x home vs. away	3	0.054	2.58 $^{(*)}$	1	0.024	2.02
Residual	1533	0.021		330	0.012	

Note: The table presents the results of Type I analyses. $^{(*)}$ $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

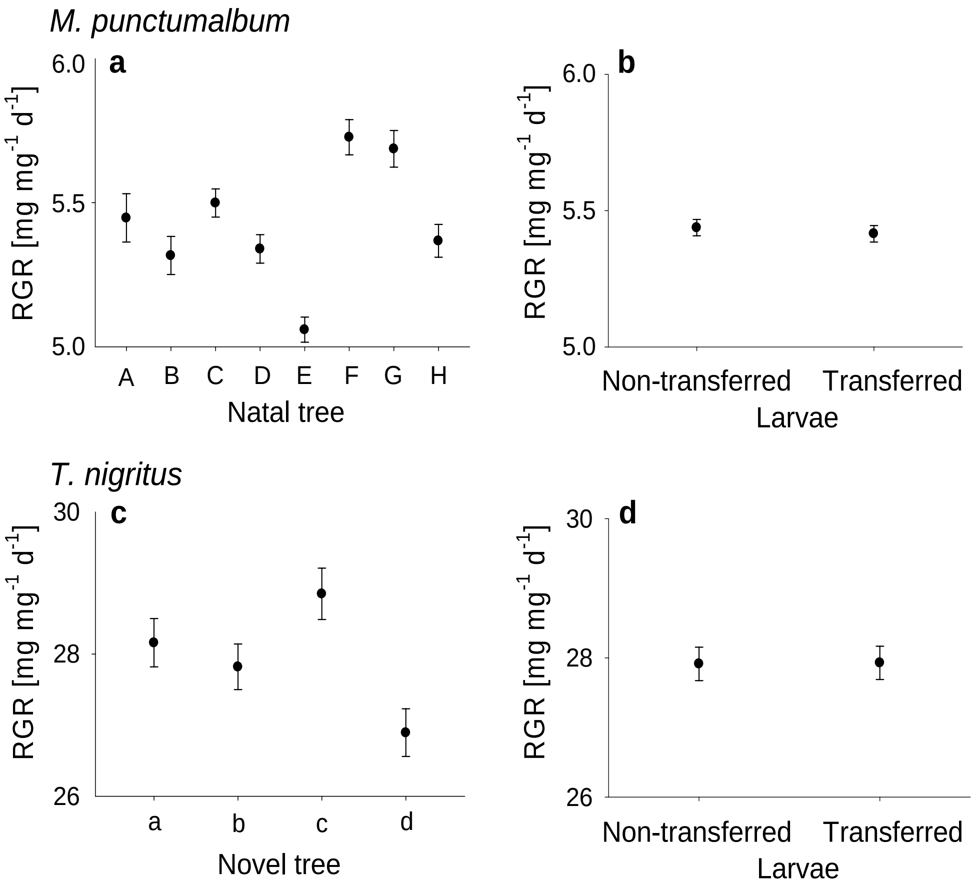


Fig. 1. Effect of natal tree, novel tree, and host transfer on the relative growth rate of larvae of *Macrophya punctumalbum* (a, b) and *Tomostethus nigritus* (c, d). We give relative growth rates (RGR) measured across 24 hours from an ANCOVA (back-transformed least squares means; error bars give back-transformed values of mean ± the standard error; hence, error bars are not symmetric).

larvae had no negative effect on relative growth rate (ANCOVA, home versus away; Table 1, Fig. 1d).

6.5. Discussion

We tested the adaptive deme formation hypothesis (Edmunds and Alstad, 1978) with two species of sawflies. In reciprocal transfer experiments with larvae of *T. nigritus* and *M. punctumalbum* on ash trees, we did not observe any effect of the transfer: for both species, relative growth rate did not differ significantly between transferred and non-transferred larvae. Therefore, we found no evidence for local adaptation of the insects to individual host plants. Our results are in line with those of several previous studies that tested the adaptive deme formation hypothesis using transfer experiments (Table 2).

As noted in the Introduction, the ability to form locally adapted demes may depend on the mobility, the feeding mode, and the breeding system of the herbivorous species (Edmunds and Alstad, 1978). The adaptive deme formation hypothesis was originally

Table 2. Results of published experiments that tested the formation of adaptive demes.

Herbivorous insect	Host plant	Local adaptation	Source
Apterothrips seticornis	*Erigeron glaucus*	Yes	Karban (1989)
Stilbosis quadricustatella	*Quercus geminata*	Yes	Mopper *et al.* (1995)
Asphodylia borrichiae	*Borrichia frutescens*	Yes	Stiling and Rossi (1998)
Cryptococcus fagisuga	*Fagus sylvatica*	Yes /No	Wainhouse and Howell (1983)
Pseudaulacaspis pentagona	*Morus alba*	Yes /No	Hanks and Denno (1994)
Nuculaspis californica	*Pinus lambertiana*	No	Rice (1983)
Matsucoccus acalyptus	*Pinus monophylla*	No	Unruh and Luck (1987)
Matsucoccus acalyptus	*Pinus edulis*	No	Cobb and Whitham (1993, 1998)
Cinara cupressi	*Cupressus lusitanica*	No	Memmott *et al.* (1995)
Daktulosphaira vitifoliae	*Vitis arizonica*	No	Kimberling and Price (1996)
Adelges japonicus	*Picea jezoensis*	No	Ozaki and Itahana (1997)
Blepharida rhois	*Rhus glabra*	No	Strauss (1997)
Chionaspis pinifoliae	*Pinus resinosa*	No	Glynn and Herms (2004)

formulated for herbivores with low dispersal. Therefore, half of the published studies used sedentary scale insects. However, in their meta-analysis van Zandt and Mopper (1998) found no evidence that adaptive demes are more common in sedentary than in mobile herbivorous insects. Nevertheless, some authors consider spatial segregation as an important precondition for the formation of local demes (Hanks and Denno, 1994). The species we used in our experiments have the potential to fly. The prepupae of the two species hibernate in the soil. After hatching, adults need to fly into the canopy for oviposition. Thus, active dispersal or passive transport may cause a mixing of populations among nearby host individuals. Nevertheless, when the selection pressure is sufficiently high, adaptive demes may form despite some gene flow (see Feder *et al.*, 1994). For *M. punctumalbum*, our results are consistent with genetic differentiation between populations on different trees, but there was no evidence for specific adaptations of these demes. Hence, genetic differentiation is not necessarily an indicator for the adaptive deme formation hypothesis. The processes that generate this genetic differentiation are not necessarily related to differences in the selection regime among tree individuals. Microclimatic differences or microclimatic barriers together with variations in host plant phenology may isolate insect populations living on different host individuals, and genetic differences between host trees are due to genetic drift.

Edmunds and Alstad (1978) developed their hypothesis for a specialist and, indeed, a limited host range has been shown to be an important precondition for adaptive demes. Parasites and herbivores with a broad host range are less likely to show local adaptation than parasites with a narrow host range (Lajeunesse and Forbes, 2002). The feeding mode of herbivorous insects is also thought to affect the probability of the formation of adaptive demes. Endophagous insects are not able to escape from unfavourable plant tissues.

Therefore, they should have a higher probability to form adaptive demes than exophagous insects. However, van Zandt and Mopper (1998) found no significant effect of feeding mode on the probability of adaptation. Nevertheless, the effect size for the endophagous insects was larger than that for exophagous species and van Zandt and Mopper (1998) interpreted this as tentative support for the influence of feeding mode on the formation of adapted demes. In our experiments, we used larvae both of a monophagous and an oligophagous sawfly species. Females of the two species of sawflies oviposit under the epidermis of leaves. The larvae are, however, free-living leaf chewers. Thus, the species may display an intermediate mode of feeding behaviour. Although the sawflies used in our experiments should have had a considerable propensity to form adaptive demes, we found no evidence for the formation of locally adapted populations.

Compared with parthenogenetic species, a sexual breeding system may increase genetic variation and should thereby allow for an effective evolution of adaptations by selection (Weismann, 1904; Strauss and Karban, 1994; Goddard *et al.*, 2005; but see Lushai *et al.*, 2003). In addition, haplodiploid insects are usually assumed to adapt more easily than diplodiploid insects (Alstad, 1998; Haccou and Schneider, 2004; Schoustra *et al.*, 2005). In contrast to these expectations, van Zandt and Mopper (1998) found no significant effect of the breeding system in their meta-analysis. Moreover, they suggest from the magnitude of the effect sizes that parthenogenetic and diplodiploid insects may even be more likely to form locally adapted demes than haplodiploid herbivores. During our experiments we used the haplodiploid *T. nigritus* and the probably parthenogenetic *M. punctumalbum* (Hoebeke and Johnson, 1985; Chevin, 1995). *Tomostethus nigritus* has not only a haplodiploid breeding system but also tends to have eruptive population dynamics with fast development of the larvae (Mrkva, 1965), whereas larvae of *M. punctumalbum* have a slower growth rate. Together, these differences between the two species should mean that it is less likely for adaptive demes to be formed by *T. nigritus* than *M. punctumalbum* (cf. Price *et al.*, 1990; van Zandt and Mopper, 1998). However, we found no evidence for differences in the formation of adaptive demes in the two species.

An important prerequisite for the formation of demes adapted to host individuals is that resource heterogeneity within individual hosts has to be much lower than among host individuals. In addition, the quality of leaf tissue of host individuals needs to be predictable for the herbivores over generations (see Edmunds and Alstad, 1978). Mopper *et al*. (2000) reported for a leafminer that it could take ten generations to form demes. At least across such temporal scales host individuals should have predictable properties to which the insects can adapt. But there may be variation in leaf tissue quality within plant individuals (Hollinger, 1989; Dudt and Shure, 1994) and these variations may affect the feeding behaviour and development of associated herbivorous insects (Howard, 1990; Fortin and Mauffette, 2002). Furthermore, large variations in leaf quality occur within and between years (Laitinen *et al.*, 2000; Osier *et al.*, 2000b; Covelo and Gallardo, 2001), although the ranking among host individuals is sometimes consistent across years (Riipi *et al.*, 2004). The leaf quality of a single plant can rapidly change with age such that a tenfold change in resistance can occur over a 2-year period (Kearsley and Whitham, 1989). Overall, the published evidence suggests that fluctuations in host quality within and between years may prevent local adaptations. Instead, the variability of leaf quality may select for genotypes that can cope with a variety of leaf qualities (Whitham, 1983; Cobb and Whitham, 1998).

In our opinion, the variability of leaf quality within and between years, together with the contradicting results from transfer experiments, indicate that generalizations about the formation of adaptive demes are premature. Following Hanks and Denno (1994), we conclude that local adaptation may fine-tune herbivore populations to host individuals under very specific conditions. However, it appears that adaptive demes do not play a general role in the evolution of herbivorous insects. Mopper (2005) suggested recently that demes will evolve when variation in host phenology is coupled with endophagy of the herbivore. Further studies are needed to evaluate this hypothesis.

Acknowledgements

We thank Manfred Kraus (Nuremberg, Germany) for identifying the larvae of the sawflies. Andreas Sickert and Lothar Fischer provided us with information about insect gradations in the forests of Leipzig (Leipzig, Germany). We are grateful to our colleagues in the Department of Community Ecology at the UFZ (Halle, Germany) for assistance and to Helmut Zwölfer (Bayreuth, Germany) for helpful comments on the manuscript. Thanks to Peter Horchler and Martin Unterseher, the coordinators for the Leipzig Crane Project (Leipzig, Germany).

6.6. References

Alstad, D. 1998. Population structure and the conundrum of local adaptation. In *Genetic Structure and Local Adaptation in Natural Insect Populations* (S. Mopper and S.Y. Strauss, eds.), pp. 3–21. New York: Chapman & Hall.

Alstad, D.N. and Corbin, K.W. 1990. Scale insect allozyme differentiation within and between host trees. *Evol. Ecol.*, **4**: 43–56.

Ayres, M.P., Suomela, J. and MacLean, S.F., Jr. 1987. Growth performance of *Epirrita autumnata* (Lepidoptera: Geometridae) on mountain birch: trees, broods, and tree x brood interactions. *Oecologia*, **74**: 450–457.

Boecklen, W.J. and Mopper, S. 1998. Local adaptation in specialist herbivores: theory and evidence. In *Genetic Structure and Local Adaptation in Natural Insect Populations* (S. Mopper and S.Y. Strauss, eds.), pp. 64–88. New York: Chapman & Hall.

Chevin, H. 1995. Biologie de *Macrophya punctumalbum* (Linné) (Hymenoptera: Symphyta, Tenthredinidae). *L'Entomologiste*, **51**: 279–285.

Cobb, N.S. and Whitham, T.G. 1993. Herbivore deme formation on individual trees: a test case. *Oecologia*, **94**: 496–502.

Cobb, N.S. and Whitham, T.G. 1998. 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* (S. Mopper and S.Y. Strauss, eds.), pp. 37–63. New York: Chapman & Hall.

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

Dudt, J.F. and Shure, D.J. 1994. The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, **75**: 86–98.

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

Feder, J.L., Chilcote, C.A. and Bush, G.L. 1994. Regional, local and microgeographic allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in western Michigan. *Evolution*, **44**: 595–608.

Fortin, M. and Mauffette, Y. 2002. The suitability of leaves from different canopy layers for a generalist herbivore (Lepidoptera: Lasiocampidae) foraging on sugar maple. *Can. J. Forest Res.*, **32**: 379–389.

Glynn, C. and Herms, D.A. 2004. Local adaptation in pine needle scale (*Chionaspis pinifoliae*): natal and novel host quality as tests for specialization within and among red and Scots pine. *Environ. Entomol.*, **33**: 748–755.

Goddard, M.R., Charles, H., Godfray, J. and Burt, A. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. *Nature*, **434**: 636–640.

Haccou, P. and Schneider, M.V. 2004. Modes of reproduction and the accumulation of deleterious mutations with multiplicative fitness effects. *Genetics*, **166**: 1093–1104.

Hanks, L.M. and Denno, R.F. 1994. Local adaptation in the amored scale insect *Pseudaulacaspis pentagona* (Homoptera: Diaspididae). *Ecology*, **75**: 2301–2310.

Hoebeke. E.R. and Johnson, W.T. 1985. A European privet sawfly, *Macrophya punctumalbum* (L.): North American distribution, host plants, seasonal history and descriptions of the immature stages (Hymenoptera, Tenthredinidae). *Proc. Entomol. Soc. Wash.*, **85**: 25–33.

Hollinger, D.Y. 1989. Canopy organization and foliage photosynthetic capacity in a broad-leaved evergreen montane forest. *Funct. Ecol.*, **3**: 53–62.

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

Howard, J.J. 1990. Infidelity of leafcutting ants to host plants: resource heterogeneity or defense induction? *Oecologia*, **82**: 394–401.

Karban, R. 1989. Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature*, **340**: 60–61

Kearsley, M.J.C. and Whitham, T.G. 1989. Developmental changes in resistance to herbivory: implications for individuals and populations. *Ecology*, **70**: 422–434.

Kimberling, D.N. and Price, P.W. 1996. Variability in grape phylloxera preference and performance on canyon grape (*Vitis arizonica*). *Oecologia*, **4**: 553–559.

Kleiner, K.W. 1991. Stability of phenolic and protein measures in excised oak foliage. *J. Chem. Ecol.*, **17**: 1243–1251.

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

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

Lorenz, H. and Kraus, M. 1957. Die Larvalsystematik der Blattwespen. *Abh. Larvalsystematik Insekten*, **1**: 1–339.

Lushai, G., Loxdale, H.D. and Allen, J.A. 2003. The dynamic clonal genome and its adaptive potential. *Biol. J. Linn. Soc.*, **79**: 193–208.

Memmott, J., Day, R.K. and Godfray, H.C.J. 1995. Intraspecific variation in host-plant quality: the aphid *Cinara cupressi* on the Mexican cypress, *Cupressus lusitanica*. *Ecol. Entomol.*, **20**: 153–158.

Mopper, S. 1996. Adaptive genetic structure in phytophagous insect populations. *Trends Ecol. Evol.*, **11**: 235–238.

Mopper, S. 2005. Phenology – how time creates spatial structure in endophagous insect populations. *Ann. Zool. Fenn.*, **42**: 327–333.

Mopper, S., Beck, M., Simberloff, D. and Stiling, P. 1995. Local adaptation and agents of selection in a mobile insect. *Evolution*, **49**: 810–815.

Mopper, S., Stiling, P., Landau, K., Simberloff, D. and van Zandt, P. 2000. Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology*, **81**: 1577–1587.

Morawetz, W. and Horchler, P.J. 2004. Leipzig Canopy Crane Project (LAK), Germany. In *Studying Forest Canopies from Above: The International Canopy Crane Network* (Y. Basset, V. Horlyck and S.J. Wright, eds.), pp. 79–85. Bogotá: Editorial Panamericana de Colombia, United Nations Environment Programme.

Mrkva, R. 1965. [Beitrag zur Kenntnis der Morphologie, Bionomie und der Parasiten der schwarzen Eschenblattwespe (*Tomostethus nigritus* [Fabr.])]. *Práce výzkumných ústavu lesnických CSSR*, **30**: 33–64 (in Czech).

Osier, T.L., Hwang, S.Y. and Lindroth, R.L. 2000a. Effects of phytochemical variation in quaking aspen *Populus tremuloides* clones on gypsy moth *Lymantria dispar* performance in the field and laboratory. *Ecol. Entomol.*, **25**: 197–207.

Osier, T.L., Hwang, S.Y. and Lindroth, R.L. 2000b. Within- and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. *Biochem. Syst. Ecol.*, **28**: 197–208.

Ozaki, K. and Itahana, N. 1997. Deme formation in gall-making aphids *Adelges japonicus* (Homoptera: Adelgidae). *Res. Popul. Ecol.*, **39**: 17–22.

Price, P.W., Cobb, N., Craig, T.P., Fernandes, G.W., Itami, J.K., Mopper, S. *et al.* 1990. Insect herbivore population dynamics on trees and shrubs: new approaches relevant to latent and eruptive species and life table development. In *Insect–Plant Interactions* (E.A. Bernays, ed.), pp. 1–38. Boca Raton, FL: CRC Press.

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

Rice, W.R. 1983. Sexual reproduction: an adaptation reducing parent–offspring contagion. *Evolution*, **37**: 1317–1320.

Riipi, M., Haukioja, E., Lempa, K., Ossipov, V., Ossipova, S. and Pihlaja, K. 2004. Ranking of individual mountain birch trees in terms of leaf chemistry: seasonal and annual variation. *Chemoecology*, **14**: 31–43.

Schoustra, S.E., Slakhorst, M., Debets, A.J.M. and Hoekstra, R.F. 2005. Comparing artificial and natural selection in rate of adaptation to genetic stress in *Aspergillus nidulans*. *J. Evol. Biol.*, **18**: 771–778.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science*, **236**: 787–792.

Stiling, P. and Rossi, A.M. 1998. Deme formation in a dispersive gall-forming midge. In *Genetic Structure and Local Adaptation in Natural Insect Populations* (S. Mopper and S.Y. Strauss, eds.), pp. 22–36. New York: Chapman & Hall.

Strauss, S.Y. 1997. Lack of evidence for local adaptation to individual plant clones or site by a mobile specialist herbivore. *Oecologia*, **110**: 77–85.

Strauss, S.Y. and Karban, R. 1994. The significance of outcrossing in an intimate plant–herbivore relationship. II. Does outcrossing pose a problem for thrips adapted to the host-plant clone? *Evolution*, **48**: 465–476.

Unruh, T.R. and Luck, R.F. 1987. Deme formation in scale insects: a test with the pinyon needle scale and a review of other evidence. *Ecol. Entomol.*, **12**: 439–449.

van Zandt, P.A. and Mopper, S. 1998. A meta-analysis of adaptive deme formation in phytophagous insect populations. *Am. Nat.*, **152**: 595–604.

Wainhouse, D. and Howell, R.S. 1983. Intraspecific variation in beech scale populations and in susceptibility of their host *Fagus sylvatica*. *Ecol. Entomol.*, **8**: 351–359.

Weismann, A. 1904. *The Evolution Theory*. London: Edward Arnold.

Whitham, T.G. 1983. Host manipulation of parasites: within-plant variation as a defense against rapidly evolving pests. In *Variable Plants and Herbivores in Natural and Managed Systems* (R.F. Denno and M.S. McClure, eds.), pp. 15–41. New York: Academic Press.

7. Synthesis

7.1. Resource heterogeneity and fine-scale adaptations of herbivorous insects

Insects should optimise their performance for the specific leaf quality that they encounter during their lifetime, thus, possibly optimising the fitness and evolutionary success of the adapted individuals. If the next generation uses the same host individual, passing these abilities on to that generation would minimise costs for managing with host individuals. Thereby, the insects would gain an evolutionary advantage. However, local adaptabilities of phytophagous insects have been discussed controversially (Van Zandt & Mopper, 1998; Cobb & Whitham, 1998) and their significance is still not clear.

The aim of this thesis is to research into adaptabilities of herbivorous insects to host individuals. For that, the resource heterogeneity for herbivores, physiological adaptations of larvae during their developmental period and the formation of locally adapted demes were investigated.

Resource heterogeneity and their temporal predictability are essential prerequisites for local adaptations. In this thesis, leaf tissue quality, herbivore attack in the field and leaf utilization by larvae of a polyphagous herbivore (*Spodoptera littoralis*, Lepidoptera: Noctuidae) in bioassays were estimated in lime (*Tilia cordata*), ash (*Fraxinus excelsior*), sycamore (*Acer pseudoplatanus*) and common oak (*Quercus robur*) across two years. The leaf tissue quality, measured as C/N-ratio and water content, differed among as well as within individual trees in all four species. Levels of leaf damage by herbivores in the field differed among tree individuals in lime, oak and sycamore, but not in ash. Further, it varied in all four tree species within tree individuals between the upper and the lower tree layer. The utilization of leaf tissue by larvae of *S. littoralis* differed among and within tree individuals in all investigated tree species. Therefore, the resource heterogeneity as prerequisite for local adaptations of herbivores is fulfilled in each of the tree species. However, the pattern of resource quality for herbivorous insects among host individuals changed in lime and oak already across two years. In ash and sycamore, the relative ranking of tree individuals in terms of insect performance remained constant, thus satisfying the second prerequisite for long-term local adaptations, the predictability of host quality. Therefore, ash and sycamore individuals may be more suitable hosts for forming adapted demes of herbivores than lime or oak. However, two consecutive years may represent rather an indication of long-term variability and are unlikely to show the full range of variations.

Resource heterogeneity for herbivorous insects among host individuals was found in several studies (e.g. Ayres *et al*., 1987; Hemming & Lindroth, 1995; Glynn *et al*., 2004) and seems to be common (but see Cipollini *et al*., 2002). However, leaf tissue quality may often vary over time (Haukioja *et al*., 1985; Riipi *et al*., 2004; Ruusila *et al*., 2005). Even though the relative ranking of tree individuals remains constant for some compounds (Haukioja *et al*., 1985; Osier *et al*., 2000; Laitinen *et al*., 2004; Riipi *et al*., 2004), this is usually not true for all primary and secondary metabolites (Riipi *et al*., 2004). Therefore, the resistance of individual plants and the ranking within a population might vary across years. Though, the relative ranking of individual trees remains sometimes relatively constant in terms of larval growth rates of herbivores despite overall variability (Senn *et al.*, 1992; Hanimäki *et al.*, 1995). The predictability of resource quality for herbivores may therefore depend on the specific plant species and the environmental conditions.

An efficient physiological adaptation of larvae to the specific leaf tissue quality that they encounter during their developmental period may facilitate gradual adaptations of insects over many generations. It should even be possible if the resource quality for herbivores changes across years. In this study, reciprocal transfer experiments with larvae of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), showed that the oak individuals differed in the quality of their leaves and that phenotypic variation in growth on different oak individuals existed among families of larvae. Both are requirements for local adaptations to host individuals. However, the transfer of the larvae to novel host individuals did not reveal a significant effect on their relative growth rate. Hence, there was no evidence for physiological adaptation to individual hosts during larval development. This was the first study on this specific form of adaptation; further studies are needed to draw a general conclusion.

Even if physiological adaptations of larvae to host individuals are not verifiable, long-term genetic adaptations may take place. The adaptive deme formation hypothesis of Edmunds & Alstad (1978) assumes that over generations herbivorous insects form ecologically and genetically distinct groups within species – the demes. The hypothesis has been discussed controversially (Van Zandt & Mopper, 1998; Cobb & Whitham, 1998) and experimental tests have been equivocal. Three of the known studies on adaptive deme formation supported the hypothesis (Karban, 1989; Mopper *et al.*, 1995; Stiling & Rossi, 1998). Two further studies found partial confirmation (Wainhouse & Howell, 1983; Hanks & Denno, 1994), but more than half of all studies could not support the hypothesis at all (Rice, 1983; Unruh & Luck, 1987; Cobb & Whitham, 1993; Memmott *et al.*, 1995; Kimberling & Price, 1996; Ozaki & Itahana, 1997; Strauss, 1997; Glynn & Herms, 2004). Most studies tested the hypothesis using piercing-sucking herbivores (Rice, 1983; Wainhouse & Howell, 1983; Unruh & Luck, 1987; Karban, 1989; Cobb & Whitham, 1993; Hanks & Denno, 1994; Memmott *et al.*, 1995; Kimberling & Price, 1996; Ozaki & Itahana, 1997; Glynn & Herms, 2004). However, leaf chewers might respond differently to leaf tissue quality (Peeters, 2002). In this thesis, the adaptive deme formation hypothesis was tested with two leaf-chewing species of sawflies, *Tomostethus nigritus* and *Macrophya punctumalbum* (Hymenoptera: Tenthredinidae) on ash (*Fraxinus excelsior*). Ash has been previously shown to be a suitable host for forming adapted demes of herbivores. In reciprocal transfer experiments with the larvae on ash individuals, there was no effect of the transfer: the relative growth rate did not differ significantly between transferred and non-transferred larvae. Therefore, there was no evidence for local adaptation of the herbivores to individual host plants and leaf chewers may not be more likely to adapt to individual hosts than piercing-sucking herbivores.

For forming locally adapted demes, insects should oviposite on their natal host. However, this might often not be the case (cf. Rojas & Wyatt, 1999). Further, just slightly more than half of the known studies support a positive correlation between adult oviposition and offspring performance (Mayhew, 1997). Moreover, the host-plant preference of herbivorous insects may change between years (Cronin *et al.*, 2001).

Immobility as well as on-site mating and oviposition should facilitate deme formation. However, in their meta-analysis Van Zandt & Mopper (1998) found no evidence that adaptive demes are more common in sedentary than in mobile herbivorous insects. Parthenogenetic reproduction of the insect was assumed to favour genetically manifested adaptations (Van Zandt & Mopper, 1998; Scheirs *et al*., 2005), though there was no evidence of local adaptation in tests with the as parthenogenetic considered (Hoebeke & Johnson, 1985) sawfly *M. punctumalbum* in this study. In general, it is still not clear which traits of the insect's biology are related to the formation of locally adapted demes (cf. Van Zandt & Mopper, 1998). Further, the results on resource heterogeneity and published evidence (Haukioja *et al*., 1985; Riipi *et al*., 2004; Ruusila *et al*., 2005) suggest that hosts act often as "moving targets" for herbivores by varying leaf quality over time. This could prevent adaptations to host individuals and the variability of leaf quality may select for insect genotypes that can cope with a variety of leaf qualities (Whitham, 1983; Cobb & Whitham, 1998). Heterogeneity *per se* could be a plant defence against herbivory (Hartley & Jones, 1997).

Local adaptations should provide adapted populations an advantage under their local environmental conditions. If herbivorous insects adapt to individual plants, however, they should nevertheless maintain their ability to colonise new hosts. The extinction of plant individuals may otherwise obliterate the adapted deme as a whole. Resource heterogeneity is only one aspect of the environmental conditions that influences herbivores considerably and selection processes for other abiotic or biotic conditions may overlay the effect of different resource quality of host individuals. Adaptations to plant individuals may therefore give locally adapted demes only a moderate advantage and may not be a driving force in evolution. In a changing environment, this form of adaptation might even be a hurdle or not feasible at all.

To summarise, the variation of resource heterogeneity for herbivorous insects and its temporal predictability differs among plant species. Therefore, the probability that insects form locally adapted demes might depend on the specific plant-insect system. Together with the contradicting results from transfer experiments, this indicates that generalisations about fine-scale adaptations are premature. It is suggested that local adaptation may fine-tune herbivore populations to host individuals under very specific conditions. However, it may not play a general role in the evolution of herbivorous insects.

7.2. References

Ayres, M.P., Suomela, J. & MacLean, S.F. Jr. (1987) Growth performance of *Epirrita autumnata* (Lepidoptera: Geometridae) on mountain birch: trees, broods, and tree x brood interactions. *Oecologia*, **74**, 450-457.

Cipollini, M.L., Paulk, E. & Cipollini, D.F. (2002) Effects of nitrogen and water treatment on leaf chemistry in horsenettle (*Solanum carolinense*), and relationship to herbivory by flea beetles (*Epitrix* spp.) and tabacco hornworm (*Manduca sexta*). *Journal of Chemical Ecology*, **28**, 2377-2398.

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

Cronin, J.T., Abrahamson, W.G. & Craig, T.P. (2001) Temporal variation in herbivore host-plant preference and performance: constraints on host-plant adaptation. *Oikos*, **93**, 312–320.

Edmunds, G.F. & Alstad, D.N. (1978) Coevolution in insect herbivores and conifers. *Science*, **199**, 941-945.

Glynn, C. & Herms, D.A. (2004) Local adaptation in pine needle scale (*Chionaspis pinifoliae*): natal and novel host quality as tests for specialization within and among red and Scots pine. *Environmental Entomology*, **33**, 748–755.

Glynn, C., Ronnberg-Wastljung, A.C., Julkunen-Tiitto, R. & Weih, M. (2004) Willow genotype, but not drought treatment, affects foliar phenolic concentrations and leaf-beetle resistance. *Entomologia Experimentalis et Applicata*, **113**, 1-14.

Hanimäki, S., Senn, J., Haukioja, E. (1995) The convergence in growth of foliage-chewing insect species on individual mountain birch trees. *Journal of Animal Ecology*, **64**, 543-552.

Hanks, L.M. & Denno, R.F. (1994) Local adaptation in the amored scale insect *Pseudaulacaspis pentagona* (Homoptera: Diaspididae). *Ecology*, **75**: 2301–2310.

Hartley, S.E. & Jones, C.G. (1997) Plant chemistry and herbivory, or why the world is green. In: Crawley, M.J. (ed.) *Plant ecology*. 2nd ed., pp. 284-324, Blackwell Science, Oxford.

Haukioja, E., Niemelä, P. & Sirén, S. (1985) Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the mountain birch *Betula pubescens* ssp *tortuosa*. *Oecologia*, **65**, 214-222.

Hemming, J.D.C. & Lindroth, R.L. (1995) Intraspecific variation in aspen phytochemistry - Effects on performance of gypsy moths and forest tent caterpillars. *Oecologia*, **103**, 79-88.

Hoebeke. E.R. & Johnson, W.T. (1985) A European privet sawfly, *Macrophya punctumalbum* (L.): North American distribution, host plants, seasonal history and descriptions of the immature stages (Hymenoptera, Tenthredinidae). *Proceedings of the Entomological Society Washington*, **85**, 25–33.

Karban, R. (1989) Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature*, **340**, 60–61.

Kimberling, D.N. & Price, P.W. (1996) Variability in grape phylloxera preference and performance on canyon grape (*Vitis arizonica*). *Oecologia*, **4**, 553–559.

Laitinen, M.L., Julkunen-Tiitto, R. & Rousi, M. (2000) Variation in phenolic compounds within a birch (*Betula pendula*) population. *Journal of Chemical Ecology*, **26**, 1609-1622.

Mayhew, P.J. (1997) Adaptive patterns of host-plant selection by phytophagous insects. *Oikos*, **79**, 417-428.

Memmott, J., Day, R.K. & Godfray, H.C.J. (1995) Intraspecific variation in host-plant quality: the aphid *Cinara cupressi* on the Mexican cypress, *Cupressus lusitanica*. *Ecological Entomology*, **20**, 153–158.

Mopper, S., Beck, M., Simberloff, D. & Stiling, P. (1995) Local adaptation and agents of selection in a mobile insect. *Evolution,* **49**, 810–815.

Osier, T.L., Hwang, S.Y. & Lindroth, R.L. (2000) Within- and between-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. *Biochemical Systematics and Ecology,* **28**, 197-208.

Ozaki, K. & Itahana, N. (1997) Deme formation in gall-making aphids *Adelges japonicus* (Homoptera: Adelgidae). *Researches on Population Ecology*, **39**, 17–22.

Peeters, P.J. (2002) Correlations between leaf constituent levels and the densities of herbivorous insect guilds in an Australian forest. *Austral Ecology*, **27**, 658-671.

Rice, W.R. (1983) Sexual reproduction: an adaptation reducing parent–offspring contagion. *Evolution*, **37**, 1317–1320.

Riipi, M., Haukioja, E., Lempa, K., Ossipov, V., Ossipova, S. & Pihlaja, K. (2004) Ranking of individual mountain birch trees in terms of leaf chemistry: seasonal and annual variation. *Chemoecology*, **14**, 31-43.

Rojas, J.C. & Wyatt, T.D. (1999) The role of pre- and post-imaginal experience in the host-finding and oviposition behaviour of the cabbage moth. *Physiological Entomology*, **24**, 83-89.

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

Scheirs, J., Jordaens, K. & De Bruyn, L. (2005) Have genetic trade-offs in host use been overlooked in arthropods? *Evolutionary Ecology*, **19**, 551-561.

Senn, J., Hanhimäki, S., Haukioja, E. (1992) Among-tree variation in leaf phenology and morphology and its correlation with insect performance in the mountain birch. *Oikos*, **63**, 215-222.

Stiling, P. & Rossi, A.M. (1998) Deme formation in a dispersive gall-forming midge. In: Mopper, S. & Strauss, S.Y. (eds.) *Genetic Structure and Local Adaptation in Natural Insect Populations*. pp. 22–36. Chapman & Hall, New York.

Strauss, S.Y. (1997) Lack of evidence for local adaptation to individual plant clones or site by a mobile specialist herbivore. *Oecologia*, **110**, 77–85.

Unruh, T.R. & Luck, R.F. (1987) Deme formation in scale insects: a test with the pinyon needle scale and a review of other evidence. *Ecological Entomology*, **12**, 439–449.

Van Zandt, P.A. & Mopper, S. (1998) A meta-analysis of adaptive deme formation in phytophagous insect populations. *American Naturalist,* **152**, 595–604.

Wainhouse, D. & Howell, R.S. (1983) Intraspecific variation in beech scale populations and in susceptibility of their host *Fagus sylvatica. Ecological Entomology*, **8,** 351–359.

Whitham, T.G. (1983) Host manipulation of parasites: within-plant variation as a defense against rapidly evolving pests. In: Denno, R.F. & McClure, M.S. (eds.) *Variable Plants and Herbivores in Natural and Managed Systems*, pp. 15–41. New York: Academic Press.

8. Summary - Zusammenfassung

Die Eigenschaften von Blättern und ihre Effekte auf pflanzenfressende Insekten stehen seit langem im Blickpunkt der Wissenschaft. Blattmerkmale, wie zum Beispiel die Konzentration verschiedener Pflanzeninhaltsstoffe, variieren nicht nur zwischen Arten, sondern oftmals auch zwischen den Individuen einer Pflanzenart. Diese Heterogenität sollte das Fressverhalten und Wachstum der an ihnen vorkommenden Insekten beeinflussen. Insektenpopulationen passen sich möglicherweise an die Wirtsheterogenität an und adaptieren sich an die Blattqualität einzelner Wirtsindividuen. Dabei könnte es zur Ausbildung ökologisch und genetisch distinkter Subpopulationen (*demes*) kommen, wie es die *adaptive deme formation*-Hypothese annimmt. Voraussetzungen für die *deme*-Bildung sind neben der Langlebigkeit der Wirte, die Fähigkeit der Insekten zwischen den Individuen einer Wirtsart zu unterscheiden sowie eine zeitliche Vorhersagbarkeit der Blattqualität. Das heißt, die Insekten sollten über Generationen hinweg eine ähnliche Nahrungsqualität an einem einzelnen Wirtsindividuum vorfinden, welche sich von der eines anderen Individuums unterscheidet. Untersuchungen zur Induktion der Nahrungspräferenz und Nahrungskonditionierung legen die Vermutung nahe, dass herbivore Insekten in der Lage sind, sich bereits während ihrer Larvalentwicklung physiologisch an die spezifische Blattqualität anzupassen. Diese Fähigkeit sollte die Bildung lokal adaptierter Subpopulationen (*demes*) beeinflussen.

Ziel der vorliegenden Arbeit ist es, die Adaptionsmöglichkeiten herbivorer Insekten an Wirtsindividuen zu erforschen. Hierfür wurde als erstes ermittelt, ob die Voraussetzungen für lokale Anpassungen im Untersuchungsgebiet, die Ressourcenheterogenität und ihre zeitliche Vorhersagbarkeit, gegeben sind. Als weiterer Schritt wurde die physiologische Anpassungsfähigkeit von Insekten während ihrer Larvalentwicklung an einzelne Baumindividuen untersucht. Drittens wurde die *adaptive deme formation*-Hypothese getestet und ermittelt, ob sich Insektenpopulationen über Generationen an Wirtsindividuen adaptieren. Alle Untersuchungen wurden mit Material aus dem Leipziger Auwald (Sachsen, Deutschland) durchgeführt. Lediglich die für Fraßtests verwendeten Larven von *Spodoptera littoralis* und *Lymantria dispar* (Lepidoptera) stammen aus Laborzuchten.

Der erste Teil der Dissertation (Kapitel 3 und 4) beschäftigt sich mit der Ressourcenheterogenität für herbivore Insekten, die in zwei aufeinanderfolgenden Jahren untersucht wurde. Im Kapitel 3 wird hierbei auf die Ressourcenheterogenität am Bergahorn eingegangen. An drei Baumindividuen wurden Blattmerkmale, die Variabilität der Herbivorie im Freiland sowie die Blattverwertung in Fraßexperimenten untersucht. Der Grad der Herbivorie im Freiland, die Blattnutzung durch einen generalistischen Phytophagen (*Spodoptera littoralis*) in Labortests sowie einfache Blattmerkmale (Wassergehalt, Kohlenstoff/Stickstoff-Verhältnis) unterschieden sich zwischen den untersuchten Individuen des Bergahorns. Innerhalb der Baumkrone variierte der Grad der Herbivorie lediglich im zweiten Untersuchungsjahr. Im Fraßexperiment zeigten die Larven von *S. littoralis* hingegen nur im ersten Untersuchungsjahr Unterschiede in der Umsatzeffizienz des aufgenommenen Blattgewebes innerhalb der Baumkrone. Alle ermittelten Merkmale deuten auf eine erhebliche Heterogenität in der Ressourcenqualität zwischen den untersuchten Baumindividuen hin. Die Variabilität innerhalb der Bäume war hingegen gering. Im Fraßexperiment blieb die Rangfolge der Baumindividuen durch einen generalistischen Phytophagen, in Bezug auf ihre Ressourcenqualität, zwischen den beiden Jahren konstant. Die gefressene Blattmenge sowie die relative Wachstumsrate und die Umsatzeffizienz der Larven der *S. littoralis* korrelierten wie erwartet negativ mit dem Kohlenstoff/Stickstoff-Verhältnis im Blattgewebe. Wir konnten hingegen keinen Zusammenhang zwischen den Daten aus dem Fraßexperiment und dem Grad der Herbivorie im Freiland nachweisen. Die räumlichen und zeitlichen Muster der Herbivorie im Freiland lassen sich anscheinend aufgrund von Wechselwirkungen mit Umweltfaktoren nicht direkt aus den Mustern der Ressourcenqualität ableiten. Zusammenfassend ist festzustellen, dass die Ergebnisse zwei grundlegende Voraussetzungen der *adaptive deme formation*-Hypothese bestätigen, die Heterogenität und die zeitliche Vorhersagbarkeit der Ressourcenqualität zwischen Wirtsindividuen.

Im Kapitel 4 wird die Ressourcenheterogenität für herbivore Insekten an jeweils drei Individuen der Esche (*Fraxinus excelsior*), der Winterlinde (*Tilia cordata*) und der Stieleiche (*Quercus robur*) zusammen mit den Ergebnissen am Bergahorn (*Acer pseudoplatanus*) dargestellt. Neben Blattmerkmalen wurde der Grad der Herbivorie im Freiland sowie die Verwertbarkeit der Blätter in Fraßtests mit *S. littoralis* bestimmt. Der Wassergehalt und das Kohlenstoff/Stickstoff-Verhältnis des Blattgewebes variierten zwischen sowie innerhalb der untersuchten Individuen der vier Baumarten. Der Grad der Herbivorie im Freiland unterschied sich zwischen den Individuen der Linde, Eiche und des Bergahorns, nicht jedoch bei der Esche. Innerhalb der Bäume variierte die durch herbivore Insekten konsumierte Blattmenge im Freiland bei allen untersuchten Baumarten. Die Fraßtests im Labor ergaben Unterschiede der Nutzung des Blattmaterials durch die Insekten zwischen sowie innerhalb der Baumindividuen bei allen vier Pflanzenarten. Die im Freiland ermittelte Heterogenität der Herbivorie sowie die in Fraßtests gezeigte Ressourcenheterogenität korrelierten nicht in jedem Fall mit den ermittelten Blattmerkmalen. Das innerartliche Muster der Verwertbarkeit des Blattgewebes für herbivore Insekten änderte sich zwischen den Untersuchungsjahren an Linde und Eiche. Somit stellen diese Baumarten vermutlich *moving targets* für ihre Parasiten dar, was die Bildung lokaler Adaptionen hemmen sollte. Esche und Bergahorn scheinen geeignetere Wirte für die Bildung lokal adaptierter Subpopulationen darzustellen. Bei diesen Baumarten blieb das Muster der Verwertbarkeit des Blattmaterials für die Insekten zwischen zwei untersuchten Jahren gleich.

Der zweite Teil der Arbeit (Kapitel 5) untersucht die physiologische Anpassungsfähigkeit von Insekten. Im Allgemeinen wird angenommen, dass Anpassungen über viele Generationen hinweg stattfinden. Die Induktion der Nahrungspräferenz, die Konditionierung sowie die Nahrungsprägung treten jedoch während der Larvalentwicklung der Insekten auf. Daher erscheint es möglich, dass Insekten sich ebenso physiologisch innerhalb der Larvalentwicklung an die unterschiedliche Verwertbarkeit einzelner Wirtsindividuen adaptieren. Dieses wurde bisher nicht untersucht. Mittels reziproker Transferexperimente mit dem Schwammspinner (*Lymantria dispar*) an Eiche (*Quercus robur*) wurde die physiologische Anpassungsfähigkeit sowie die genetische Variabilität der phänotypischen Adaptionsfähigkeit getestet. Die Ergebnisse zeigen, dass die relative Wachstumsrate an den einzelnen Eichen zwischen den Familien des Schwammspinners differiert. Die drei Eichen waren für die Larven unterschiedlich verwertbar. Jedoch war kein signifikanter Effekt des Transfers zwischen Eichenindividuen auf die relative Wachstumsrate der Tiere nachweisbar. Damit bestätigen die Ergebnisse nicht die Idee einer physiologischen Adaption eines polyphagen Insekts an Wirtsindividuen während dessen Larvalentwicklung.

Im dritten Teil der Arbeit (Kapitel 6) wird die *adaptive deme formation*-Hypothese an zwei Blattwespenarten (*Tomostethus nigritus* und *Macrophya album*) an Esche (*Fraxinus excelsior*) getestet. Beide Insektenarten unterscheiden sich in ihrer Biologie. *M. album* gilt als parthenogenetisch, während *T. nigritus* eine haplodiploide Blattwespe mit gelegentlichen Massenausbrüchen ist. In reziproken Transferexperimenten erhielten Larven beider Arten Blätter unterschiedlicher Eschenindividuen als Nahrungsquelle. Nach 24 Stunden wurde die relative Wachstumsrate der Larven bestimmt. Trotz unterschiedlicher Biologie der Blattwespenarten unterschieden sich die Ergebnisse der Tests nicht. Der Transfer der Larven zwischen Eschenindividuen zeigte keinen signifikanten Einfluss auf ihre Wachstumsrate. Somit war die Bildung lokal adaptierter *demes* nicht nachweisbar und die Ergebnisse bestätigen nicht die *adaptive deme formation*-Hypothese. Jedoch war eine genetische Differenzierung der Population der *M. album* zwischen den Eschenindividuen nachweisbar. Diese kann durch mikroklimatische Unterschiede und Barrieren zwischen den Wirtsindividuen sowie Unterschiede im Blattaustrieb der Eschen hervorgerufen worden sein.

Aus den hier vorgestellten Ergebnissen sowie publizierten Daten ist zu schließen, dass Ressourcenheterogenität für herbivore Insekten zwischen Baumindividuen weit verbreitet ist. Ihre zeitliche Vorhersagbarkeit ist jedoch nicht immer gegeben und scheint zwischen Pflanzenarten zu differieren. Somit hängt die Möglichkeit herbivorer Insekten sich an einzelne Wirtsindividuen anzupassen von dem jeweiligen Pflanze-Insekt-Sytem ab, z. B. scheinen Esche und Bergahorn geeignetere Wirtsarten für lokale Adaptionen darzustellen als Winterlinde und Stieleiche. Dennoch konnten wir im Untersuchungsgebiet keine Adaption zweier Pflanzenwespenarten an einzelne Eschenindividuen nachweisen. Ebenfalls war keine physiologische Adaption von Insekten während ihrer Larvalentwicklung an Wirtsindividuen nachweisbar. Allgemein ist festzustellen, dass zwar unter bestimmten Bedingungen Adaptionen herbivorer Insekten an Pflanzenindividuen stattfinden können, aber die Bildung lokal adaptierter Subpopulationen kein generelles Phänomen bei herbivoren Insekten darstellt.

9. Acknowledgements

I would like to thank Prof. Dr. Roland Brandl (University of Marburg, Germany) for his supervision of my thesis. He was always very interested in the progress of the thesis and gave invaluable advice. Many thanks to Prof. Dr. Diethart Matthies (University of Marburg) for his endless patience in explaining statistical issues and his helpful comments on manuscripts. I could always consult Dr. Martin Schädler (University of Marburg) when I had some tricky questions. I want to express my gratitude for that.

The study was carried out at the Department of Community Ecology at the Helmholtz Centre for Environmental Research - UFZ (Halle, Germany). I would like to thank all my colleagues of the Department. They provided a pleasing working ambience as well as enjoyable distractions. I am especially grateful to Dr. Stefan Klotz, the head of the Department, who gave me the opportunity to work in such a pleasant environment. Dr. Daniel Prati kindly helped with statistics and the excellent technicians Hannelore Jany, Antje Thondorf, Hannelore Kobs and our former apprentice Ines Stolpe gave assiduous assistance.

Many thanks to Prof. Dr. Peter Proksch and Dr. Gero Eck (University of Düsseldorf, Germany) for providing *Spodoptera littoralis* and Prof. Dr. Andreas Linde and Dr. Dörte Goertz (University of Applied Sciences Eberswalde, Germany) for providing egg batches of the gypsy moth. Dr. Manfred Kraus (Nuremberg, Germany) kindly identified the larvae of the sawflies. Andreas Sickert and Lothar Fischer (Leipzig, Germany) provided me with information about insect gradations in the forests of Leipzig. Thanks to Peter Horchler and Martin Unterseher (Leipzig), the coordinators for the Leipzig Crane Project. They were very valuable in arranging working time on the canopy crane.

I am grateful to all my friends. To be with them was always encouraging and enjoyable. Some of them helped improving my English. In addition, Robert Paxton (Belfast, U.K.) and Jake Alexander (Zurich, Switzerland) made texts better readable and Karsten Seidelmann and Christel Roß (both Halle, Germany) gave invaluable advice. Thanks to all of you for your great support.

Halle, 2007 Haike Ruhnke

10. Appendix

10.1. Pictures of the study area and used insect species

Fig. 10.1. The study area: The canopy crane in the Leipzig floodplain forest.

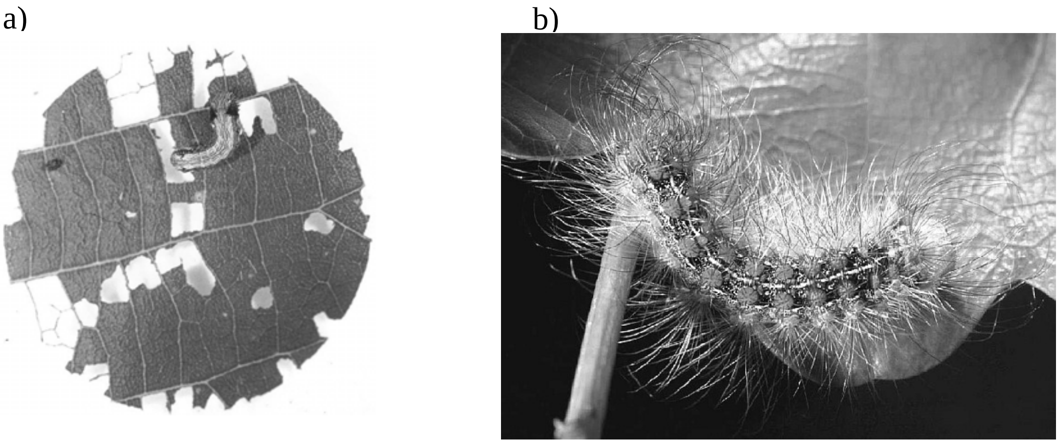
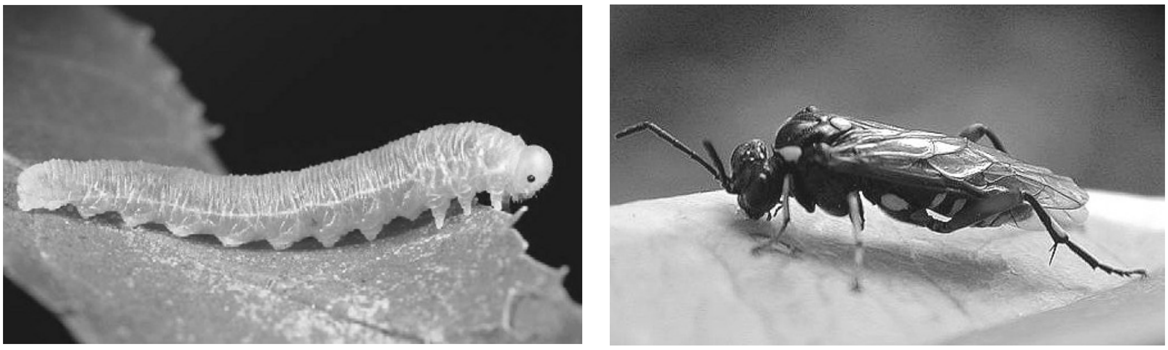


Fig. 10.2. Larvae of the (a) African cotton leafworm, *Spodoptera littoralis,* and (b) the gypsy moth, *Lymantria dispar*. The larvae of *S. littoralis* helped to investigate the resource heterogeneity among and within host individuals for herbivorous insects. With larvae of *L. dispar,* experiments investigating the physiological adaptability of generalistic herbivores to host individuals were performed.

a) *Tomostethus nigritus*



b) *Macrophya punctumalbum*

c) *Macrophya punctumalbum*

Fig. 10.3. Larvae (a, b) and imago (c) of the black sawfly *Tomostethus nigritus* (a), and the privet sawfly *Macrophya punctumalbum* (b, c). The adaptive deme formation hypothesis was tested with larvae of these two species feeding on ash.

(c) Picture by courtesy of Hania Arentsen, www.gardensafari.net

10.2. Declaration of self-contained work

Herewith I affirm that I composed the Dissertation

Effects of resource heterogeneity in trees upon insect herbivory

single handed without utilising illegitimate resources. I used no other than the cited references and facilities. This work has not been previously handed in to another university and was not subject to miscellaneous examinations.

Halle, March 2007 Haike Ruhnke

10.3. Curriculum vitae

Contact information

Name	Haike Ruhnke
Address	Helmholtz Centre for Environmental Research – UFZ Department of Community Ecology Theodor-Lieser-Str. 4, D-06120, Halle, Germany
Telephone	+49 – (0)345 – 55 85 302
E-mail	ruhnke.ufz@web.de

Personal information

Date of birth	10.04.1971
City, Country	Wismar, Germany

Education

Since 04/2004	External PhD student at the Department of Animal Ecology, University of Marburg, Germany
03/1998	Diploma degree in biology (equivalent to a master’s degree), graduated with honours at the Department of Zoology, University of Halle, Germany
10/1991 to 03/1998	Studies of biology at the University of Halle, Germany

Research experience

- Since 02/2007 Scientific officer at the Helmholtz Centre for Environmental Research - UFZ in Halle, Germany
    - Generating of data bases
- 12/2006 to 01/2007 Job-seeking
- 09/2006 to 11/2006 Work placement at Aston University in Birmingham, U.K.; Experiments with microbes as well as breast cancer research
    - Transformation of plasmid DNA into competent *E. coli*, DNA extraction, restriction digestion of DNA, cell culture
    - Isolation and culture of soil microbes
    - Tutoring of students
- 08/2006 Freelance work as biologist: acquisition of potential virus vectors on roses at the *Rosarium* in Sangerhausen, Germany
    - Field samplings of bees, bugs, cicada, sawflies, weevils, thrips
    - Identification of bee species,
    - Co-ordination of the processing of identification of the other insect taxa
    - Writing of an expertise

- 04/2002 to 08/2006 Working at the Helmholtz Centre for Environmental Research - UFZ in Halle, Germany
    - Feeding experiments with larvae of moths and sawflies on leaves of tree species: estimating the leaf consumption, relative growth rate and conversion efficiency of the larvae
    - Measurements of leaf traits
    - Responsible for stock breedings of different insect species
- 08/2001 to 03/2002 Scientific officer at the asthma research group of the University of Halle, Germany
    - Immunisation and respiratory measurements on mice
    - Estimates of the content of several cytokines with ELISA
    - Cell culture
- 07/2000 to 07/2001 Graduate assistant at the Department of Developmental Physiology, University of Tübingen, Germany
    - Molecular genetic investigations on the grade of polyandry of a stingless bee
    - Microsatellite analyses, AFLP
    - Teaching assistant
- 04/1998 to 12/2000 Freelance work as biologist in environmental consultancy, bee field surveys in Germany
    - Field samplings
    - Identification of bee species
    - Writing of expertises
- 03/1998 Diploma thesis in animal ecology: “Distribution, biology and endangering of the leafcutter bee *Megachile genalis* Mor. (Hymenoptera: Apidae)”