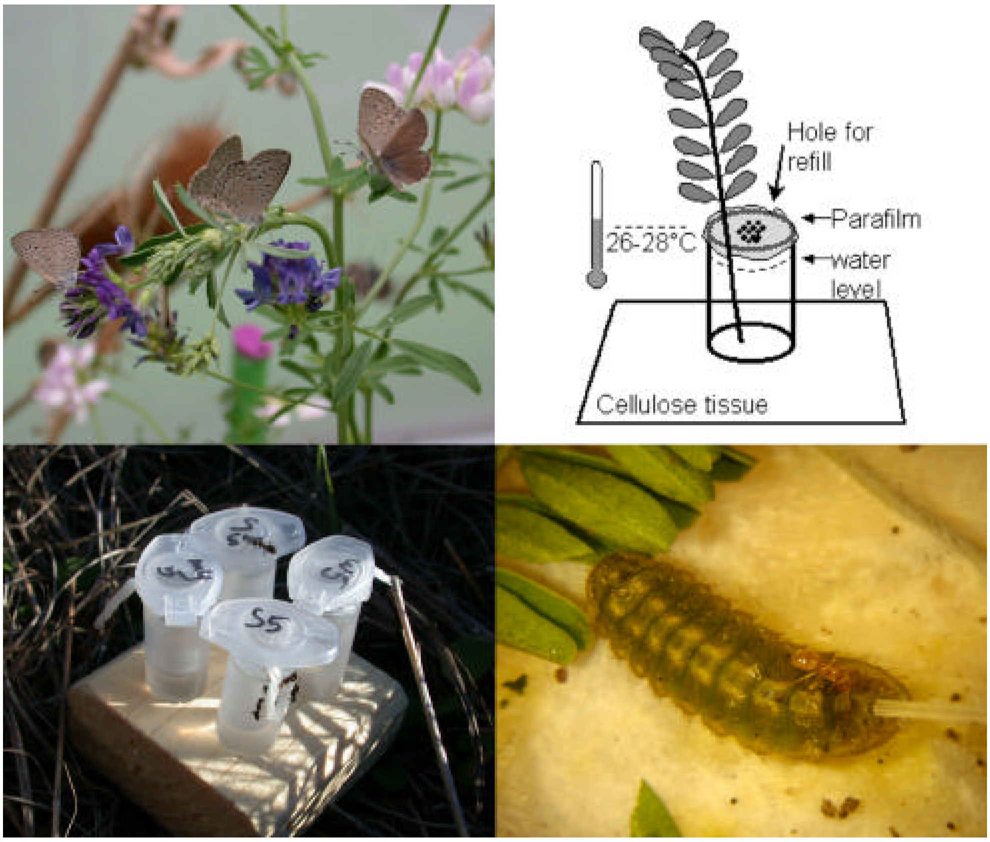
Facultative butterfly-ant interactions – the role of variation in composition of nectar secretions



Facultative butterfly-ant interactions – the role of variation in composition of nectar secretions

Dissertation

zur Erlangung des Grades eines
Doktors der Naturwissenschaften
– Dr. rer. nat. –
der Fakultät Biologie / Chemie / Geowissenschaften
der Universität Bayreuth

Vorgelegt von
Holger Daniels

Bayreuth, Mai 2004

Die vorliegende Arbeit wurde am Lehrstuhl Tierökologie I der Universität Bayreuth in der Arbeitsgruppe von Herrn Prof. Dr. Konrad Fiedler angefertigt.

Gefördert durch die Deutsche Forschungsgemeinschaft im Rahmen des Graduiertenkollegs 678: „Ökologische Bedeutung von Wirk- und Signalstoffen bei Insekten - von der Struktur zur Funktion“.

Vollständiger Abdruck der von der Fakultät für Biologie, Chemie und Geowissenschaften der Universität Bayreuth genehmigten Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.).

Tag der Einreichung: 04. Mai 2004
Tag des Promotionskolloquiums: 21. Juli 2004

Prüfungsausschuß:
Prof. Dr. K. Fiedler (Erstgutachter)
Prof. Dr. G. Rambold (Zweitgutachter)

Prof. Dr. E. Komor (Vorsitzender)
Prof. Dr. K. Dettner
Prof. Dr. K. Seifert

Dissertation als pdf-Datei und Bilder aus den Farbtafeln in höherer Auflösung auf Nachfrage verfügbar.

Thesis available as pdf-file, likewise single pictures out of the colour plates in high resolution.

Kontakt / contact: HolgerDaniels@web.de

Sie geben um zu leben
Khalil Gibran (Vom Geben)

Gewidmet dem Andenken an
Walter Peter

1 Introduction

H. Daniels

Department of Animal Ecology I, University of Bayreuth, D-95440 Bayreuth, Germany

Interspecific mutualisms are ubiquitous and fundamentally influential at all levels of biological organization (Boucher 1982; Boucher 1985; Bronstein 1994; Bronstein 2001; Herre et al. 1999 and references therein). All species on earth have been argued to be involved in at least one kind of interspecific mutualism (Bronstein 2001), and often many more. Mutualisms are linked to key events in evolution such as the development of the eukaryotic cell or the radiation of the angiosperms. From the mitochondria or chloroplasts in eukaryotic cell lineages, pollinator systems, lichens, mycorrhiza, coral reef build-up, deep sea vent systems, which rely entirely on mutualism, to the countless microorganisms involved in nitrogen fixation or digestion, mutualism can be found everywhere, and more such cases will be found still, especially as only a very small percentage of microbial diversity is known so far (Madigan et al. 2000). In this regard it is even more surprising that the importance of mutualism was largely ignored for the most part of the 20th century as opposed to antagonistic forces like predation and competition. The term mutualism was defined already in 1873/75 by Pierre van Beneden in the following way: in addition to parasites "we find others, who mutually provide each other services, it would be most unflattering to call them all parasites or commensals. We consider it fairer to call them *Mutualists*, mutualism takes place beside *commensalism* and *parasitism*". The term was possibly coined in analogy to the French worker societies Mutualité (Boucher 1982). An illustrious character such as the Russian Prince Peter Kropotkin, published an influential book on mutualism ("Mutual aid: a factor in evolution" 1902) but was simultaneously an influential anarchist. These political implications might have contributed to the fact that mutualism remained an unpopular topic. From about 1970 onwards (Boucher 1982; Boucher 1985) mutualisms entered the centre stage again, and these types of interaction were more and more intensively studied in the last decades (Bronstein 1994; Bronstein 1998). Mutualisms, here defined as interactions which increase the potential fitness of both partners, can be intimate, space-confined close interactions such as between gut bacteria in mammals or termites with their hosts. Alternatively there are highly integrated, specialized and probably coevolved interactions between spatially separated organisms, or more loose associations, up to diffuse and indirect interactions (Herre et al. 1999). Thus with regard to intimacy and specificity mutualisms cover a whole continuum from obligate and partner specific to facultative and unspecific interactions.

In terrestrial ecosystems ants occupy a large number of different terrestrial ecological niches, and due to their sheer biomass and number, they are an important component of such systems (Hölldobler & Wilson 1990). Ants are the leading invertebrate predators, with very strong impact on the structure and diversity of many arthropod communities (Hölldobler 1996). But they also engage in a multitude of mutualistic interactions, with over 465 plant species from more than 52 families (Jolivet 1996), with fungi (Schultz & McGlynn 2000) or with trophobiont species such as homopterans (Bristow 1991). Although in general the number of studies on mutualisms is rising, mutualisms involving ants and especially ant-insect protection mutualisms are still proportionally under-studied (Bronstein 1998). Among the trophobionts also lepidopteran larvae from the butterfly families Lycaenidae and Riodinidae can be found. Such a mutualistic relationship might be surprising, as ‘typical’ lepidopteran larvae with their relatively slow moving soft bodies, full of nutrients, are appealing targets for predators. The evolutionary rise and subsequent stability of mutualisms between organisms, which would normally be expected to behave antagonistically, is especially interesting and rewarding to study, both experimentally and theoretically. Model systems and theoretical frameworks for the description and analysis of these interactions were developed from game theory, resulting in the prisoner’s dilemma and its iterated form (IPD) (Axelrod & Hamilton 1981; Trivers 1971, but see Bendor & Swistak 1995; Boyd & Lorberbaum 1987, reviewed e.g. Brembs 1996; Dugatkin 1997, an IPD designed for mutualisms is Doebeli & Knowlton 1998). Another class of models, which stress more the exchange of services than the question of possibilities of defection, are the biological markets (Noe & Hammerstein 1994; Noe & Hammerstein 1995, also Hoeksema & Bruna 2000; Schwartz & Hoeksema 1998). These and other models are hotly debated in recent literature, and it remains to be addressed how well predictions derived from these models can be matched to trophobiotic mutualisms between ants and producers of honeydew or nectar. Ant attendants of trophobionts are mainly from the families of Formicinae and Dolichoderinae, but to a lesser degree also members of Myrmicinae, Ponerinae and a few other subfamilies are involved (DeVries 1991b; Eastwood & Fraser 1999; Fiedler 1991; Fiedler 2001; Hölldobler & Wilson 1990). Tending ant species, which comprise only a small part of the whole ant diversity, typically collect liquid carbohydrates from insects as well as from plants (extrafloral nectar, infrequently floral nectar, plant wound sap), and are characterized by widespread geographical distribution and ecological dominance.

Mutualisms between riodinid butterfly larvae and ants are ancient, as evidenced by fossils (DeVries & Poinar 1997). The Lycaenidae are a family with about 4500 species (if the Riodinidae are excluded) and thus comprise almost a third of the butterfly species (Ackery et al. 1999). More than half of the estimated lycaenid species associate with ants at least during part of their development (Fiedler 1991; Pierce et al. 2002), while larvae of the remaining half of species are mostly ignored by ants. The habit of lycaenid larvae (and of many other arthropods) to associate with ants is termed myrmecophily (Hölldobler & Wilson 1990). The peaceful interactions of the lycaenid larvae and ants can be classified according to their degree of intimacy. Those species which are ignored or elicit only a very short inspection by the ants are termed myrmecoxenous (Kitching & Luke 1985). If the ants are required essentially, as protectors or as a food source, the interaction is termed obligate myrmecophily. Only 20% of all lycaenid species worldwide appear to be obligate myrmecophiles (Fiedler 1991; Fiedler 1997b). However, the incidence of obligate myrmecophily differs significantly between phylogenetic lineages and biogeographical regions. While in Australia and Southern Africa large fractions of the unique lycaenid faunas depend on specific host ants (Heath & Claassens 2003; Pierce 1987; Pierce et al. 2002), such intimate associations are rare in the West Palearctic region and nearly absent in the Nearctic (Fiedler 1991; Fiedler 1997a; Fiedler 2001).

A special case of obligate myrmecophiles are the ant-parasitic lycaenids, e.g. the European *Maculinea* species, which parasitise *Myrmica* ant colonies (Akino et al. 1999; Thomas & Elmes 1998), or the famous ‘tank-like‘ *Liphyra brassolis* which preys on the brood of the

Australian ant *Oecophylla smaragdina* (Braby 2000; Chapman 1902). Although predominant in biology text books (e.g. (Begon et al. 1996), the parasites comprise only a very small fraction of the lycaenid species (37 confirmed cases worldwide (Fiedler 1998)). They are regarded to have a relatively recent origin in evolutionary terms (Fiedler 1998) and are possibly an evolutionary dead end (Pierce 2001). The large remaining majority of ant-associated lycaenid species can thrive also without ants and engage in rather loose associations. Such associations are generally assumed as being mutualistic, but evidence in this regard remains scant (see below).

In lycaenid-ant associations food (see below) is offered to the ants. The lycaenid caterpillars, in turn, can at least enter habitats in which predacious ants exclude (most other) lepidopteran caterpillars, thus gaining an enemy free space (Atsatt 1981; Lenz 1917). Furthermore, they might gain protection against enemies (predators and parasitoids) as initially suggested by Thomann (1901). This protection has indeed been shown for some obligately myrmecophilous and very few facultatively myrmecophilous species (DeVries 1991a; Fiedler & Maschwitz 1988; Pierce & Easteal 1986; Pierce et al. 1987; Pierce & Mead 1981; Seufert & Fiedler 1996a; Wagner 1993). Costs may exist for the larvae of obligate myrmecophiles in form of reduced adult size, since the copious amounts of nutrients delivered in nectar are lacking for the caterpillar's development (Baylis & Pierce 1992; Pierce et al. 1987). For obligately myrmecophilous species also the parasitation risk can be enhanced if attendant ants are used as host-finding cues by parasitoids (Nash 1989; Pierce 1987; Seufert & Fiedler 1996b; Seufert & Fiedler 1999). In facultative myrmecophiles costs are low or the caterpillars might even overcompensate for losses of nutrients to ants, as the caterpillars grow larger if tended by ants (Fiedler & Hölldobler 1992; Fiedler & Saam 1994; Robbins 1991; Wagner 1993; Wagner & Martínez del Rio 1997). Whether the food rewards which ants can harvest really translate into fitness benefits has been scarcely investigated. Workers of some ant species do live longer, if access to nectar-producing lycaenid larvae is granted (Cushman et al. 1994; Fiedler & Saam 1995), and enhanced colony growth was shown in one case, where caterpillars produce copious food rewards (Nash 1989). The costs for the ants accruing from participation in these interactions are unclear.

Lycaenid caterpillars possess a whole array of morphological and behavioural adaptations to the life with ants. The first systematic comparative analysis was performed by Malicky 1969 & 1970 (all older reviews cited therein), and an extensive review was published by Cottrell (1984). Lycaenid larvae are peculiar onisciform shaped (woodlice-like appearance) and possess an exceptionally thick cuticle, with pronounced protrusions. All important organs are protected deeply within the body, secured against bite attacks by ants. Scattered over the integument, and concentrated at certain taxon-specific locations, are pore cupola organs (PCO, nomenclature after Cottrell (1984)). These are minute hair derived glands (Fiedler et al. 1996; Kitching 1983; Kitching & Luke 1985; Malicky 1969). They were already detected by Thomann (1901), but not identified as such. They share some anatomical and functional similarity to the lenticles of certain Hesperiidae larvae (Franzl et al. 1984). PCOs are secretory and innervated. Their secretions might contain amino acids in the Lycaenidae (Pierce 1983), but the topic still awaits an exact chemical analysis. Furthermore specialized dendritic setae are found on the integument (Kitching 1983; Kitching & Luke 1985). Dendritic setae have been related to ant-attendance and attractiveness based on behavioural evidence (Ballmer & Pratt 1991), whereas other authors showed them to have mechanoreceptive properties (Tautz & Fiedler 1992). Many lycaenid species possess on the eighth segment one pair of tube-like eversible tentacles, unfolding a corona of spiny bristles at the top if fully everted. Detected already in the 18th century (Denis 1785), and characterised for the first time 100 years later (Edwards 1878), these structures are strongly suspected to emit alarm pheromone mimics (Henning 1983), although again the secretions still await a clear chemical identification. Eversions of the tentacle organs induce ants to follow the caterpillar, or release alarmed behaviour in attending ants upon disturbance of the caterpillar (Ballmer & Pratt 1991; Claassens & Dickson 1977; Fiedler 1991; Fiedler & Maschwitz 1987). Thus the tentacle organs serve to bind ants especially at the initiation of an association and to regulate the ant number and attention (Axén et al. 1996; DeVries 1988; Fiedler & Maschwitz 1987).

The most important organ related to myrmecophily in the Lycaeninae is the dorsal nectar organ (DNO, nomenclature after Cottrell (1984)) (Fiedler 1991; Fiedler & Maschwitz 1989; Leimar & Axén 1993). The gland was first described by Guenée (1867), analysed anatomically almost in parallel by Newcomer (1912) and Ehrhardt (1914) (hence the term Newcomer gland) and was later reanalysed by Fiori (1958), and especially exhaustively by Malicky (1969). This gland is situated dorsally on the seventh abdominal segment and, upon solicitation by the attendant ants, secretes a fluid, which the ants readily imbibe. Nectar secretions are essential to induce food recruitment behaviour in ants which have found a lycaenid caterpillar (Fiedler & Maschwitz 1989). Comparative data also emphasize the importance of a dorsal nectar organ for maintaining stable ant associations (Fiedler 1991; Kitching & Luke 1985).

The exudate from this gland sometimes called ‘honeydew’ in analogy to the homopteran excretion. But Homoptera suck in most cases at phloem or xylem vessels or cell contents of their host plants and thereby ingest large amounts of sugar and only slight amounts of amino acids. The surplus of sugars passes the gut and is excreted as honeydew, up to 90% of the ingested energy being jettisoned (Muthukrishnan & Pandian 1987). Therefore honeydew is an excrement produced regardless of the presence of any ants, whereas almost all lycaenid caterpillars release the fluid from the organ only after stimulation by ants. Lycaenid nectar thus is an exocrine secretion (Malicky 1970), not just an (modified) excretion. Honeydew is excreted in large amounts. Earliest chemical characterizations date back to the 19th century (Berthelot 1859) and a vast body of literature regarding the composition is available (Bernays & Klein 2002; Hendrix et al. 1992; Nemec & Stary 1990; Woodring et al. in press; Yao & Akimoto 2001; Yao & Akimoto 2002). Lycaenid caterpillars, in contrast, secrete minute amounts in a strongly controlled manner dependent on their nutritional, behavioural social and ecological conditions (Agrawal & Fordyce 2000; Axén 2000; Axén & Pierce 1998; Axén et al. 1996; Burghardt & Fiedler 1996; Fiedler & Hagemann 1995; Leimar & Axén 1993). Few attempts to identify the composition of the nectar of lycaenid larvae have been published (DeVries 1988; Maschwitz et al. 1975; Nomura et al. 1992; Pierce 1983; Pierce & Nash 1999; Wagner 1994) and usually few quantitative data were reported at all, especially for facultative myrmecophiles (Maschwitz et al. 1975; Wagner 1994).

As mentioned before, the lycaenid-ant interactions show considerable variability, ranging from facultative to obligate myrmecophily. Caterpillars of different butterfly species are differently favoured by different ant species (Malicky 1969). Obligately myrmecophilous species are tended by specific ant species. For example various *Iridomyrmex* species tend the larvae of Australian *Jalmenus* species (Pierce & Nash 1999), and in SE Asia the weaver ant *Oecophylla smaragdina* specifically attends a couple of obligate associates, but hardly any other lycaenid larvae (Seufert & Fiedler 1996a). Chemical communication by the caterpillars on the one hand and oviposition behaviour of the female, which selects the hostplants according to the presence of the right ant species (Pierce & Elgar 1985; Seufert & Fiedler 1996a; Seufert & Fiedler 1996b; Smiley et al. 1988; van Dyck et al. 2000, but see Thomas & Elmes 2001), on the other, mediate this partner specificity in obligate interactions.

In contrast, facultative ant associations are not characterized by species or genus specificity (Fiedler 1991; Fiedler 2001; Malicky 1969; Peterson 1993; Schmidt & Rice 2002; Seufert & Fiedler 1996a). Nevertheless certain ant species are more often found tending these lycaenids than other ants. Furthermore facultative myrmecophily in the Lycaenidae can range from rather loose associations, which are maintained only for short time periods and easily break apart, to strong associations, which can be maintained for longer periods of time (albeit the lycaenids are still able to thrive without ant protection). These differences might possibly be attributed to the contents of the nectar secretions of the respective caterpillars (Pierce 1987).

This leads to the major aim of this thesis. Can the observed variability in ant attendance among facultatively myrmecophilous species be related to the contents and composition of their nectar secretions from the dorsal glands (sugars and free amino acids)?

Closely related to this question are the effects of larval nutrition on the contents of the nectar secretion. Are content and composition of the secretions largely independent of the nutrients a caterpillar has available, or does the transfer of nutrients to the secretion strongly depend on the ingested food?

Does nectar composition only reflect ecological attributes of the lycaenid species, or do taxonomic patterns also exist?

To address these questions a comparison between three facultatively myrmecophilous species of varying intimacy in ant-attendance was attempted here for the first time. For the comparative analyses a strongly myrmecophilous species was used, the European *Polyommatus coridon*, and two only moderately myrmecophilous species, the European *P. icarus* and the African *Zizeeria knysna*. Both *Polyommatus* species are rather closely related (Wiemers 2003), whereas *Z. knysna* is a far more distant relative (Eliot 1973; Scott & Wright 1990).

The results of the secretion analyses are subsequently validated in bioassays with ants. Do the preferences exhibited by ants match the nectar composition of their favoured lycaenid trophobionts respective what renders nectar secretions attractive?

The gustatory preferences of ants for components and whole analoga of the nectar were tested. For this purpose ant species were chosen from three different subfamilies, which regularly engage in trophobiotic interactions. As representative for the Formicinae *Lasius niger* was chosen. This very common ant species is also quantitatively one of the most important visitors of the lycaenids *Polyommatus coridon* and *P. icarus* in Central Europe (Fiedler 1991). *Myrmica rubra* was selected as representative of the Myrmicinae, several related species tend both *Polyommatus* species, too (Fiedler 1991). As a Dolichoderinae, *Tapinoma melanocephalum* was chosen, which is known to tend larvae of *Zizeeria knysna* and other (sub-)tropical lycaenid species (Fiedler 1991; Fiedler 2001).

Combining the results obtained from the nectar analyses with gravimetric data of caterpillars evaluation of the benefit for the ants is possible. Do ants really benefit from tending facultatively myrmecophilous lycaenid larvae, and to which extent does harvesting of nectar secretion really translate into fitness gains?

The answers to these questions enhance the understanding of the balance between the ant and butterfly partners in these facultative mutualisms, and are furthermore used to explore the evolutionary ecology of myrmecophily in the butterfly family Lycaenidae.

References

Ackery, P. R., de Jong, R. & Vane-Wright, R. I. 1999: The butterflies: Hedyloidea, Hesperioidea and Papilionoidea. In: Lepidoptera, Moths and Butterflies, Vol. 1 (Kristensen, N. P. ed). W deGruyter, Berlin, New York. pp. 263-300.

Agrawal, A. A. & Fordyce, J. A. 2000: Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B **267**, 1857-1861.

Akino, T., Knapp, J. J., Thomas, J. A. & Elmes, G. W. 1999: Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. Proc R Soc Lond B **266**, 1419-1426.

Atsatt, P. R. 1981: Lycaenid butterflies and ants: selection for enemy-free space. Am Nat **118**, 638-654.

Axelrod, R. & Hamilton, W. D. 1981: The evolution of cooperation. Science **211**, 1390-1396.

Axén, A. H. 2000: Variation in behavior of lycaenid larvae when attended by different ant species. Evol Ecol **14**, 611-625.

Axén, A. H. & Pierce, N. E. 1998: Aggregation as a cost-reducing strategy for lycaenid larvae. Behav Ecol **9**, 109-115.

Axén, H. A., Leimar, O. & Hoffman, V. 1996: Signalling in a mutualistic interaction. Anim Behav **52**, 321-333.

Ballmer, G. R. & Pratt, G. F. 1991: Quantification of ant attendance (myrmecophily) of lycaenid larvae. J. Res Lepid **30**, 95-112.

Baylis, M. & Pierce, N. E. 1992: Lack of compensation by final instar larvae of the myrmecophilous lycaenid butterfly, *Jalmenus evagoras*, for the loss of nutrients. Physiol Entomol **17**, 107-114.

Begon, M., Harper, J. L. & Townsend, C. R. 1996: Ecology. Blackwell Science, Oxford.

Bendor, J. & Swistak, P. 1995: Types of evolutionary stability and the problem of cooperation. Proc Natl Acad Sci USA **92**, 3596-3600.

Bernays, E. A. & Klein, B. A. 2002: Quantifying the symbiont contribution to essential amino acids in aphids: the importance of tryptophan for *Uroleucon ambrosiae*. Physiol Entomol **27**, 275-284.

Berthelot, M. 1859: Nouvelles recherches sur les corps analogues au sucre de canne. Ann chim et phys, 269-296.

Boucher, D. H. 1982: The ecology of mutualism. Ann Rev Ecol Syst **13**, 315-347.

-. 1985: The idea of mutualism, past and future. In: The Biology of Mutualism - Ecology and Evolution (Boucher, D. H. ed). Crown Helm, London. pp. 1-28.

Boyd, R. & Lorberbaum, J. P. 1987: No pure strategy is evolutionary stable in the repeated Prisoner's Dilemma game. Nature **327**, 58-59.

Braby, M. F. 2000: Butterflies of Australia, their identification, biology and distribution. CSIRO, Melbourne.

Brembs, B. 1996: Chaos, cheating and cooperation: potential solutions to the prisoner's dilemma. Oikos **76**, 14-24.

Bristow, C. M. 1991: Why are so few aphids ant-tended. In: Ant-plant interactions (Huxley, C. R. & Cutler, D. F. eds). Oxford University Press, Oxford. pp. 104-119.

Bronstein, J. L. 1994: Conditional outcomes in mutualistic interactions. Trends Ecol Evol **9**, 214-217.

-. 1998: The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica **30**, 150-161.

-. 2001: The exploitation of mutualism. Ecol Lett **4**, 277-287.

Burghardt, F. & Fiedler, K. 1996: Myrmecophilous behaviours in caterpillars of the butterfly, *Polyommatus icarus* (Rottemburg, 1775): temporal patterns and age dependency (Lepidoptera: Lycaenidae). Nota lepid **19**, 212-229.

Chapman, T. A. 1902: On the larva of *Liphyra brassolis* Westw. Entomologist **35**, 225-228+252-255.

Claassens, A. J. M. & Dickson, C. G. C. 1977: A study of the myrmecophilous behaviour of the immature stages of *Aloides thyra* (L.) (Lep.: Lycaenidae) with special reference to the function of the retractile tubercles and with additional notes on the general biology of the species. Entomologist's Record **89**, 225-231.

Cottrell, G. B. 1984: Aphytophagy in butterflies: its relationship to myrmecophily. Zool J Linnean Soc **79**, 1-57.

Cushman, J. H., Rashbrook, V. K. & Beattie, A. J. 1994: Assessing benefits to both participants in a lycaenid-ant association. Ecology **75**, 1031-1041.

Denis, M. 1785: Entomologische Fragmente. In: Neues Magazin für Liebhaber der Entomologie (Fuessly, J. C. ed). pp. 370-378.

DeVries, P. J. 1988: The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants. Zool J Linnean Soc **94**, 379-393.

-. 1991a: Evolutionary and ecological patterns in myrmecophilous ridodinid butterflies. In: Ant plant interactions (Huxley, C. R. & Cutler, D. F. eds). Oxford University Press, Oxford. pp. 143-171.

-. 1991b: Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. Biol J Linnean Soc **43**, 179-195.

DeVries, P. J. & Poinar, G. O. 1997: Ancient butterfly ant symbiosis: direct evidence from Dominican amber. Proc R Soc Lond B **264**, 1137-1140.

Doebeli, M. & Knowlton, N. 1998: The evolution of interspecific mutualism. Proc Natl Acad Sci USA **95**, 8676-8680.

Dugatkin, L. A. 1997: Cooperation among animals. Oxford University Press, New York.

Eastwood, R. & Fraser, A. M. 1999: Associations between lycaenid butterflies and ants in Australia. Aust Ecol **24**, 503-537.

Edwards, W. H. 1878: Notes on *Lycaena pseudargiolus* and its larval history. Can Entomol **10**, 1-14.

Ehrhardt, R. 1914: Über die Biologie und Histologie der myrmekophilen Organe von *Lycaena orion*. Ber naturf Ges Freiburg **20**, 90-98.

Eliot, J. N. 1973: The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. Bull Brit Mus Nat Hist (Entomol) **28**, 371-505.

Fiedler, K. 1991: Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionidae). Bonner Zoologische Monographien **31**.

-. 1997a: Geographical patterns in life-history trait of Lycaenidae butterflies - ecological and evolutionary implications. Zoology **100**, 336-347.

-. 1997b: Life-history patterns of myrmecophilous butterflies and other insects: their implications on tropical species diversity. In: Proceedings of the International Symposium on Biodiversity and Systematics in Tropical Ecosystems (Ulrich, H. ed). Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

-. 1998: Lycaenid-ant interactions of the *Maculinea* type: tracing their historical roots in a comparative framework. J Insect Conservation **2**, 3-14.

-. 2001: Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. Divers Distrib **7**, 45-60.

Fiedler, K. & Hagemann, D. [1995]: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K. & Hölldobler, B. 1992: Ants and *Polyommatus icarus* immatures (Lycaenidae): sex related developmental benefits and costs of ant attendance. Oecologia **91**, 468-476.

Fiedler, K., Hölldobler, B. & Seufert, P. 1996: Butterflies and ants: the communicative domain. Experientia **52**, 14-24.

Fiedler, K. & Maschwitz, U. 1987: Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) III. New aspects of the function of the retractile tentacular organs of lycaenid larvae. Zool Beitr N F **31**, 409-416.

-. 1988: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) II. Lycaenid larvae as trophobiotic partners of ants - an quantitative approach. Oecologia **75**, 204-206.

-. 1989: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. Ethology **80**, 71-80.

Fiedler, K. & Saam, C. 1994: Does ant-attendance influence development in 5 European Lycaenide butterfly species ? (Lepidoptera). Nota lepid **17**, 5-24.

-. 1995: Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study. Oecologia **104**, 316-322.

Fiori, G. 1958: "Strymon ilicis" Esp. (lepid. Lycaenidae). Boll Ist Ent Univ Bologna **22**, 202-256.

Franzl, S., Locke, M. & Huie, P. 1984: Lenticles: innervated secretory structures that are expressed at every other larval moult. Tissue Cell **16**, 251-268.

Heath, A. & Claassens, A. J. M. 2003: Ant-association among Southern African Lycaenidae. J Lepid Soc **57**, 1-16.

Hendrix, D. L., Wei, Y.-A. & Leggett, J. E. 1992: Homopteran honeydew sugar composition is determined by both the insect and plant species. Comp Biochem Physiol B **101**, 1/2.

Henning, S. F. 1983: Chemical communication between lycaenid larvae (Lepidoptera: Lycaenide) and ants (Hymenoptera: Formicidae). J ent Soc sth Afr **46**, 341-366.

Herre, E. A., Knowlton, N., Mueller, U. G. & Rehner, S. A. 1999: The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol Evol **14**, 49-53.

Hoeksema, J. D. & Bruna, E. M. 2000: Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. Oecologia **125**, 321-330.

Hölldobler, B. 1996: Verständigung im Ameisenstaat. Verh Dtsch Zool Ges **89.2**, 7-24.

Hölldobler, B. & Wilson, E. O. 1990: The ants. Harvard University Press / Springer, Heidelberg.

Jolivet, P. 1996: Ants and plants: an example of coevolution (enlarged edition). Backhuys Publishers, Leiden.

Kitching, R. L. 1983: Myrmecophilous organs of the larvae and pupae of the lycaenid butterfly *Jalmenus evagoras*. J Nat Hist **17**, 471-481.

Kitching, R. L. & Luke, B. 1985: The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist **19**, 259-276.

Kropotkin, P. 1902: Mutual aid: a factor in evolution. Heinemann, London.

Leimar, O. & Axén, A. H. 1993: Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav **46**, 1177-1182.

Lenz, F. 1917: Der Erhaltungsgrad der Myrmekophilie. Z indukt Abstammungs Vererbungsl **19**, 44-46.

Madigan, M. T., Martinko, J. M. & Parker, J. 2000: Brock - Biology of Microorganisms 9th ed. Prentice-Hall, London.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

-. 1970: New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymneoptera). J Lep Soc **24**, 190-202.

Maschwitz, U., Wüst, M. & Schurian, K. 1975: Bläulingsraupen als Zuckerlieferanten. Oecologia **18**, 17-21.

Muthukrishnan, J. & Pandian, T. J. 1987: Insecta. In: Animal Energetics (Pandian, T. J. & Vernberg, F. J. eds). Academic Press, New York.

Nash, D. R. 1989: Cost-benefit analysis of a mutualism between lycaenid butterflies and ants. Ph.D. thesis, University of Oxford, Oxford.

Nemec, V. & Stary, P. 1990: Sugars in honeydew. Biologia Bratislava **45**, 259-264.

Newcomer, E. J. 1912: Some observations on the relations of ants and lycaenid caterpillars, and a description of the relational organs of the latter. J NY Entomol Soc **20**, 31-36.

Noe, R. & Hammerstein, P. 1994: Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behav Ecol Sociobiol **35**, 1-11.

-. 1995: Biological markets. Trends Ecol Evol **10**, 336-339.

Nomura, K., Hirukawa, N., Yamaoka, R. & Imafuku, M. 1992: Problems on the symbiosis between the lycaenid butterfly larva, *Niphanda fusca shijimia* and the ant *Camponotus* (*Camponotus*) *japonicus*. Tyo to Ga **43**, 138-143.

Peterson, M. A. 1993: The nature of ant attendance and the survival of larval *Icaricia acmon* (Lycaenidae). J Lepid Soc **47**, 8-16.

Pierce, N. E. 1983: The ecology and evolution of the symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University.

-. 1987: The evolution and biogeography of associations between lycaenid butterflies and ants. Oxford Surveys in Evolutionary Biology **4**, 89-116.

-. 2001: Peeling the onion: Symbioses between ants and blue butterflies. In: Model Systems in Behavioral Ecology (Dugatkin, L. A. ed). Princeton University Press, Princeton. pp. 41-56.

Pierce, N. E., Braby, M. F., Huxley, C. R., Lohman, D. J., Mathew, J., Rand, D. B. & Travassos, M. A. 2002: The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol **47**, 733-771.

Pierce, N. E. & Easteal, S. 1986: The selective advantage of attendant ants for the larvae of a lycaenid butterfly, *Glaucopsyche lygdamus*. J Anim Ecol **55**, 451-462.

Pierce, N. E. & Elgar, M. A. 1985: The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. Behav Ecol Sociobiol **16**, 209-222.

Pierce, N. E., Kitching, R. L., Buckley, R., Taylor, M. F. J. & Benbow, K. F. 1987: The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. Behav Ecol Sociobiol **21**, 237-248.

Pierce, N. E. & Mead, P. S. 1981: Parasitoids as selective agents in the symbiosis between lycaenid butterfly larvae and ants. Science **21**, 1185-1187.

Pierce, N. E. & Nash, D. R. 1999: The Imperial Blue, *Jalmenus evagoras* (Lycaenide). In: Biology of Australian Butterflies, Vol. 6 (Kitching, R. L., Scheermeyer, E., Jones, R. E. & Pierce, N. E. eds). CSIRO Publishing, Melbourne. pp. 279-317.

Robbins, R. K. 1991: Cost and evolution of a facultative mutualism between ants and lycaenid larvae (Lepidoptera). Oikos **62**, 363-369.

Schmidt, D. J. & Rice, S. J. 2002: Association of ants with juvenile *Ogyris amaryllis amaryllis* (Hewitson) (Lepidoptera: Lycaenidae) in south-eastern Queensland. Aust J Entomol **41**, 164-169.

Schultz, T. R. & McGlynn, T. P. 2000: The interactions of ants with other organisms. In: Ants - standard methods for measuring and monitoring biodiversity (Agosti, D., Majer, J. D., Alonso, L. E. & Schultz, T. R. eds). Smithsonian Institution Press, Washington, London.

Schwartz, M. W. & Hoeksema, J. D. 1998: Specialization and resource trade: biological markets as a model of mutualisms. Ecology **79**, 1029-1038.

Scott, J. A. & Wright, D. M. 1990: Butterfly phylogeny and fossils. In: Butterflies of Europe Vol 2: Introduction to lepidopterology (Kudrna, O. ed). Aula Verlag, Wiesbaden.

Seufert, P. & Fiedler, K. 1996a: The influence of ants on the patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a south-east Asian tropical rain forest. Oecologia **106**, 127-136.

-. 1996b: Life history diversity and local coexistence of three closely related lycaenid butterflies (Lepidoptera: Lycaenidae) in Malaysian rainforests. Zool Anz **234**, 229-239.

-. 1999: Myrmecophily and parasitoid infestation of south-east asian lycaenid butterfly larvae. Ecotropica **5**, 56-94.

Smiley, J. T., Atsatt, P. R. & Pierce, N. E. 1988: Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. Oecologia **76**, 416-422.

Tautz, J. & Fiedler, K. 1992: Mechanoreceptive properties of caterpillar hairs involved in mediation of butterfly-ant symbioses. Naturwissenschaften **79**, 561-563.

Thomas, J. A. & Elmes, G. W. 1998: Higher productivity at the cost of increased host specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. Ecol Entomol **23**, 457-464.

-. 2001: Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. Proc R Soc Lond B **268**, 471-477.

Trivers, R. L. 1971: The evolution of reciprocal altruism. Q Rev Biol **46**, 35-57.

van Beneden, P. J. 1875: Les Commensaux et les parasites. Biblio Sci Int, Paris.

van Dyck, H., Oostermeijer, J. G. B., Talloen, W., Feenstra, V., van der Hidde, A. & Wynhoff, I. 2000: Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? Proc R Soc Lond B **267**, 861-866.

Wagner, D. 1993: Species-specific effects of tending ants on the development of lycaenid butterfly larvae. Oecologia **96**, 276-281.

-. 1994: Species-specific effects of tending ants on the life history of *Hemiargus isola*, a North American lycaenid butterfly. Ph.D. thesis, Princeton University, Princeton.

Wagner, D. & Martínez del Rio, C. 1997: Experimental tests of the mechanism for ant-enhanced growth in an ant-tended lycaenid butterfly. Oecologia **112**, 424-429.

Wiemers, M. 2003: Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*) - a molecular phylogenetic approach. Ph.D. thesis, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn.

Woodring, J., Wiedemann, R., Fischer, M. K., Hoffmann, K. H. & Völkl, W. in press: Honeydew amino acids in relation to sugars and their role in the establishment of ant-attendance hierarchy in eight species of aphids feeding on tansy (*Tanacetum vulgare*). Physiol Entomol.

Yao, I. & Akimoto, S. 2001: Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Oecologia **128**, 36-43.

-. 2002: Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Ecol Entomol **27**, 745-752.

2 General life-history traits of the species investigated

H. Daniels

Department of Animal Ecology I, University of Bayreuth, D-95440 Bayreuth, Germany

2.1 Lycaenid butterflies

***Polyommatus* (*Lysandra*) *coridon* (Poda 1761)**

The "Chalk Hill Blue" occurs from Northern Spain to the Ural river in Russia and from the southern coast of the Baltic Sea to Sardinia and central Italy, respectively (Ebert & Rennwald 1991). It is widespread on calcareous grasslands, and can reach very high population densities on suitable sites, e.g. warm, often southerly oriented hill slopes. In western Europe the caterpillars feed only on *Hippocrepis comosa* under natural conditions, from Poland on eastwards they switch to *Securigera* (*Coronilla*) *varia*. Both plants occur only on calcareous soils, therefore *P. coridon* cannot thrive on acidic soils. In central Europe the flight period of the only generation per year extends from the end of June till early September. Females oviposit either directly on the host plants or in the immediate vicinity on the soil or stones or on leaves of dead grass. The eggs undergo an extended diapause during autumn and winter and the larvae hatch very early in the following spring. Most populations are strictly univoltine, but a bivoltine population occurs in a few places in Slovakia (Tolman & Lewington 1998). Apart from that, due to different glacial refugia, subsequent postglacial migration and area expansion there seem to be two major genetic lineages, one in Western Germany and westwards and one east of NE-Germany and the German-Czech Republic border (Schmitt et al. 2002; Schmitt & Seitz 2001). *P. coridon* is one of the six most characteristic butterfly species of European calcareous grasslands (van Swaay 2002). These semi-natural calcareous grasslands suffered severe fragmentation in Europe in the last few decades, which reducing the limited dispersal from or into the mostly closed populations (Schmitt & Seitz 2002) further. *P. coridon* is listed in some of the regional Red Data books of Germany (Ebert & Rennwald 1991).

P. coridon is a highly, but facultatively myrmecophilous species. Its caterpillars have been reported to associate with the following ant species: *Lasius niger*, *L. paralienus*, *L. alienus*, *L. flavus*, *L. fuliginosus*(?), *Plagiolepis vindobonensis*, *Formica rufa*, *F. lemani*, *F. cunicularia* (Formicinae), *Myrmica scabrinodis*, *M. sabuleti*, *M. schencki*, *Aphaenogaster subterranea*, *Leptothorax nigriceps*, *Tetramorium caespitum* (Myrmicinae), *Tapinoma ambiguum* and *T. erraticum* (Dolichoderinae) (Fiedler 1987; Fiedler & Rosciszewski 1990; Kitching & Luke 1985; Malicky 1969; Pfeuffer 2000; Tolman & Lewington 1998, K. Fiedler pers. comm.) This list of mutualistic ant associates is certainly not yet exhaustive.

***Polyommatus* (*Polyommatus*) *icarus* (Rottemburg 1775)**

The “Common Blue” can be found from western Europe to East Asia (North Korea and Sakhalin) and from the northern polar circle to northern Africa and non-tropical Asia (Ebert & Rennwald 1991; Lukhtanov & Lukhtanov 1994). Since it is highly generalized in its habitat requirements, it settles in a wide variety of habitats, preferring open vegetation (Ebert & Rennwald 1991 ; van Swaay 2002). In Germany *P. icarus* usually occurs in two generations, from about June to mid July and from end of July to August. In the summer generation the population sizes are usually greater. The offspring of the second generation overwinters in different larval stages. In very warm areas or in hot summers a third generation is possible. On the Canary Islands they even occur the whole year round (Tolman & Lewington 1998). Its larvae feed on a number of herbaceous Fabaceae (Ebert & Rennwald 1991; Martín Cano 1984), often preferring *Lotus corniculatus*. The eggs are mostly laid in the flowers or on young buds. Due to its versatility it is not endangered, although population losses occurred as a consequence of land-use changes, e.g. the intensification of agricultural practices.

P. icarus is a moderately myrmecophilous species, less strongly ant associated than *P. coridon* (Fiedler 1991). Its caterpillars associate with the following ant species: *Lasius niger*, *L. alienus*, *L. flavus*, *L. fuliginosus*(?), *Plagiolepis pygmaea*, *Formica subrufa*, *F. rufibarbis*, *F. subpilosa*, *F. cinerea*(?) (Formicinae), *Myrmica sabuleti*, *M. lobicornis* and *M. tenuispina* (Myrmicinae) (Emmet & Heath 1990; Jutzeler 1989; Kitching & Luke 1985; Malicky 1969; Martín Cano 1984; SBN 1987; Tolman & Lewington 1998).

***Zizeeria knysna* (Trimen 1862)**

The “African Grass Blue” is distributed from the Iberian Peninsula and the Canary Islands southwards through most parts of Africa, including Madagascar, and eastwards to Arabia (Wiemers 1995). The small butterfly prefers open, xeric habitats with moist spots, as e.g. coastal canyons or oases. It is polyvoltine, with up to whole-year-round-occurrence under favourable conditions, as the larvae have a short development time (32 days from egg to adult at 26°C constant and 18:6 LD cycle, unpublished data). The caterpillars feed on a variety of plants from the families Fabaceae, Amaranthaceae, Zygophyllaceae, Oxalidaceae, Chenopodiaceae and Euphorbiaceae (Clark & Dickson 1971; Tolman & Lewington 1998). The eggs are mostly laid in the flowers or on young buds. *Z. knysna* is seemingly not (yet) endangered.

It is a facultatively and rather strongly myrmecophilous species (Fiedler 1991; Heath & Claassens 2003), although literature records regarding identifications of tending ants are almost unavailable. Its caterpillars are known to be visited by the ant species *Tapinoma melanocephalum* (Dolichoderinae) and *Pheidole* sp. (Myrmicinae) (Tolman & Lewington 1998). In the laboratory associations may also be formed with *Lasius niger*, *L. flavus* and *Myrmica rubra*, although with the latter these associations tend to be unstable (own observations, K. Fiedler pers. comm.).

2.2 Food plants

Medicago sativa **(Linnaeus)**

The perennial herb "Alfalfa" is one of the earliest cultivated food plants, originally originating from the east Mediterranean, but today it is occurring worldwide .Its common name is derived from the Arabian al-fac-facah, the "father of all foods". The plant is still widely used as food for cattle. In northern America its specialized pollinator bee is even industrially rentable for pollination. It occurs also in meadows and along road sides. Due to its symbiotic nitrogen fixation it has a relative high nutritive value, although some lineages can be toxic (Small 1996).

M. sativa is a natural food plant of *P. icarus*, although if the caterpillars are fed on foliage alone rather than flowers and foliage, they develop slowly and reach lower pupal weights (Burghardt & Fiedler 1996). *M. sativa* is also used as food plant by other Lycaenids, e.g. *Plebejus argus*, *Celastrina argiolus*, *Cupido argiades*, and by *Z. knysna* in the lab (Fiedler & Hagemann 1995, own observations), thus contradicting Tolman (1998).

Hippocrepis comosa **(Linnaeus)**

The "Horseshoe Vetch" is a long-living perennial herb, the base often woody and deeply rooting (Fearn 1973). It is distributed in middle and south Europe. The cyanogenous plant is typical for xerophilous grasslands and rocky cliffs, naturally preferring limestone ground, although it can be raised even on siliceous substrates.

Due to its remarkable frost tolerance (Hennenberg & Bruelheide 2003) the shoots and a large part of the leaves stay green throughout the winter and are thus available as food for the early spring hatching *P. coridon* caterpillars (see above). *Polyommatus bellargus* and *Plebejus argus* feed also from *H. comosa* (Fiedler 1991).

Securigera **(*Coronilla*)** ***varia*** **(Linnaeus) Lassen**

The "Crown Vetch" is a perennial herb with trailing to ascendent stems, reaching up to one meter height. This European species is abundant on ruderalized ground, road sides and it can be used for erosion control. For the latter usage it was also introduced in North America. The cyanogenous plant contains other toxins as well (Shenk et al. 1976). *S. varia* is an alternative food plant for *P. coridon* (see above). A number of other Lycaenids (*Cupido argiades*, *Glaucopsyche alexis*, *Plebejus argus, Polyommatus bellargus*, *P. daphnis*) use it as well and the plant can also be fed to *P. icarus* (Burghardt & Fiedler 1996; Fiedler 1990) or *Z. knysna* (unpublished data).

2.3 Ants

Lasius (Cautolasius) flavus **(Fabricius 1781)**

Lasius flavus is a very common holarctic Formicinae species, which lives subterraneously, preferring dry and moist grasslands, but is sometimes also found in bogs or light woods. Due to heavy nitrogen fertilization its abundance is decreasing. Its grass-overgrown mounds are typical for "cobbled" cattle pastures, often neighbouring several other nests within few meters. The hills are highly resistant to trampling damage. They may reach ages of over 150 years and are therefore used as a dating method in biogeography (King 1981; Kovár et al. 2000). *L. flavus* as a superb "master builder" moves tremendous amounts of earth, it is therefore of importance for soil development. Its hills grow with one litre per year and in one hectar up to seven tons of earth are moved per year (King 1981; Seifert 1996). The mounds house huge monogynous nests with up to 100 000 workers.

Due to its lifestyle *L. flavus* is a weakly aggressive ant and actually preyed upon by other ants (De Vroey 1979; De Vroey & Pasteels 1978). It lives almost exclusively subterraneously, harvesting the honeydew excretions of up to 22 different root aphid species (Mordwilko 1907; Pontin 1963; Pontin 1978; Seifert 1996). Due to this lifestyle *L. flavus* rarely encounters lycaenid larvae in nature, but if larvae are presented, it readily enters into highly successful and stable trophobiotic interactions (Fiedler 1990; Fiedler 1991; Rayward 1906). As it is such an effective tender of trophobiont and relatively easily cultivable in the laboratory, it is used as a standard ant for bio assays with Lycaenids (Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler & Saam 1994; Fiedler & Saam 1995).

Lasius **(*****Lasius*** **s. str.)** ***niger*** **(Linnaeus 1758)**

Lasius niger is a pioneer Formicinae species (Bonte et al. 2003) accepting moderate xerothermic to mesophilic habitats. Next to natural habitats it also readily settles at road banks, in parks, cities and industrial areas. Due to its versatility it is not endangered. The monogynous nests house up to over 10 000 workers and are build into the ground, often under stones or sometimes in rotten wood. In summer the nests are extended with hills composed of loose dry earth. This material is also used for the building of roofs over the ants' roads, or as shelters over aphids (Mordwilko 1907) and lycaenid larvae (Fiedler & Maschwitz 1988; Pfeuffer 2000, own observations).

L. niger is a relatively aggressive ant species (Mabelis 1984) and one of the dominant species in middle-Europe (Fiedler 2001). It is highly omnivorous, even eating seeds and its own species (Pontin 1961; Stradling 1978). It often occurs as a pest in buildings, but does not act as disease vector. It enters trophobiotic interactions with a high number of aphid species and several lycaenid species (Fiedler 1991). *L. niger* and the closely related *L. alienus*, *L. paralienus* and *L. platythorax* are quantitatively the most important ant visitors of larvae of *P. coridon* and *P. icarus* in many places in Central Europe.

Myrmica rubra **(Linnaeus 1758)**

The Myrmicinae *Myrmica rubra* is the most common and ecologically most versatile of the European *Myrmica* species. It can settle in almost all habitats, although preferring mesophilic to moist conditions. It can be the dominating ant species in meadows and is not endangered. It nests in rotten wood, plant cushions, in the ground or under stones. The nests are highly polygynous and can house up to 600 queens with more than 20 000 workers.

Myrmica species are weak competitors and on food sources often subordinate to some *Formica* species and to *Lasius niger* (De Vroey 1979; Fiedler 2001; Vepsäläinen & Savolainen 1990). However it is highly aggressive against other *M. rubra* colonies and *L. flavus* (De Vroey 1979; De Vroey & Pasteels 1978; Seifert 1996).

Myrmica species avoid foraging around midday and lay weak pheromone trails (van Dyck et al. 2000). The omnivorous *M. rubra* forages heavily on trees and bushes, preying or scavenging on arthropods, harvesting plant nectar and tending aphids and coccids (Seifert 1988).

Several species of the ant-parasite lycaenid genus *Maculinea* use *M. rubra* as host (Als et al. 2002; Ehrhardt 2002; Elmes et al. 2002; Schönrogge et al. 2004; Stankiewicz & Sielezniew 2002). *M. rubra* has voluntary trophobiotic interactions with non parasitic lycaenid species as well, e.g. *Polyommatus* (*Aricia*) *eumedon*, *Cupido minimus*. Other species of the genus *Myrmica* in general are relatively often associated with a high number of lycaenid species (Fiedler 1991).

Tapinoma melanocephalum **(Fabricius, 1793)**

The exact geographical origin of this Dolichoderinae species is unknown, perhaps Africa or the orient (Thompson 1990). It was widely distributed with human trading and therefore occurs in tropical to mid-latitude areas in America, Asia and Africa. It was introduced into Europe for the first time already in 1883 (Donisthorpe 1927) but can only live inside buildings, as it needs a nesting temperature of 25-32°C and a relative humidity of 70-90%. If the environment gets too dry, the ants start to migrate. *T. melanocephalum* is very catholic in its nest site choice (soil, beneath stones, detritus or tree bark, in plant cavities, rotten wood, hollow twigs, closets, clothing piles, building crevices). The polygynous nests house 100-1000 workers. Colonies typically divide in subunits, which can further retain contact. There seems to be no real nuptial flight (Harada 1990; Scheurer 1989; Scheurer & Liebig 1998). It is seen as an annoying pest in buildings, but seems not to have hospitalic significance. On plants it secondarily causes damage by caring for myrmecophilic pest insects (Fowler et al. 1990; Veeresh 1990). The workers run relatively fast (up to 3.5cm/s) in erratic movements and forage in nature on honeydew as well as on living and dead insects. In buildings they feed on raw meat, dead insects, *Musca domestica* offspring, egg-yolk, bakery products, fruits and kitchen refuse (Harada 1990; Scheurer 1984). It reportedly associates with various lycaenid species such as *Z. knysna*, *Z. karsandra* and *Lampides boeticus*. Other *Tapinoma* species are also regularly recorded as visitors of various lycaenid species; e.g. *Glaucopsyche alexis*, *Iolana iolas*, *Scolitantides orion*, *Plebejus* sp, *Polyommatus* sp (Fiedler 1991).

Colour plates

Legends:

Plate 1 (next page)

Life-stages of *Polyommatus coridon*: **A** copula, male to the left, female to the right **B** caterpillar, early 2nd instar on *Hippocrepis comosa* **C** caterpillar, early 3rd instar on *Hippocrepis comosa* **D** caterpillar, 3rd instar on *Hippocrepis comosa* **E** two caterpillars, 4th instar at the base of a *Hippocrepis comosa* rosette **F** non-feeding, non mobile prepupa tended by two *Lasius flavus* workers **G** three day old pupa **H** *Cotesia cupreus* (Hymenoptera) cocoons at the ventral side of a still living 4th instar caterpillar on *Securigera varia* **I** caterpillar carcass, 4th instar, puparium of emerged *Aplomya* sp. (Diptera) to the top **J** latter half of abdomen, dorsal view, dorsal nectar organ (DNO) visible as slit central on segment 7, paired tentacle organs everted on segment 8 **K** caterpillar, 4th instar tended by several *Lasius flavus* workers, one droplet secreted at DNO

Plate 2 (page 21)

Life stages of *Polyommatus icarus*: **A** female to the left, male to the right, eggs to the right on *Lotus corniculatus* **B** two males (one to the front, one in the back) and five females sucking on an artificial flower **C** caterpillar, 4th instar on *Medicago sativa*

Life stages of *Zizeeria knysna*: **D** males and females on *Medicago sativa* in the flight cage **E** caterpillars displaying different colours, 4th instar **F** fresh pupa **G** *Tapinoma melanocephalum* workers tending 4th instar caterpillar **H** all stages in comparison: on top eggs and feeding spot of 1st instar caterpillar on *Medicago sativa* leaf, top right early 1st instar caterpillar, below 2nd instar caterpillar, bottom right 3rd instar caterpillar, central 4th instar caterpillar, bottom central non-feeding, still mobile prepupa, central left girdled prepupa, bottom left pupa

food plants: **I** *Hippocrepis comosa* **J** bunch of nectar plants, mainly *Securigera varia* in flight cage **K** *Medicago sativa*

Plate 3 (page 22)

Methods: **A** raising an artificial second generation of *Polyommatus coridon*, some of the glass vials with eggs and *Hippocrepis comosa* plant pots under continuous light **B** several glass vials on moist cellulose tissue **C** dissected abdomen of *Lasius flavus* worker, crop thoroughly filled with coloured sucrose solution **D** *Cotesia cupreus* female with *P. icarus* caterpillar **E** artificial ant, relay with *Homo sapiens* hair in silicone rubber tube to the front, in the back circuit board with resistor array **F** *Lasius flavus* nest in the laboratory **G** *Lasius flavus* worker tending *Polyommatus coridon* caterpillar, 4th instar, microcapillary ready to harvest the tiny nectar secretions droplets **H** *Myrmica rubra* nest in the laboratory **I** *Tapinoma melanocephalum* nest in the laboratory **J** Bioassay with *Lasius niger* **K** Bioassay with *Myrmica rubra* in the laboratory **L** Bioassay with *Tapinoma melanocephalum* in the laboratory

Plate 1: *Polyommatus coridon*

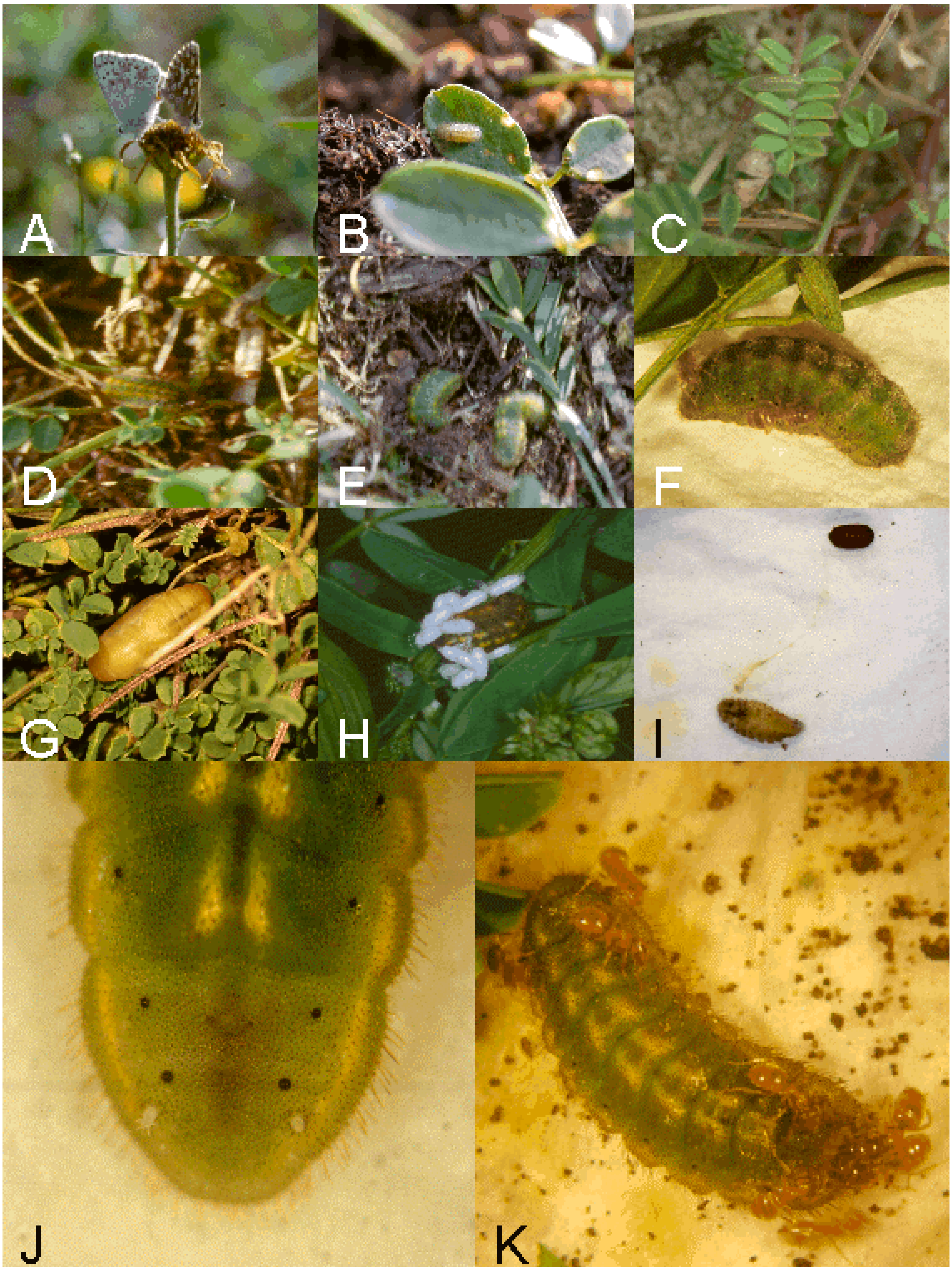


Plate 2: *Polyommatus icarus*, *Zizeeria knysna* and different food plants

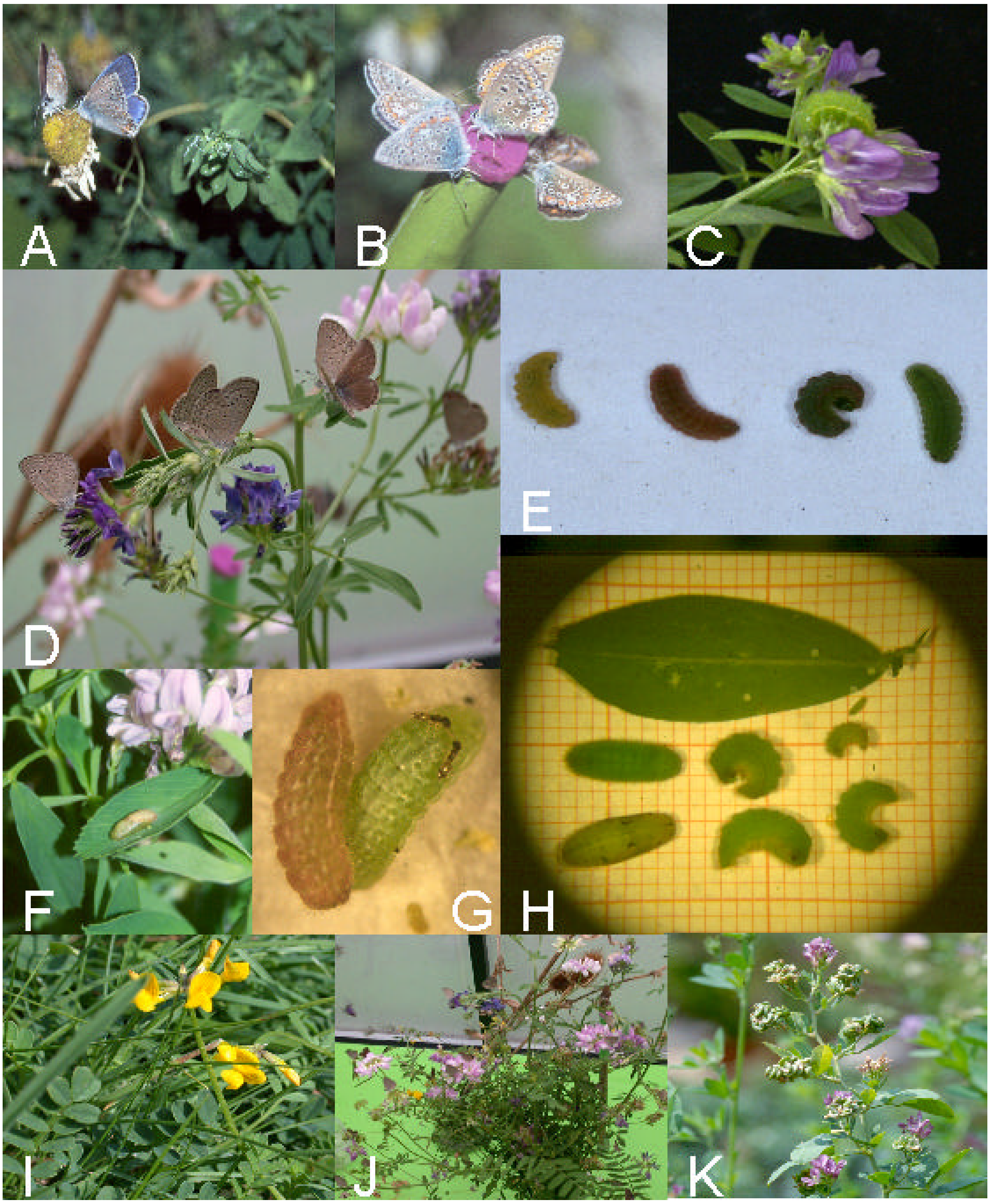
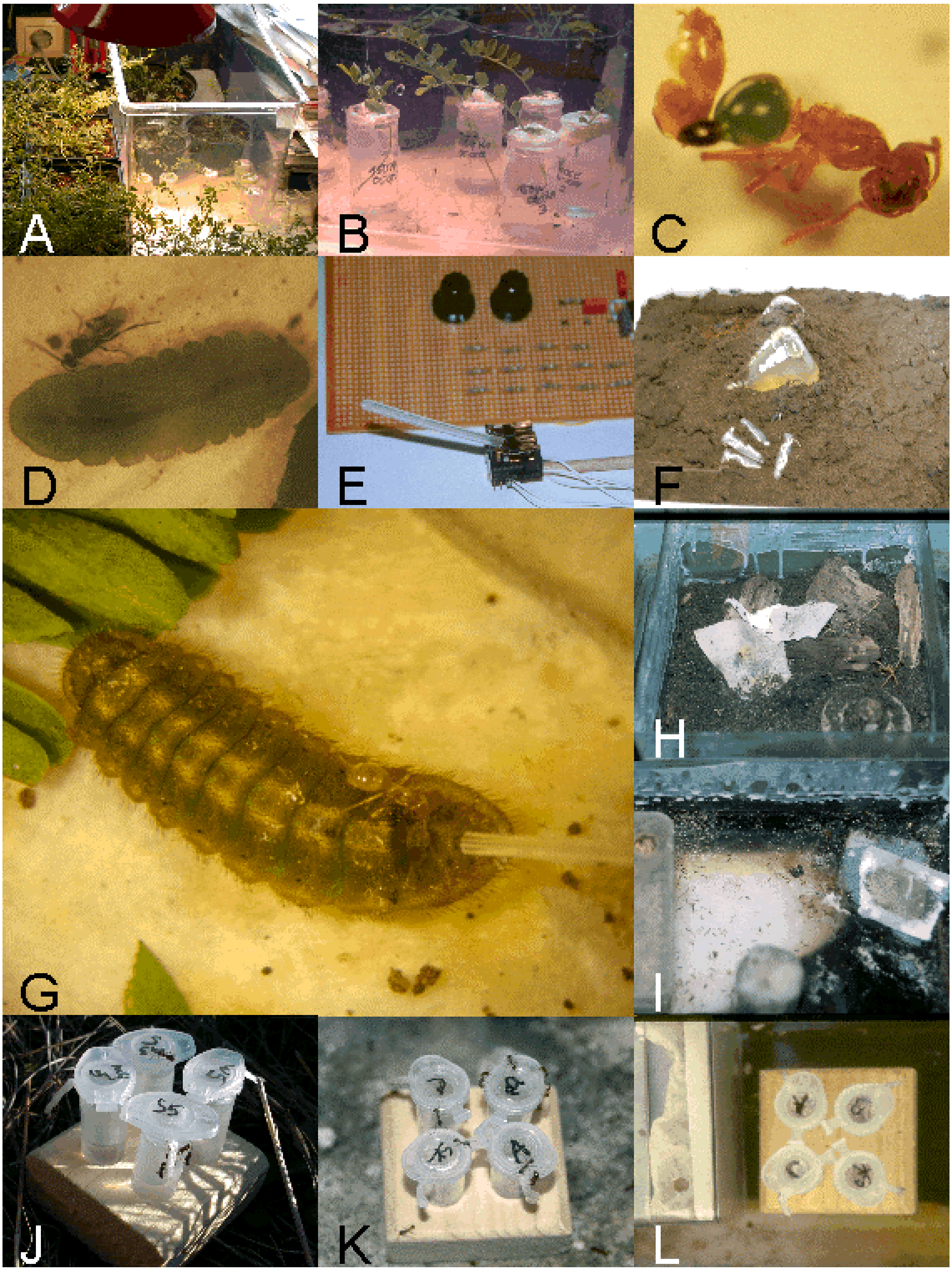


Plate 3: Methods



References

Als, T. D., Nash, D. R. & Boomsma, J. J. 2002: Geographical variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. Ecol Entomol **27**, 403-414.

Bonte, D., Dekonick, W., Provoost, S., Cosijns, E. & Hoffmann, M. 2003: Microgeographical distribution of ants (Hymenoptera: Formicidae) in coastal dune grassland and their relation to the soil structure and vegetation. Anim Biol **53**, 367-377.

Burghardt, F. & Fiedler, K. 1996: The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). Ecol Entomol **21**, 1-8.

Clark, G. C. & Dickson, C. G. C. 1971: Life histories of South African lycaenid butterflies. Purnell, Cape Town.

De Vroey, C. 1979: Aggression and Gause's Law in Ants. Physiol Entomol **4**, 217-222.

De Vroey, C. & Pasteels, J. M. 1978: Agonistic Behavior of *Myrmica rubra*. Insectes Sociaux **25**, 247-265.

Donisthorpe, H. S. 1927: British Ants. Routledge & Sons, London.

Ebert, G. & Rennwald, E. 1991: Die Schmetterlinge Baden-Württembergs. Eugen Ulmer Verlag, Stuttgart.

Ehrhardt, W. 2002: Über einen erfolgreichen Aussetzungsversuch bei *Glaucopsyche* (*Maculinea*) *alcon* ([Denis & Schiffermüller], 1775)(Lepidoptera: Lycaenide). Nachr entomol Ver Apollo Frankfurt N F **22**, 245-250.

Elmes, G. W., Akino, T., Thomas, J. A., Clarke, R. T. & Knapp, J. J. 2002: Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. Oecologia **130**, 525-535.

Emmet, A. M. & Heath, A. 1990: The butterflies. Harley books, Colchester.

Fearn, G. M. 1973: Biological flora of the British Isles. *Hippocrepis comosa* L. J Ecol **61**, 915-926.

Fiedler, K. 1987: *Tetramorium caespitum* (Linnaeus 1758)(Hymenoptera: Formicidae), eine weitere mit *Polyommatus coridon* (Poda 1761)(Lepidoptera: Lycaenidae) vergesellschaftete Ameisenart. Nachr entomol Ver Apollo Frankfurt N F **8**, 60.

-. 1990: Effects of larval diet on myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenide). Oecologia **83**, 284-287.

-. 1991: Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionidae). Bonner Zoologische Monographien **31**.

-. 2001: Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. Divers Distrib **7**, 45-60.

Fiedler, K. & Hagemann, D. 1995: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K. & Hummel, V. 1995: Myrmecophily in the brown argus butterfly, *Aricia agestis* (Lepidoptera: Lycaenidae): Effects of larval age, ant number and persistence of contact with ants. Zoology **99**, 128-137.

Fiedler, K. & Maschwitz, U. 1988: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) II. Lycaenid larvae as trophobiotic partners of ants - an quantitative approach. Oecologia **75**, 204-206.

Fiedler, K. & Rosciszewski, K. 1990: *Myrmica sabuleti* (Hym., Formicidae) - ein weiterer Symbiosepartner von *Polyommatus coridon* (Lep., Lycaenidae). Nachr entomol Ver Apollo Frankfurt N F **11**, 141-142.

Fiedler, K. & Saam, C. 1994: Does ant-attendance influence development in 5 European Lycaenide butterfly species ? (Lepidoptera). Nota lepid **17**, 5-24.

-. 1995: Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study. Oecologia **104**, 316-322.

Fowler, H. G., Bernardi, J. V. E., Delabie, J. C., Forti, L. C. & Pareira-da-Silva, V. 1990: Major ant problems in South America. In: Applied Myrmecology - A world perspective (Vander Meer, R. K., Jaffe, K. & Cedeno, A. eds). Westview Press, Boulder. pp. 3-14.

Harada, A. Y. 1990: Ant pests of the Tapinomini tribe. In: Applied Myrmecology - A world perspective (Vander Meer, R. K., Jaffe, K. & Cedeno, A. eds). Westview Press, Boulder. pp. 298-311.

Heath, A. & Claassens, A. J. M. 2003: Ant-association among Southern African Lycaenidae. J Lepid Soc **57**, 1-16.

Hennenberg, K. J. & Bruelheide, H. 2003: Ecological investigations on the northern distribution range of *Hippocrepis comosa* L. in Germany. Plant Ecol **166**, 167-188.

Jutzeler, D. 1989: Weibchen der Bläulingsart *Lycaeides idas* L. riechen ihre Wirtsameisen (Lepidoptera: Lycaenidae). Mitt entomol Ges Basel N F **39**, 95-118.

King, T. J. 1981: Ant hills and grassland history. J Biogeogr **8**, 329-334.

Kitching, R. L. & Luke, B. 1985: The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist **19**, 259-276.

Kovár, P., Kovárova, M., Dostál, P. & Herben, T. 2000: Vegetation of ant-hills in a mountain grassland: effects of mound history and of dominant ant species. Plant Ecology **00**, 1-13.

Lassen, P. 1989: Om kroniller, släktet *Coronilla*, i Norden. Sven Bot Tidskr **83**, 83-86.

Lukhtanov, V. & Lukhtanov, A. 1994: Die Tagfalter Nordwestasiens. In: Herbipoliana: Buchreihe zur Lepidopterologie, Vol. 3 (Eitschberger, U. ed). Verlag Dr Ulf Eitschberger, Marktleuthen.

Mabelis, A. A. 1984: Interference between Wood Ants and Other Ant Species (Hymenoptera Formicidae). Neth J Zool **34**, 1-20.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

Martín Cano, J. 1984: Biología comparada de Lampides boeticus (L.), *Syntarucus pirithous* (L.) y *Polyommatus icarus* (Rot.) (Lep., Lycaenidae). Graellsia **40**, 163-193.

Mordwilko, A. 1907: Die Ameisen und Blattläuse in ihren gegenseitigen Beziehungen und das Zusammenleben von Lebewesen überhaupt. Biol Zbl **27**, 212-224.

Pfeuffer, E. 2000: Zur Ökologie der Präimaginalstadien des Himmelblauen Bläulings (*Lysandra bellargus* ROTTEMBURG 1775) und des Silbergrünen Bläulings (*Lysandra coridon* PODA 1761), unter besonderer Berücksichtigung der Myrmekophilie. Ber Naturwiss Ver Schwaben **104**, 72-98.

Pontin, A. J. 1961: The prey of *Lasius niger* (L.) and *L. flavus* (F.) (Hym., Formicidae). Entomol Mon Mag **97**, 135-137.

-. 1963: Further considerations of competition and the ecology of the ants *Lasius flavus* (F.) and *L. niger* (L.). J Anim Ecol **32**, 565-574.

-. 1978: The numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr). Ecol Entomol **3**, 203-207.

Rayward, A. L. 1906: Larvae of *Polyommatus icarus* and their connection with ants. Entomologist's Record **19**, 108-110.

SBN. 1987: Tagfalter und ihre Lebensräume. Schweizerischer Bund für Naturschutz. Fotorotar AG Egg/ZH, Basel.

Scheurer, S. 1984: Erstnachweis des Hygieneschädlings *Tapinoma melanocephalum* (Hymenoptera, Formicidae) in der DDR. Angew Parasitol **25**, 96-99.

-. 1989: Morphologie, Koloniegründung und hygienische Bedeutung von *Tapinoma melanocephalum* FABRICIUS, einer tropischen Ameise im menschlichen Siedlungsbereich in der DDR. Paper pres. Verhandlungen IX. SIEEC Gotha 1986 (Dresden).

Scheurer, S. & Liebig, G. 1998: *Tapinoma melanocephalum* Fabr (Formicidae, Dolichoderinae) in Gebäuden - Beobachtungen zu ihrer Biologie und Bekämpfung. Anz Schädlingskde, Pflanzenschutz, Umweltschutz **71**, 145-148.

Schmitt, T., Gießl, A. & Seitz, A. 2002: Postglacial colonisation of western Central Europe by *Polyommatus coridon* (Poda 1761) (Lepidoptera: Lycaenidae): evidence from population genetics. Heredity **88**, 26-34.

Schmitt, T. & Seitz, A. 2001: Allozyme variation in *Polyommatus coridon* (Lepidoptera: Lycaenidae): identification of ice-age refugia and reconstruction of post-glacial expansion. J Biogeogr **28**, 1129-1136.

-. 2002: Influence of habitat fragmentation on the genetic structure of *Polyommatus coridon* (Lepidoptera:Lycaenidae): implications for conservation. Biol Conservation **107**, 291-297.

Schönrogge, K., Wardlaw, J. C., Peters, A. J., Everett, S., Thomas, J. A. & Elmes, G. W. 2004: Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. J Chem Ecol **30**, 91-107.

Seifert, B. 1988: A taxonomic revision of the *Myrmica* species of Europe, Asia Minor, and Caucasia (Hymenoptera: Formicidae). Abh Ber Naturkundemuseum Görlitz **62**, 1-75.

-. 1996: Ameisen: beobachten, bestimmen. Naturbuch Verlag, Augsburg.

Shenk, J. S., Wangsness, P. J., Leach, R. M., Gustine, D. L., Gobble, J. L. & Barnes, R. F. 1976: Relationship between Beta Nitro Propionic-Acid Content of Crownvetch and Toxicity in Nonruminant Animals. J Anim Sci **42**.

Small, E. 1996: Adaptations to herbivory in alfalfa (*Medicago sativa*). Can J Bot **74**, 807-822.

Stankiewicz, A. & Sielezniew, M. 2002: Host specificity of *Maculinea teleius* BGSTR. and *M. nausithous* BGSTR. (Lepidoptera: Lycaenidae) the new insight. Annales Zoologici (Warszawa) **52**, 403-408.

Stradling, D. J. 1978: Food and feeding habits of ants. In: Production ecology of ants and termites (Brian, M. V. ed). Cambrigde University Press, Cambridge.

Thompson, C. R. 1990: Ants that have a pest status in the United States. In: Applied Myrmecology - A world perspective (Vander Meer, R. K., Jaffe, K. & Cedeno, A. eds). Westview Press, Boulder. pp. 51-67.

Tolman, T. & Lewington, R. 1998: Die Tagfalter Europas und Nordwestafrikas. Franckh-Kosmos, Stuttgart.

van Dyck, H., Oostermeijer, J. G. B., Talloen, W., Feenstra, V., van der Hidde, A. & Wynhoff, I. 2000: Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? Proc R Soc Lond B **267**, 861-866.

van Swaay, C. A. M. 2002: The importance of calcareous grasslands for butterflies in Europe. Biol Conservation **104**, 315-318.

Veeresh, G. K. 1990: Pest ants of India. In: Applied Myrmecology - A world perspective (Vander Meer, R. K., Jaffe, K. & Cedeno, A. eds). Westview Press, Boulder. pp. 15-24.

Vepsäläinen, K. & Savolainen, R. 1990: The effect of interference by formicine ants on the foraging of *Myrmica*. J Anim Ecol **59**, 643-654.

Wiemers, M. 1995: The butterflies of the Canary Islands. A survey on their distribution, biology & ecology (Lepidoptera and Hesperioidea)(second part). Linn Belg **15**, 87-118

.

3 Development of Sampling Methods

H. Daniels

Department of Animal Ecology I, D-95440 Bayreuth, Germany

3.1 Abstract

For the first time comparative chemical analyses of the larval nectar secretions from unspecific and facultatively myrmecophilous lycaenid caterpillars were to be obtained. To achieve this goal new rearing and stimulation techniques were tested. An effective method for breaking the egg diapause of *Polyommatus coridon* requiring but moderate work effort is presented. In an attempt to harvest nectar secretions without the presence of ants at the caterpillars the feasibility of several artificial stimulations of caterpillars was explored. For this purpose the palpating behaviour of *Lasius flavus* worker ants displayed at *P. coridon* caterpillars was analysed in detail. Based on these data the construction of an “artificial ant” was attempted. This “artificial ant” was then tested with caterpillars of *Zizeeria knysna*. Eversions of tentacle organs could be elicited frequently, but nectar secretions occurred only very rarely after artificial stimulation. Hence, the only practical way to harvest secretions remained the uptake of droplets using microcapillaries in established caterpillar-ant associations. Mean size of the droplets from the dorsal nectar gland in three facultatively myrmecophilous species was determined as 3.7nl for *P. coridon*, 2.0nl for *P. icarus* and 1.4nl for *Z. knysna* respectively.

3.2 Introduction

A considerable fraction of the nutrition of many ant species in the subfamilies Formicinae, Dolichoderinae, Myrmicinae is derived from trophobiotic interactions (Degen et al. 1986; Hölldobler & Wilson 1990; Stradling 1978; Tobin 1994). Most of these associations are with Homopteran species, but there are also interactions with a vast array of lycaenid species possible. The chemical contents of homopteran exudates are rather well known from many species (newer literature sources: (Bernays & Klein 2002; Byrne et al. 2003; Douglas 1993; Engel et al. 2001; Febvay et al. 1999; Fischer & Shingleton 2001; Fischer et al. 2002; Nemec & Stary 1990; Sandström & Moran 2001; Woodring et al. in press; Yao & Akimoto 2001; Yao & Akimoto 2002), whereas lycaenid species were studied much more rarely (Cushman et al. 1994; DeVries & Baker 1989; Maschwitz et al. 1975; Nomura et al. 1992; Pierce 1983). The exudates from aphids (“honeydew”) are more or less excretions, even though certain carbohydrates occur in honeydew, that are not present in phloem or xylem sap (Bacon & Dickinson 1957; Wilkinson et al. 1997; Woodring et al. in press). Lycaenid caterpillars do actually actively secrete the exudates from specialized epidermal glands (=”nectar”). Moreover they are able to finely tune the secretion behaviour to certain situations. Secretions are delivered more frequently at the beginning of an interaction (Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler et al. 1994), the amount varies dependent on the presence of different numbers of ants (Axén & Pierce 1998; Axén et al. 1996; Fiedler & Hagemann 1995; Leimar & Axén 1993), or the type of ant species present

(Axén 2000). Secretions are also presented in larger amounts under the threat of enemies, when additional ant attendants might be required (Agrawal & Fordyce 2000; Leimar & Axén 1993).

The few published studies on chemical composition of lycaenid nectar were either concerned with obligately myrmecophilous species that produce large quantities of nectar ((Cushman et al. 1994; Nomura et al. 1992; Pierce 1983; Pierce 1989; Pierce & Nash 1999), for a riodinid species (DeVries 1988; DeVries & Baker 1989)). Or, if addressing facultatively myrmecophilous species, the analyses were based on extremely small sample sizes (Maschwitz et al. 1975; Wagner 1994) due to the difficulties in harvesting sufficient amounts of nectar. In order to reliable obtain larval nectar secretions from facultatively myrmecophilous species, which tend to spend much less secretions than obligate associated species, a number of new techniques were tested, respectively had to be established. *Polyommatus coridon* develops relatively slowly and is an univoltine species. To obtain enough larvae for experiments and nectar sampling, the egg diapause, which the species generally undergoes, had to be broken. This issue is dealt with in the paragraph 3.3.

The next section (3.4) describes the attempt to develop a method, which effectively and with a considerably smaller investment of man-hours would allow to sample secretion amounts sufficient for subsequent chemical analysis. Furthermore measurements of droplet sizes of the dorsal nectar glands in three facultatively lycaenid species are included. Closely related to this topic is the endeavour to construct an ”artificial ant“ (3.5). Such a machinery would render the harvesting of nectar possible independent of the motivation of ants and would also circumvent the potential loss of secretion droplets to ants. Note that lycaenid caterpillars usually do never secrete nectar spontaneously, without being mechanically stimulated by ants (Malicky 1969; Malicky 1970). Furthermore, the successful implementation of an “artificial ant” might allow to more precisely examine the palpation pattern preferred by the caterpillars. It might even be possible to completely “milk” a caterpillar, thus deriving an estimate of the total gland bladder contents at a certain point of time.

3.3 Obtaining an artificial second generation of *P. coridon*

3.3.1 Material & methods

Second and third instar caterpillars of *P. coridon* were collected in the field at two sites in Northern Bavaria (49°48’N 11°24’E 540m NN & 49°46’N 11°27’E 460m NN) in spring in each of the years 2001-2003. The caterpillars were directly placed in plastic boxes (volume: 1000cm^3), lined with moistened cellulose tissue for optimal humidity. These boxes were kept at 25°C (L:D 16:8), cleaned from the faeces of the caterpillars and old food every day and afterwards filled with new shoots from *Hippocrepis comosa* or *Securigera varia*.

Upon pupation, pupae were removed and fixed with double-sided glue strips (Tesa) in a new box. These boxes were placed in a flight cage (1.95x0.88x1.15m) in an environmental cabinet where the adults could eclose and mate (26°C:15°C, 75% r.h., L:D 16:8). Food for the adult butterflies was provided in “artificial flowers”: plastic microcaps (Eppendorf 2ml) with a coloured lid with several holes drilled into the lid (Colours: violet, yellow) (chapter 2 Plate 2, B), and filled with sucrose solution (67.5% w/v). For the males cellulose plugged glass vials with salt water were provided, since in many butterflies sodium ions appear to be important for reproductive success (“mud puddling” (Beck et al. 1999; Schmidt-Nielsen 1997)). A small bunch of nectar plants (as available from the field in the respective season, primarily Asteraceae) was also offered, as not all males accepted the artificial flowers. On several days with good, sunny weather conditions *P. coridon* adults were placed in a gauze cage outside in the sun, as they mated only reluctantly under the artificial light (8xOsram L58W, 2xL36W UV, Osram Quicktronic frequency enhancer). Some additional mated females were caught at the aforementioned sites in July and August.

Mated females were placed either as a group in clear plastic boxes (volume: 10l) or separately in glass vials (volume: 2l) with netting as lid. Shoots or pots of *H. comosa* were provided for oviposition and artificial flowers for nutrition. Every three days the oviposition substrates were retrieved, the eggs counted and stripped off the shoots. Glass vials (volume: 15ml) were filled with water and sealed with Parafilm (American National Can). The Parafilm seals were gently pushed in, to obtain a concave surface. Two very small holes were pierced into the film and small fresh shoots of *H. comosa* placed in these, so that the Parafilm intimately surrounded the shoots. A third hole on the edge was made, to allow refill of water by means of a Pasteur pipette. The eggs removed from the oviposition substrates were transferred with a small brush to these vials. Up to 150 eggs were placed in the concave depressions. All glasses were placed in a plastic box (volume: 10l), lined with cellulose tissue at the bottom (moistened at least daily), and a light bulb (60W frosted) was placed 26cm above the bottom of the box (L:D 24:0). This resulted in a temperature of 26-28°C (laboratory thermometer DTM 3010 with thermocouple) at the height of the eggs when kept at room temperature (Fig. 3.1). Every morning until midday hatching first instar larvae were collected and placed into rearing boxes. Plant shoots were replaced every four days. In 2003 some smaller oviposition pots (volume 0.5l) with eggs were directly placed under the lamp, thus sparing the transfer of eggs to vials.

Basic calculations and data sorting were done in MS Excel™, statistical analysis with Statistica 6.0™ (Statsoft 2003) and SPSS 11.5™ (SPSS 2002). If not mentioned otherwise, values are given as mean±s.d. Not normally distributed data was either transformed to achieve this or an appropriate non-parametric statistic was used.

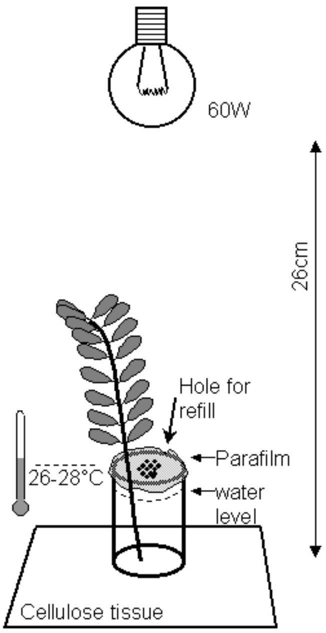


Fig. 3.1 Experimental design for breaking the diapause of *P. coridon* eggs. Glass vials with Parafilm lids and *Hippocrepis comosa* shoots arranged on moistened cellulose tissue, so that a temperature of 26-28°C at height of the eggs results through continuous light of a 60W frosted bulb

3.3.2 Results

On ten occasions (out of 1368 eggs, 0.7%) in 2002 larvae hatched already from eggs in the flight cage, without further treatment. In 2001 first instar larvae hatched from 58±33.5% of the eggs placed on six vials kept at room temperature, in 2002 69.5±32.1% (n=38 vials). Altogether, in both years 1253 larvae were obtained from 2103 eggs without diapause (59.6%). At temperatures of about 23°C at the height of the eggs no larvae hatched, at higher temperatures of about 29-30°C only 1.4% hatched (n=144 eggs, seven vials), and most eggs desiccated. There was almost no influence of the number of eggs placed on the vials on the proportion of larvae hatching detectable, as shown by the linear relationship between eggs placed on the lid and larvae hatching (Fig. 3.2). However for handling purposes less than 40 eggs per vial were optimal, since too high numbers of hatching larvae on some days resulted in a rapid devouring of the *H. comosa* shoots and subsequent migration of the larvae. In all three years 2001-2003 it was tried to mate the adults of the second generation in autumn (October & November), to obtain a third (laboratory) generation, but no successful mating could be initiated.

In 2003 first instar larvae hatched from 49.9±36.4% of the eggs (n=10 pots, total 186 eggs) the females had placed on the shoots, soil or the edge of the pots, which were placed under the lamp.

Trials with cellulose tissue as lids above the glass vials resulted in the loss of some larvae, which crept downwards along the stalks of the *H. comosa* shoots and drowned in the water. The Parafilm lids closed tightly around the shoots, so that the larvae were not able to pass downwards. The cellulose tissue was also less stable, tending to tear apart if the vials were used for longer periods of time. Due to evaporation through the tissue, the water of the shoots had to be refilled more often, but the larvae had to endure a less dry environment than larvae hatching on the watertight Parafilm lids.

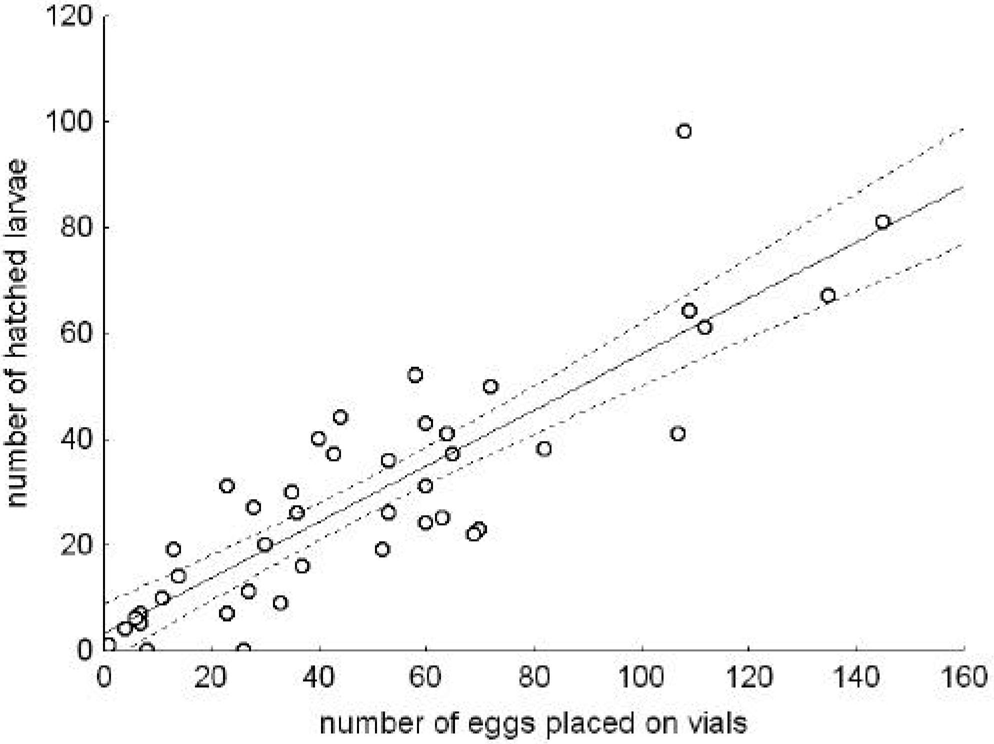


Fig. 3.2 Number of hatched *P. coridon* larvae compared to the number of eggs placed on the glass vials used in experiments to break diapause (r=0.87 p<0.05 n=44, solid line: regression, 95%confidence interval in dashed lines)

Interestingly in 2001 of 18 females, which were provided with *S. varia* shoots for oviposition for five days, none laid any eggs on these. One female laid an egg on a *M. sativa* nectar plant. After switching to *H. comosa* all females readily laid eggs.

3.3.3 Discussion

A temperature of 26-28°C under continuous light yielded the highest number of hatching larvae of an artificial second generation. Lower temperatures seem not to suffice to actually break the diapause. At higher temperatures the embryos in the eggs died due to drought stress. Some individuals obviously do not need the continuous light conditions, but these were very rare. However, the occurrence of a few subitaneously developing individuals under long-day conditions shows that even in the northern Bavarian *P. coridon* some potential exists to develop without diapause. Further east, i.e. in Slovakia, even bivoltine populations exist locally (originally described as a separate species, *P. slovacus*) (Tolman & Lewington 1998; Vítaz et al. 1997). In another univoltine lycaenid butterfly, *Lycaena hippothoe*, temperature and light conditions can be manipulated, so that no larval diapause occurs (Fischer & Fiedler 2002), but only a small fraction of subitaneous developers could be observed. Earlier attempts to rear *P. coridon* without diapause (Schurian 1989) were also only partly successful. The rearing method presented here for the first time allows for a reliable production of a larger number of individuals of a second laboratory generation of *P. coridon*.

The highest losses are to ascribed to a distinct behaviour of the *P. coridon* larvae. After hatching, the larvae feed upon the shoots, but most of them have a tendency to climb downwards the stems. In nature *P. coridon* feeds mainly in the evening or at night, staying at the ground hidden in the *H. comosa* rosettes during daytime. In the diapause breaking arrangement this leads to drowning if the lids are not sealed tightly around the stems. One might avoid this when using plant pots, as it was done for some of the eggs in 2003. These yielded a smaller number of larvae, probably since the light intensity in the deeper plant parts was not high enough. Also one has to search for the larvae on the plant, whereas on the glass vial arrangement they are quite easily visible.

So, with limited work effort it is possible to obtain a large second generation, although for further generations the critical point remains, as in most butterfly rearings, the mating of the adults.

Interesting is the observation of the females' refusal to oviposit on *S. varia*. This was also assumed by Pfeuffer (2000). Whereas larvae of the Bavarian *P. coridon* thrive well on *S. varia* (see chapter 4), females ignore it. Further east the populations have *H. comosa* no longer available, thus there is a preference for *S. varia* exhibited. Thus this might indicate a difference in oviposition preferences between the two genetic lineages of *P. coridon* (Schmitt & Seitz 2001) separated along the Czech-German border.

Under natural conditions *P. coridon* seems to be almost strictly univoltine, whereas the closely related *P. bellargus* regularly is bivoltine. The difference might be due to the relatively slow development of *P. coridon*. First, larvae of the second generation would have less food plant biomatter available, as *H. comosa* is growing and flowering only in spring under natural condition (a second flowering can be initiated, unpublished data). *P. bellargus*' second generation has also a smaller population size (Roy & Thomas 2003). This shortage further enhances the second considerable risk getting caught by early frost without being able to mate and initiate the following generation. *P. coridon* (artificial second generation individuals) needed from hatching until pupation 38.5±1.9d (n=6) under a permanent temperature of 26°C (L:D 16:8), under simulated day-night temperature change (26°C:15°C, L:D 16:8) 42.5±6.1d (n=15). Pupal duration was additional 15.4±1.4days (n=124, 26°C:15°C, L:D 16:8). These values are from climate chamber reared individuals, where conditions are much more favourable than they would be outside (compare also with values of *P. bellargus* (Fiedler & Saam 1994)). Unfortunately there is not enough precise data available about the possible second generation in Slovakia . But for bivoltine generations relative growth rates have to increase considerably (Fischer & Fiedler 2002), and it is questionable, if *P. coridon* might be able to realise these. *P. coridon* thus seems to follow a strategy different than *P. bellargus*, although it has enough phylogenetic plasticity left to exhibit different life-history pathways, as shown with the artificial second generation.

3.4 Stimulation of the dorsal nectar organ

For basic calculations and data sorting MS Excel™ was used, statistical analysis was done with Statistica 6.0™ (Statsoft 2003). If not mentioned otherwise, values are given as mean±s.d. Not normally distributed data was either transformed to achieve this or an appropriate non-parametric statistic was used. Bonferroni correction, if required, follows

Hochberg (1988) and is explicitly noted, where applicable. Wilcoxon-Wilcox test for the localisation of significant effects after a Friedman-ANOVA was calculated as in Sachs (1999).

3.4.1 Techniques tested

Lasius flavus nests were collected in 2001 and 2002 at two sites in Northern Bavaria (49°46'N 11°27'E 460m NN and 49°58'N 11°39'E 480m NN). Nest boxes (0.54x0.35x0.30m) for experiments were placed in environmental chambers (26°C:15°C, 75% r.h., L:D 16:8), further reserve nests were maintained at room temperature. Colonies were provided with honey, sucrose solution with added vitamins (Multi-Sanostol™, Altana) *ad libitum* and chopped *Gryllus bimaculatus* once a week. Water was provided at all times *ad libitum*.

According to literature sources nectar secretions can be obtained by various mechanical stimulations (Ehrhardt 1914; Schurian et al. 1993; Wagner 1994), K. Schurian pers. comm.). However, in none of these sources precise technical details or data about the success of such stimulations are reported.

Ants willing to enter a trophobiotic interactions with a lycaenid butterfly larvae (maybe after being appeased), generally start with a short period of slow groping behaviour, followed by a period of faster palpating of the caterpillar surface. This initiates in a normal behavioural sequence the secretion of one or several droplets from the dorsal nectar organ, which are readily imbibed by the ant (Malicky 1969; Malicky 1970).

Tactile stimulation with *Homo sapiens* female brown hairs, male blond hairs, beard hairs (either moustache or chin), *Canis lupus* hairs, brushes, wires, forceps or amputated ant heads (*L. flavus* workers) or heads plus thoraxes were all not suitable to elicit secretions from either *P. coridon* or *Polyommatus icarus* third or fourth instars or prepupal larvae (all on n=5 caterpillars for a duration of 5 minutes each). Often they just generated a contraction of the area around the dorsal nectar organ (DNO) or escape behaviour of the caterpillar, suggesting that the touch was too hard. The movement of an electrical tooth brush (Oral B) also just skimmed the caterpillars around (*P. coridon*, *Zizeeria knysna*), as it was too fast. Living ants, hold by forceps, did in some cases palpate, and sometimes even elicit movement of, the DNO, but no secretion occurred. Also it was difficult to hold the ants at the right pressure and angle for longer times, and the ants lost motivation rather fast. Carbon dioxide anaesthetised ants were glued into glass rods or onto wooden sticks (glue: Uhu™ Sekunden Alleskleber Gel). After awakening almost all of these ants inevitably just tried to free themselves and could not be motivated to palpate any caterpillars or show other normal behaviour.

Ehrhardt (1914) claimed to having obtained secretions, by means of "der elektrische Strom" (electrical current), without any further mention of used voltage or current. *P. coridon* and *P. icarus* third or fourth instars or prepupae were placed on wet paper or on a copper plate. Electrodes were used to either touch or caress lightly the posterior end of the caterpillars. With a transformator (Siemens) first direct current (DC 1.1A, 1-14 V) was tested, but did only provoke regurgitations. Alternating current (AC 1.1-3.3A, 14V) was a bit less irritating, but also generated only avoidance behaviour. Using the electrodes at the head or thoracic regions did generate some tentacle organ activity. With a function generator (Voltcraft™ MX 2020, ~ 1Hz-1MHz, 7.7V) sinus and triangle functions were tested from 1-50Hz in 5Hz steps. The caterpillars also did in several cases just regurgitate, at best only tentacle organ activity could be elicited.

As another alternative parasitoids were tested, as they are able to elicit secretion behaviour (Schurian et al. 1993). Living *Cotesia cupreus* (Lyle 1925) females were obtained from parasitised *P. coridon* caterpillars (compare (Baumgarten & Fiedler 1998; Fiedler et al. 1995) and grouped singly with *P. icarus* caterpillars. In several cases they stung the caterpillar, which almost always showed strong defence reactions like abruptly raising their heads, flicking the abdomen, regurgitating and discharging faeces. The behaviours did not prevent stinging. In none of the eight trials the female wasps tried to elicit secretions from the dorsal nectar organ.

To test, if it is possible to obtain the gland content through forced draining of the gland reservoirs, anaesthetised fourth instars or prepupae of *P. coridon*, *P. icarus* and *Z. knysna* were gently, but considerably squeezed on the sixth abdominal segment. Rather large droplets could be obtained from the glandular opening in the case of *P. coridon*, but it was impossible to adjust the pressure used so that the nectar was not contaminated by hemolymph (cloudy appearance of the otherwise clear secretion). In the other two species almost always contamination occurred. This was also the case if fresh cadavers (died from diseases) were used.

To test if obtaining the harvested nectar secretion out of the crop of ant workers is practical, *L. flavus* workers were fed with coloured sucrose solution (50%, water colour Goldfaber) and killed subsequently with short time freezing. The cuticle of the abdomen was removed, until the crop was freely accessible. The surface of the crop was cleaned and dried with cellulose tissue. The crop was punctured with an insect needle and the outspilling fluid collected with a microcapillary (0.5µl). Successful sampling was only possible with very large and thoroughly filled crops. With limp crops no collectible fluid drops formed. Also in the latter case the risk of hemolymph contamination was too great, as the surface of the crop could not easily be cleaned.

At last several temporal patterns of associations of five to ten *L. flavus* foraging worker ants together with one caterpillar of the three lycaenid species were tested. A caterpillar was placed on a piece of moist cellulose tissue in a small Petri dish (diameter 5.5cm). The dish was secured against concussion and vibration by pieces of foam rubber which were glued to its bottom. Ants foraging at the honey-water tubes in their nest box were collected from the nest with an exhaustor (Hölldobler & Wilson 1995) and placed close to the caterpillar. The formation of an initial association was allowed for different time periods up to half an hour, then the associations were disrupted. After different periods of time had passed, the participants were grouped together again. Due to this procedure the caterpillars always had to re-establish the dsturbed association and secreted more droplets than they otherwise would have done (see below). The optimal procedure determined in the course of many trials was a short first association of up to five minutes, followed by interruptions from 15-45 minutes. The nectar had then to be harvested by means of microcapillaries (0.5µl, Hirschmann), before the ants could imbibe the secreted droplets.

The droplets secreted during each of these “secretion bouts” were counted. The length of the fluid column obtained was immediately measured with a calibrated micrometer eyepiece (Wild M5A stereomicroscope, 25x magnification). These length measurement data allowed calculation of the secretion amount in one secretion bout. Dividing the amount secreted during one secretion bout through the number of droplets secreted in this bout allows calculation of mean droplet size. The mean droplet size in the first secretion bout at the initiation of an ant-association was 3.7nl in *P. coridon* (median, quartiles 1.8-6.2nl, n=91 caterpillars, fourth instar caterpillars up to non-feeding, still moving prepupae), in *P. icarus* it was 2.0nl (median, quartiles 0.6-3.6nl, n=13, late fourth instar caterpillars and moving prepupae), in *Z. knysna* 1.4nl (median, quartiles 1.0-2.3, n=72, fourth instar up to moving prepupae (non-feeding, not girdled)). *P. coridon* caterpillars secreted highly significant greater droplets than *Z. knysna* caterpillars (Kruskal-Wallis-ANOVA, $H_{(2,176)}$=36.6, p<0.001, pairwise post-hoc comparison p<0.001), and marginally significant greater droplets than P. icarus (pairwise post-hoc comparison p=0.058), while there were no differences in droplet size between *P. icarus* and *Z. knysna* (pairwise post-hoc comparison p>0.05) (Fig. 3.3). Maximum observed droplet size was as absolutely rare events in *P. coridon* 0.3µl (in bout 2) in *P. icarus* 0.21µl (in bout 2) and in *Z. knysna* >0.05µl (in bout 1) respectively. Compared to the normal mean droplet size (35x lower) the latter declared also the high variability of *Z. knysna* mean droplet sizes in Fig. 3.3.

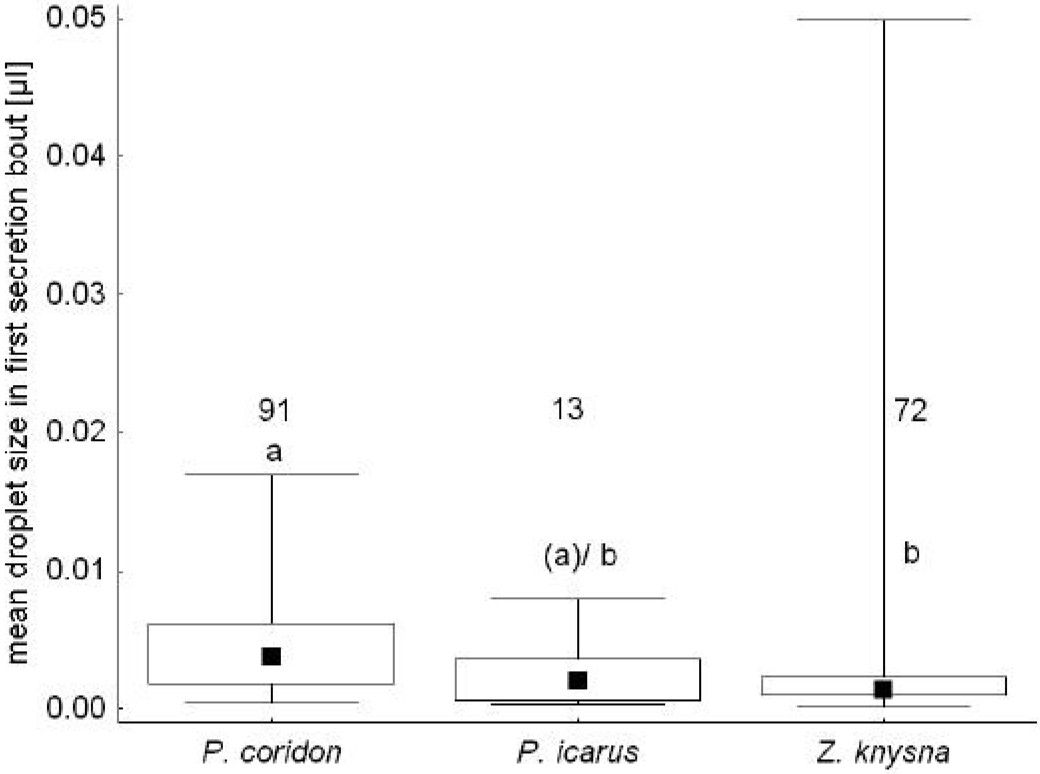


Fig. 3.3 Species differences in mean droplet size (volume of bout/number of droplets in bout) in the first secretion bout at the initiation of an ant-association (median of the mean droplet sizes, box: percentiles whiskers: min-max). Number of individuals noted above (Kruskal-Wallis-ANOVA $H_{(2,176)}$=36.6, p<0.01, multiple comparison of p-values: *P. coridon* vs. *P. icarus* p=0.058, *P. coridon* vs. *Z. knysna* p<0.001, *P. icarus* vs. *Z. knysna* p>0.5)

An influence of larval food on mean droplet size in the first bout was detected neither in *P. coridon* (Kruskal-Wallis-ANOVA, $H_{(2,32)}$=1.26, p=0.58, 8 prepupae fed with *H. comosa* vs. 12 fed *S. varia* and artificial food, respectively) nor in *Z. knysna* (Mann-Whitney test, U=124.5, p=0.35, 10 *M. sativa* vs. 31 artificial food fed prepupae). Droplets secreted by prepupal caterpillars were not significantly greater than droplets of fourth instar caterpillars (mean droplet size, first bout, *P. coridon*: Kruskal-Wallis-ANOVA, $H_{(2,88)}$=3.47, p=0.17, 35 fourth vs. 21 late fourth instars vs. 32 moving prepupae; *Z. knysna*: $H_{(2,67)}$=5.4, p=0.07, 16 fourth vs. 13 late fourth instars vs. 38 moving prepupae). As the caterpillars had to re-establish the ant-associations after each interruption, they tended to secrete greater droplets in the second and third bout (Fig. 3.4). However, this was found to be significant only for *Z. knysna* prepuae (Friedman ANOVA, ?$^2_{(3,11)}$=14.94, p=0.002; Wilcoxon-Wilcox test, k=3, n=7, mean droplet size third bout > first bout p<0.01, second > first bout p<0.05). The mean droplet size tended to decrease again in the fourth bout.

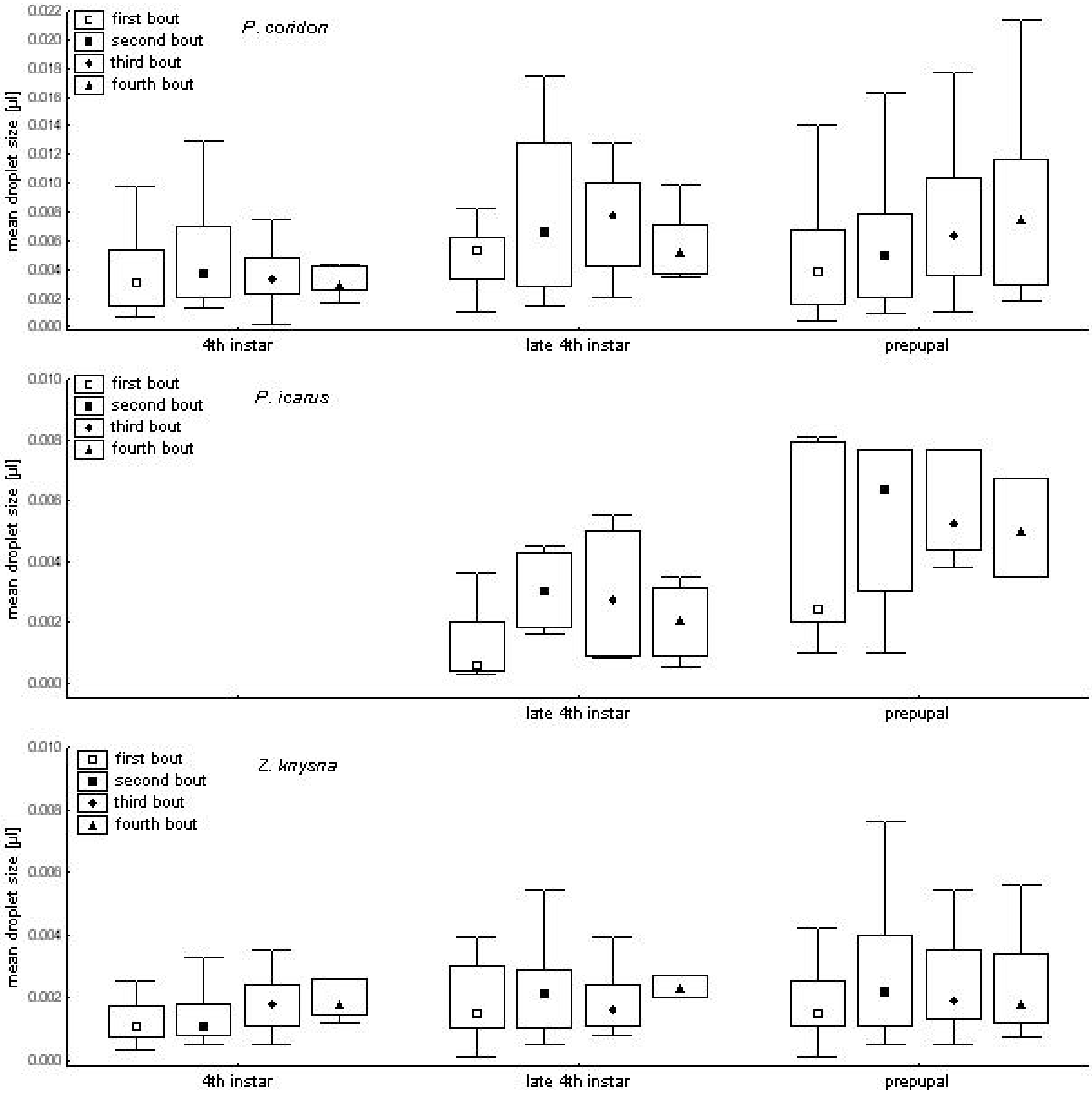


Fig. 3.4 Comparison of the mean droplet size (volume of bout/number of droplets in bout) secreted in the first to fourth secretion bouts by caterpillars of three lycaenid species tested at three different ages (median of the mean droplet sizes, box: percentiles whiskers: min-max). Ant-associations were repeatedly interrupted after each bout for about 40 minutes

There was only a slight correlation of the caterpillars' size (weight in mg) with the volume secreted in the first bout (r=0.36, p=0.04, n=32) in *P. coridon* prepupae, and no correlation in *Z. knysna* prepupae (r_s=0.13, p=0.4, n=41). Therefore, only after observation of the association one could determine the profitability of the particular caterpillars concerning the harvest of its secretions.

3.4.2 Discussion

All tests in which manual stimulation with various tools was attempted, failed to elicit secretion behaviour. Obviously it was not possible to generate the right intensity of tactile stimulation or to match the time pattern required. Lycaenid caterpillars, at least *P. icarus*, possess rather unusual mechanoreceptors surrounding the DNO, which theoretically should be able to discriminate time pattern acutely (Tautz & Fiedler 1992). The trials with electrical current were also a complete failure, obviously contradicting Ehrhardt's (1914) statement. The regurgitations clearly showed that the caterpillars perceived the stimulus as annoying. Even the tentacle organ activity in these cases, where no regurgitation under electrical "stimulation" was observed, can be interpreted as a "call for help" to possibly nearby existing ants, as tentacle organs can have an alarming function on formicine ants (Ballmer & Pratt 1991; Fiedler & Maschwitz 1987; Henning 1983).

The parasitoids did also not succeed. But even if this approach had worked, the limited availability of these animals would have been the next hindrance. The forced draining of the glands proved to be impractible, as it was not possible to do this without a high risk of hemolymph contamination of the samples. Only in *P. coridon* did the bladder walls of the DNO withstand the generated pressure from the body fluids for some (unpredictable) time, whereas in the other two species they ruptured almost immediately. The extraction of secretions harvested by the ants from their crops also was not feasible. Only with sufficient filling level and resulting pressure of the contents in the crop, a sampling without any contamination from the outer crop wall would have been possible.

So the only realistic option was the grouping of ants with caterpillars in a certain time frame. This ensures a high initial nectar secretion every time an association has to be re-established by the caterpillar. At the onset of an ant association, lycaenid larvae typically secrete more and larger droplets. This phenomenon has already been described for *P. icarus* (Burghardt & Fiedler 1996b) and *Z. knysna* (Fiedler & Hagemann 1995) and for other lycaenid species as well ((Fiedler & Hummel 1995) & Fiedler unpublished data). With sufficiently long breaks in between, the associations had to be renewed by the caterpillars, and they once again secreted more than they would otherwise have done in a prolonged association.

The droplet size measurements are to be considered with caution due to the following problems. The first droplet in a bout is always very large (Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler et al. 1994), followed by successively smaller droplets, thus the calculated mean (volume of bout/number of droplets) is rather unrepresentative. The values are overestimated, especially for the smaller two species *P. icarus* and *Z. knysna*, since often individuals secreted droplets which were smaller than the inner diameter of the microcapillary. Such droplets could not be collected and measured. Therefore the samples underlying these droplet size measurements are necessarily shifted towards a sub-sample of individuals which had secreted larger amounts. Furthermore, the whole procedure was aimed at optimising a high harvest of nectar and not towards exact measurement of droplet sizes. Collecting was stopped when for one minute no more droplets were secreted, to avoid evaporation loss from the capillary. Therefore, duration of collecting and total volume secreted in one bout are not always comparable. Moreover, for the third and following bouts only individuals were chosen, which had been profitable in the first two bouts. Nevertheless, the measurement method presented here is based on a large sample size and is far more accurate than the visual estimates of droplet size and subsequent volume calculation done so far (Burghardt & Fiedler 1996a; Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler & Maschwitz 1989). The visual measurements, although carefully calculated and conservatively handled, are still overestimations. Even so, it was corroborated, that facultatively myrmecophilous lycaenid species secrete minute droplets (Table 3.1) (see also (Maschwitz et al. 1975), maximum droplet size 0.2µl, (Wagner 1994) ca 0.05µl), compared to obligately myrmecophilous species (1-3ml, (DeVries 1988)).

Table 3.1 Comparison of droplet size measurement with visual estimates in literature, [a)]more conservative estimate

species	mean droplet size [nl] this study	optical estimate [nl]	in
P. coridon	3.7	4.0	Fiedler & Maschwitz 1988
P. icarus	2.0	8.2	Burghardt & Fiedler 1996b
Z. knysna	1.4	6.62 or 4.0[a]	Fiedler & Hagemann 1995

An effect of larval food on secretion droplet size could not be detected. An effect of larval food on secretion parameters might only be visible, if caterpillars are reared on relatively stressful food (e.g. (Fiedler 1990)), which was not intended in this study. The repeated interruption of associations tended to increase the mean droplet size in the second and third bout. This effect, however was statistically significant only in *Z. knysna* prepupae. This might be attributed to high individual variability. Interestingly, droplet size tended to decrease (though not significantly) in the fourth and following bouts, indicating that the caterpillars spend less and might save secretions. The maximum amount harvested from one individual *P. icarus* prepupa was 0.361µl over the course of five hours, which is more than the size of the reservoir of the nectar gland, which has been estimated as 0.06µl (Fiedler et al. 1995). Thus continuous nectar production seems to be at work, and possibly a certain rate cannot be exceeded. This might force the caterpillar to restrict initial investment in beginning associations after several interruptions.

Tremendous individual variability in the willingness and/or ability of the larvae to deliver nectar secretions obscured the potential effects of larval weight. A similar result was obtained for *P. icarus*, where only after pooling of caterpillars reared on very different food qualities, thus resulting in large size differences, a low correlation between secretion rate and weight could be shown (Burghardt & Fiedler 1996a). When reared on artificial food at room temperature under short-day conditions exceptionally large caterpillars of *Z. knysna* could be obtained, where an effect of body size on secretion rates was discernible (Fiedler, unpublished data 2004). Thus the amount of secretion and therefore profitability of nectar harvest could not be predicted according to the size of the tested caterpillar. Nectar yield of an individual caterpillar could only be determined in hind sight, leading often to trials with unproductive associations. So, even with this optimised harvesting protocol, completion of a nectar sample to a cumulative volume suitable for reliable chemical analysis (>0.5µl) required collecting hundreds of individual droplets under a stereo microscope, usually to be collected from numerous caterpillar individuals. In less productive species like *P. icarus* and *Z. knysna* completion of one sample not rarely took several weeks.

The best ant for the harvesting method is *Lasius flavus*, as it is a highly efficient visitor of trophobionts (Malicky 1969; Pontin 1978). *L. flavus* ants behave in a rather peaceful manner, enabling the operator to steal the secretion right away in front of the ants, if he is fast and deft enough. Still, as the ants are not always motivated and may lose motivation after some time, if repeatedly the droplets are stolen away right in front of them, an attempt was undertaken to build an “artificial ant”, which is always ready and “eager”.

3.5 Construction of an ”artificial ant“

H. Daniels*, T. Geister

Department of Animal Ecology I, D-95440 Bayreuth, Germany

* corresponding author

3.5.1 Material & methods

To characterise the antennal movement of ants at the integument of lycaenid caterpillars these behaviour patterns were videotaped. A caterpillar was placed together with four *L. flavus* ants on a piece of moist cellulose tissue in a Petri dish (diameter 9cm), with edges coated with Teflon-Emulsion (Dyneon). Pieces of foam rubber were glued to the dish’s bottom to secure it against concussion and vibration. With a video camera (Panasonic F15, time in 1/10s steps displayed) coupled with a stereomicroscope (Wild M5A) the interactions in the area of the dorsal nectar organ and the tentacle organs were filmed at 25fold magnification. From interactions filmed for about five minutes, four or five second intervals shortly before droplet secretion, in which the antennal movements were in the plane of focus, were used for detailed analysis. The sequences were played in stop motion (1/10s intervals, Panasonic NV-FS=HQ recorder with shuttle function), and movements of the antenna were transferred from the screen to transparency sheets attached to the monitor screen (one for each antenna and each second). The sequence was repeated and the actual contacts of the antenna with the caterpillar’s surface counted. Punctuated touching as well as smooth caressing were defined as contacts. If the same amount of contacts to the caterpillar’s integument were exhibited from the right and the left antennae in one 1/10s interval, this was evaluated as “synchronous”. Therefore if e.g. five out of ten were evaluated as synchronous, overall synchronization of the second was calculated as 50%.

As simulator of the ant antenna movements an astable multivibrator was built. A timer (NE555, SGS Thomson) coupled with an array of resistors, which could be added one by one with a respective switch, thus changing the overall sum of resistance, generated 18 different frequencies from 3-20Hz (see Appendix). A common magnetic relay with removed lid was connected and different materials could be attached to a nook at the magnet. The magnet of the relay moved with the frequency selected on the circuit board and the respective material followed the duty cycle with a swinging movement. As materials *Homo sapiens* hair from the head or beard fixed with glue on one end to a cardboard holder, wire from fine electronics cables (diameter 0.08mm), dead *L. flavus* ants glued with the abdomen to cardboard, and human hairs glued into a long silicone rubber tube (diameter 4mm) were tested (details in Table 3.2).

This “artificial ant” was tested with fourth instar caterpillars from *P. coridon* and *Zizeeria knysna* (rearing of the latter see 4.3.1).

Basic calculations and data sorting were done in MS Excel™, statistical analysis with Statistica 6.0™ (Statsoft 2003). If not mentioned otherwise, values are given as mean±s.d. Not normally distributed data was either transformed to achieve this or an appropriate non-parametric statistic was used. Wilcoxon-Wilcox test for the localisation of significant effects after a Friedman-ANOVA was calculated as in Sachs (1999).

3.5.2 Results

The ants' (*L. flavus*) two antenna contacted the caterpillar surface with a mean frequency of 9.57±1.63Hz per antenna. Synchronisation of the antennal contacts was low (median 44.7%, quartiles 30–52.94%, n=7), ants therefore most of the time did not produce exactly simultaneous tactile stimulations. Ants did not change the frequency or synchronisation for the duration of close observation intervals (Kruskal-Wallis-ANOVA $H_{3,28)}=2.95$, p=0.40; and $H_{(3,28)}=3.91$, p=0.27; respectively) The abdominal segment with the dorsal nectar organ (7^{th}) and the segments posterior of the latter were thoroughly palpated. The seventh segment was more often tactile stimulated than the segment anterior of it (Wilcoxon-Wilcox test k=8, n=7, p<0.05).

Based on these results the “artificial ant“ was tested at frequencies from 6-10Hz with different materials attached to it as stimulators/effectors and the caterpillars' reaction was qualitatively observed. The clicking noise of the relay did not have any observable influence on the caterpillars. In continuous operation the relay itself became hot, but not the effector materials attached to it. In most of the tests the caterpillars moved slowly while being stimulated, as it is also observable when being grouped together with ants in laboratory arenas. A slight positive stimulation was achieved with the human hairs as effectors, while wires elicited too hard contacts (Table 3.2). Hairs and wire was also tested with two relays simultaneously, therefore simulating two antennae, but no different behaviour resulted. With the dead ants exact positioning directly above the caterpillars was very difficult to achieve. Often the caterpillars were not tapped at all or at least not the whole time over, as the ants body did hinder a clear line of sight. Tests with a mirror to improve viewing also proved impractical.

Due to the short length of the hairs and the wires the touching movement was relatively hard. The hairs in the silicone rubber tubes performed a swinging movement which was retarded relative to the relay movement. Due to their length those effectors touched the caterpillars rather lightly. The caterpillars everted the tentacle organs, which in natural associations elicits following behaviour of ants (Claassens & Dickson 1977). In four different trials with *Z. knysna* one droplet secretion each could be achieved.

Table 3.2 Description of effector materials used with the "artificial ant" and resulting behaviour of *P. coridon* and *Z. knysna* caterpillars. 1: amplitude at the caterpillar 1mm like a real ant antenna, 2: tested only with *Z. knysna*, DNO: dorsal nectar organ

	description	length [mm]		caterpillar behaviour	remarks
H. sapiens hair (head, blond, male)^1	flexible, thin	hair cardboard	4 3	slow crawling, movement of DNO	relatively hard touch
H. sapiens hair (beard)^1	coarse, bristle, stable	hair cardboard	4 3	slow crawling, movement of DNO	relatively hard touch
0.08mm wire^1	rigid, stable	wire cardboard	4 3	slow crawling	hard touch
dead *L. flavus*	-	cardboard	5	slow crawling	difficult handling
silicone rubber tube + *H. sapiens* hair^{1,2}	flexible, smooth movement, soft touch	tube hair	55 25	slow crawling, movement of DNO, tentacle eversion occasionally nectar secretion	easy handling

The artificial ant construction with a hair in a silicone rubber tube was used to test if *Z. knysna* caterpillars prefer certain stimulation frequencies over others. For this purpose, the latency time from the onset of the stimulation until the first eversion of the tentacle organs was measured. No particular frequency was significantly preferred (Friedman $?^{2}_{(4,16)}=6.43$, $p=0.33$) (Fig. 3.5), although at 10Hz the latency time was reduced by 12.6s (41%) as compared to stimulation at 6Hz.

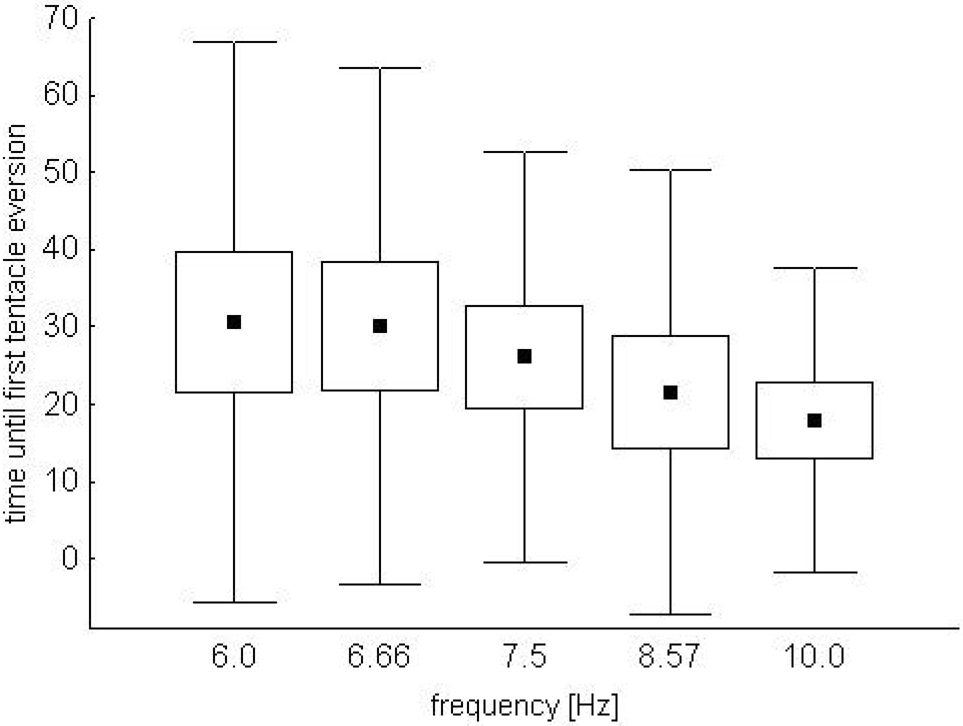


Fig. 3.5 Latency time until first tentacle eversion of *Z. knysna* caterpillars tested with the "artificial ant" at different frequencies (box: mean±s.e. whiskers: mean±s.d.) (16 caterpillars tested at five frequencies)

3.5.3 Discussion

Closer examinations of the ants' (*L. flavus*) antennal movements on the integument of *P. coridon* caterpillars revealed that these movements seemed not to follow any distinct and specified pattern. Neither did the antenna move synchronously nor did they tap the caterpillar in an exactly alternating manner. There was also no acceleration or retardation detectable in the palpating behaviour, which could be related to the actual moment of nectar droplet delivery by the attendant caterpillar. The ants did, however, prefer the latter half of the caterpillars and there especially the region of the dorsal nectar organ itself (Ballmer & Pratt 1991; Clark & Dickson 1956; Edwards 1878; Fiedler & Maschwitz 1989; Henning 1983; Leimar & Axén 1993; Malicky 1969; Malicky 1970; Tautz & Fiedler 1992; Thomann 1901). This region is also anatomically different, as the normal long setae of the caterpillars are absent and specialized receptor setae are abundant (Ballmer & Pratt 1991; Clark & Dickson 1956; Clark & Dickson 1971; Fiedler 1988; Fiedler & Maschwitz 1987; Kitching 1983; Kitching & Luke 1985). Interestingly in *P. icarus* these are able to preserve the exact time pattern of a tactile stimulation, independent of the pressure applied or the duration of the contact (Tautz & Fiedler 1992). So it does apparently not matter if the ants just touch or actually stroke the caterpillar with the antennae. *Z. knysna* did not demonstrate a particular preference of a certain palpation frequency. Possibly the latency time of tentacle eversions is a rather unsuitable measurement criterion. Another possible explanation might be that facultatively myrmecophilous species, in particular, cannot predict, which ant they might encounter. Therefore they might have just a very coarse template, matching the time patterns of antennal movements of many different ant species, without further preferences for any of these. This might also explain, why non-ant organisms occasionally can elicit secretion behaviour (Downey 1965; Gilbert 1976; Schurian et al. 1993). It might be rewarding to test obligately versus facultatively myrmecophilous species regarding the possible existence of preferences in the frequency or temporal pattern of tactile stimulation. The prediction would be that obligately myrmecophilous species do show some preferences as a result of coevolution.

Even though no obvious frequency preferences for the elicitation of nectar secretion acts could be detected, nectar delivery is under tight control of the caterpillars. Nectar secretions in response to totally artificial stimuli has only very rarely been reported in the vast literature (Ehrhardt 1914; Schurian et al. 1993; Wagner 1994), K. Schurian pers. comm.) and my own experiments in this regard totally failed (see 3.4). The far more sophisticated „artificial ant“ was in some cases able to elicit droplet secretions in *Z. knysna* caterpillars, but unfortunately not overly reliable. So this approach had to be discarded for the secretion sampling. Moreover, the relay tended to reach tremendous temperatures in the continuous switching mode. During longer usage the „artificial ant“ might overheat. This would have necessited the development of a special cooling device. Nevertheless with some further improvement regarding the type of effector used (e.g. real ant antennae glued to a suitable elongation) it might be possible to finally obtain a really capable “artificial ant” and do experiments as described above.

3.5.4 Acknowledgements

The function generator used in the artificial stimulation experiments was kindly supplied by the Department of Experimental Physics V, University Bayreuth, Prof. Dr. Georg Eska. Bernd Kornmaier, Dagmar Isheim and Thorin Geister contributed valuable discussion to several drafts (based on different construction principles) of the “artificial ant“. Thorin Geister built the final version of the “artificial ant” and performed most of the experiments with it. The district government of Oberfranken kindly issued permits to collect individuals in the field for starting of laboratory populations. Dr. Klaus G. Schurian kindly provided *Zizeeria knysna* larvae and pupae from the Canary Islands, and Annick Servant helped rearing the *P. icarus* and *Z. knysna* caterpillars.

3.6 References

Agrawal, A. A. & Fordyce, J. A. 2000: Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B **267**, 1857-1861.

Axén, A. H. 2000: Variation in behavior of lycaenid larvae when attended by different ant species. Evol Ecol **14**, 611-625.

Axén, A. H. & Pierce, N. E. 1998: Aggregation as a cost-reducing strategy for lycaenid larvae. Behav Ecol **9**, 109-115.

Axén, H. A., Leimar, O. & Hoffman, V. 1996: Signalling in a mutualistic interaction. Anim Behav **52**, 321-333.

Bacon, J. S. D. & Dickinson, B. 1957: The origin of melezitose: a biochemical relationship between the lime tree (*Tilia* spp.) and an aphis (*Eucallipterus tiliae* L.). Biochem J **66**, 289-297.

Ballmer, G. R. & Pratt, G. F. 1991: Quantification of ant attendance (myrmecophily) of lycaenid larvae. J. Res Lepid **30**, 95-112.

Baumgarten, H. T. & Fiedler, K. 1998: Parasitoids of lycaenid butterfly caterpillars: Different patterns in resources use and their impact on the Hosts' symbiosis with ants. Zool Anz **236**, 167-180.

Beck, J., Muehlenberg, E. & Fiedler, K. 1999: Mud-puddling behavior in tropical butterflies: In search of proteins or minerals? Oecologia **119**, 140-148.

Bernays, E. A. & Klein, B. A. 2002: Quantifying the symbiont contribution to essential amino acids in aphids: the importance of tryptophan for *Uroleucon ambrosiae*. Physiol Entomol **27**, 275-284.

Burghardt, F. & Fiedler, K. 1996a: The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). Ecol Entomol **21**, 1-8.

-. 1996b: Myrmecophilous behaviours in caterpillars of the butterfly, *Polyommatus icarus* (Rottemburg, 1775): temporal patterns and age dependency (Lepidoptera: Lycaenidae). Nota lepid **19**, 212-229.

Byrne, D. N., Hendrix, D. L. & Livy, H. W. I. 2003: Presence of trehalulose and other oligosaccharids in Hemiptera honeydew, particularly Aleyrodidae. Physiol Entomol **28**, 144-149.

Claassens, A. J. M. & Dickson, C. G. C. 1977: A study of the myrmecophilous behaviour of the immature stages of *Aloides thyra* (L.) (Lep.: Lycaenidae) with special reference to the function of the retractile tubercles and with additional notes on the general biology of the species. Entomologist's Record **89**, 225-231.

Clark, G. C. & Dickson, C. G. C. 1956: The honey gland and tubercles of larvae of the Lycaenidae. Lepidopterists' News **10**, 37-43.

-. 1971: Life histories of South African lycaenid butterflies. Purnell, Cape Town.

Cushman, J. H., Rashbrook, V. K. & Beattie, A. J. 1994: Assessing benefits to both participants in a lycaenid-ant association. Ecology **75**, 1031-1041.

Degen, A. A., Gersani, M., Avivi, Y. & Weisbrot, N. 1986: Honeydew intake of the weaver ant *Polyrhachis simplex* (Hymenoptera: Formicidae) attending the aphid *Chaitophorous populialbae* (Homoptera: Aphididae). Insectes Sociaux **33**, 211-215.

DeVries, P. J. 1988: The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants. Zool J Linnean Soc **94**, 379-393.

DeVries, P. J. & Baker, I. 1989: Butterfly exploitation of an ant-plant mutualism: adding insult to herbivory. J New York Entomol Soc **97**, 332-340.

Douglas, A. E. 1993: The nutritional quality of phloem sap utilized by natural aphid populations. Ecol Entomol **18**, 31-38.

Downey, J. C. 1965: Thrips utilize Exudations of Lycaenidae. Entomol News **76**, 25-27.

Edwards, W. H. 1878: Notes on *Lycaena pseudargiolus* and its larval history. Can Entomol **10**, 1-14.

Ehrhardt, R. 1914: Über die Biologie und Histologie der myrmekophilen Organe von *Lycaena orion*. Ber naturf Ges Freiburg **20**, 90-98.

Engel, V., Fischer, M. K., Wäckers, F. L. & Völkl, W. 2001: Interactions between extrafloral nectaries, aphids and ants: are there competition effects between plant and homopteran sugar sources? Oecologia **129**, 577-584.

Febvay, G., Rahbe, Y., Rynkiewicz, M., Guillaud, J. & Bonnot, G. 1999: Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, *Acyrtosiphon pisum*, reared on different diets. J exp Biol **202**, 2639-2652.

Fiedler, K. 1988: The preimaginal epidermal organs of *Lycaena tityrus* (PODA 1761) and *Polyommatus coridon* (PODA 1761) (Lepidoptera: Lycaenidae) - a comparison. Nota lepid **11**, 100-115.

-. 1990: Effects of larval diet on myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenide). Oecologia **83**, 284-287.

Fiedler, K. & Hagemann, D. [1995]: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K. & Hummel, V. 1995: Myrmecophily in the brown argus butterfly, *Aricia agestis* (Lepidoptera: Lycaenidae): Effects of larval age, ant number and persistence of contact with ants. Zoology **99**, 128-137.

Fiedler, K. & Maschwitz, U. 1987: Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) III. New aspects of the function of the retractile tentacular organs of lycaenid larvae. Zool Beitr N F **31**, 409-416.

-. 1989: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. Ethology **80**, 71-80.

Fiedler, K. & Saam, C. 1994: Does ant-attendance influence development in 5 European Lycaenide butterfly species ? (Lepidoptera). Nota lepid **17**, 5-24.

Fiedler, K., Schurian, K. G. & Hahn, M. 1994: The life history and myrmecophily of *Polyommatus candalus* (Herrich-Schäffer) from Turkey (lep., Lycaenidae). Linn Belg **14**, 315-332.

Fiedler, K., Seufert, P., Pierce, N. E., Pearson, J. G. & Baumgarten, H.-T. 1995: Exploitation of lycaenid-ant mutualisms by braconid parasitoids. J Res Lepid **31**, 153-168.

Fischer, K. & Fiedler, K. 2002: Life-history plasticity in the butterfly *Lycaena hippothoe*: local adaptions and trade-offs. Biol J Linnean Soc **75**, 173-185.

Fischer, M. K. & Shingleton, A. W. 2001: Host plants and ants influence the honeydew sugar composition of aphids. Funct Ecol **15**, 544-550.

Fischer, M. K., Völkl, W., Schopf, R. & Hoffmann, K. H. 2002: Age-specific patterns in honeydew production and honeydew composition in the aphid *Metopeurum fuscoviride*: implications for ant-attendance. J Insect Physiol **48**, 319-326.

Gilbert, L. E. 1976: Adult resources in butterflies: African Lycaenid *Megalopalpus* feeds on larval nectary. Biotropica **8**, 282-283.

Henning, S. F. 1983: Chemical communication between lycaenid larvae (Lepidoptera: Lycaenide) and ants (Hymenoptera: Formicidae). J ent Soc sth Afr **46**, 341-366.

Hochberg, Y. 1988: A sharper bonferroni procedure for multiple tests of significance. Biometrika **75**, 800-802.

Hölldobler, B. & Wilson, E. O. 1990: The ants. Harvard University Press / Springer, Heidelberg.

-. 1995: Ameisen - Die Entdeckung einer faszinierenden Welt. Birkhäuser, Basel.

Kitching, R L. 1983: Myrmecophilous organs of the larvae and pupae of the lycaenid butterfly *Jalmenus evagoras*. J Nat Hist **17**, 471-481.

Kitching, R. L. & Luke, B. 1985: The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist **19**, 259-276.

Leimar, O. & Axén, A. H. 1993: Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav **46**, 1177-1182.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

-. 1970: New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymneoptera). J Lep Soc **24**, 190-202.

Maschwitz, U., Wüst, M. & Schurian, K. 1975: Bläulingsraupen als Zuckerlieferanten. Oecologia **18**, 17-21.

Nemec, V. & Stary, P. 1990: Sugars in honeydew. Biologia Bratislava **45**, 259-264.

Nomura, K., Hirukawa, N., Yamaoka, R. & Imafuku, M. 1992: Problems on the symbiosis between the lycaenid butterfly larva, *Niphanda fusca shijimia* and the ant *Camponotus* (*Camponotus*) *japonicus*. Tyo to Ga **43**, 138-143.

Pierce, N. E. 1983: The ecology and evolution of the symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University.

-. 1989: Butterfly-ant mutualisms. In: Towards a more exact ecology (Grubb, P. J. & Whittaker, J. eds). Blackwell Scientific, Oxford. pp. 299-324.

Pierce, N. E. & Nash, D. R. 1999: The Imperial Blue, *Jalmenus evagoras* (Lycaenide). In: Biology of Australian Butterflies, Vol. 6 (Kitching, R. L., Scheermeyer, E., Jones, R. E. & Pierce, N. E. eds). CSIRO Publishing, Melbourne. pp. 279-317.

Pontin, A. J. 1978: The numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr). Ecol Entomol **3**, 203-207.

Roy, D. B. & Thomas, J. A. 2003: Seasonal variation in the niche, habitat availability and population fluctuations of a bivoltine thermophilous insect near its range margin. Oecologia **134**, 439-444.

Sachs, L. 1999: Angewandte Statistik. Springer, Heidelberg.

Sandström, J. P. & Moran, N. A. 2001: Amino acid budgets in three aphid species using the same host plant. Physiol Entomol **26**, 202-211.

Schmidt-Nielsen, A. 1997: Animal physiology. Cambridge University Press, Cambridge.

Schmitt, T. & Seitz, A. 2001: Allozyme variation in *Polyommatus coridon* (Lepidoptera: Lycaenidae): identification of ice-age refugia and reconstruction of post-glacial expansion. J Biogeogr **28**, 1129-1136.

Schurian, K. G. 1989: Revision der Lysandra-Gruppe des Genus *Polyommatus* LATR. (Lepidoptera: Lycaenide). Dr. Ulf Eitschberger Verlag, Marktleuthen.

Schurian, K. G., Fiedler, K. & Maschwitz, U. 1993: Parasitoids exploit secretions of myrmecophilous lycaenid butterfly caterpillars (Lycaenidae). J Lepid Soc **47**, 150-154.

SPSS. 2002: SPSS 11.5. SPSS Inc, Chicago.

Statsoft. 2003: Statistica 6.0. Statistica Inc, Tulsa.

Stradling, D. J. 1978: Food and feeding habits of ants. In: Production ecology of ants and termites (Brian, M. V. ed). Cambrigde University Press, Cambridge.

Tautz, J. & Fiedler, K. 1992: Mechanoreceptive properties of caterpillar hairs involved in mediation of butterfly-ant symbioses. Naturwissenschaften **79**, 561-563.

Thomann, H. 1901: Schmetterlinge und Ameisen. Beobachtungen über eine Symbiose zwischen *Lycaena argus* L. und *Formica cinerea* Mayr. Jahresber naturf Ges Graubünden **44**, 1-41.

Tobin, J. E. 1994: Ants as primary consumers: diet and abundance in the Formicidae. In: Nourishment and Evolution in Insect Society (Hunt, J. H. & Nalepa, C. A. eds). Westview Press, Oxford & IBH Publishing Co Pvt Ltd, Boulder, New Delhi.

Tolman, T. & Lewington, R. 1998: Die Tagfalter Europas und Nordwestafrikas. Franckh-Kosmos, Stuttgart.

Vítaz, L., Bálint, Z. & Žitnan, D. 1997: *Polyommatus slovacus* sp. nov. (Lepidoptera, Lycaenidae): the bivoltine relative of *Polyommatus coridon* in Slovakia. Entomol Problems **28**, 1-8.

Wagner, D. 1994: Species-specific effects of tending ants on the life history of *Hemiargus isola*, a North American lycaenid butterfly. Ph.D. thesis, Princeton University, Princeton.

Wilkinson, T. L., Ashford, D. A., Pritchard, J. & Douglas, A. E. 1997: Honeydew sugars and osmoregulation in the Pea Aphid *Acyrthosiphon pisum*. J exp Biol **200**, 2137-2143.

Woodring, J., Wiedemann, R., Fischer, M. K., Hoffmann, K. H. & Völkl, W. in press: Honeydew amino acids in relation to sugars and their role in the establishment of ant-attendance hierarchy in eight species of aphids feeding on tansy (*Tanacetum vulgare*). Physiol Entomol.

Yao, I. & Akimoto, S. 2001: Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Oecologia **128**, 36-43.

-. 2002: Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Ecol Entomol **27**, 745-752.

4 Chemical composition of larval nectar secretions from three species of myrmecophilous lycaenid butterflies

H. Daniels[a,*], G. Gottsberger[b], K. Fiedler[a]

[a] *Department of Animal Ecology I, University of Bayreuth, D-95440 Bayreuth, Germany*

[b] *Department of Systematic Botany and Ecology, University of Ulm, D-89081 Ulm, Germany*

[*] *corresponding author*

4.1 Abstract

For the first time a comparative chemical analysis of the larval nectar secretions and hemolymph from unspecifically and facultatively myrmecophilous lycaenid caterpillars was performed. Using HPLC-technique, secretions of two *Polyommatus* species and *Zizeeria knysna* were compared.

Sucrose was the main sugar component in all three species. In *P. coridon* in half of the samples it was accompanied by glucose, while other sugars occurred only rarely and in minor quantities. Melezitose was never found in *P. coridon* samples. In *P. icarus* and *Z. knysna* melezitose was the second-most important component, followed by fructose and glucose. Total sugar contents were 43.6±14.8g/l for *P. coridon*, 74.2g/l for *P. icarus* and 68.3±22.6g/l for *Z. knysna*.

Up to 14 different identified amino acids were found in *P. coridon* nectar, with a total content of 9.7±3.4g/l. Leucine was always the major component (contributing 50% of overall amino acid content). Further important amino acids were tyrosine, proline, arginine, and phenylalanine. *P. icarus* nectar contained up to six amino acids in a total content of 1.2g/l, dominated by tyrosine and phenylalanine. *Z. knysna* nectar contained alanine and proline, with only 0.3±0.17g/l the total content was much lower.

Hemolymph sugars were almost exclusively trehalose, accompanied by a smaller amount of sucrose in all three species. Up to 16 different amino acids occurred relatively regularly in the hemolymph of all three species, dominating were histidine, followed by serine and proline. The amino acid pattern in hemolymph was considerably different from that of the nectar secretions.

Larval food influenced the contents of *P. coridon* secretion. Caterpillars reared with *Securigera varia* secreted less amino acids. Caterpillars fed with semi-synthetic diet secreted more sucrose, and showed a trend towards higher total sugar content. Rearing on semi-synthetic diet furthermore generated nectar secretions with a more homogeneous amino acid pattern.

These results are compared to reports from other lycaenid species and to the chemical composition of other liquid carbohydrate and amino acid sources, which are consumed by ants in mutualistic associations. Secretions rich in amino acids are related to intimate and often obligate ant-associations, whereas facultative and unspecific myrmecophiles rely more on carbohydrates. Within the Lycaenidae, the degree of myrmecophily seems to be a better predictor of secretion content than taxonomic relatedness.

4.2 Introduction

Lepidopteran caterpillars are relatively slow moving insects, with a soft body enclosed in a rather weakly sclerotized cuticle. These fast growing larvae, accumulating resources for the adult phase of their life, are full of nutrients and therefore an attractive target for predators. Ants as the leading terrestrial invertebrate predators (Hölldobler & Wilson 1990) readily prey on caterpillars unless these are protected by mechanical (e.g. hairiness) or chemical (e.g. deterring or poisonous substances) means (Deml & Dettner 1995; Deml & Dettner 2003; Dyer 1997; Montllor & Bernays 1993). A remarkable exception are caterpillars from the family Lycaenidae, comprising with their estimated 4500 described species (excluding Riodinidae) about 30% of all butterflies (Ackery et al. 1999). Lycaenid caterpillars are either ignored by ants, or can even be observed in peaceful interactions with these aggressive social insects. More than the half of the lycaenid species associate with ants at least partly in their development (Fiedler 1991; Pierce et al. 2002). The habit of lycaenid larvae (and of many other arthropods) to associate with ants is termed myrmecophily. The intimacy of such associations is defined by the degree of dependence upon the ant partner. Species which require specific ants essentially for their survival, be it as protectors against enemies or as a food source, are labelled as obligately myrmecophilous. In contrast, species which can thrive also without ants are termed facultatively myrmecophilous. Only 20% of all lycaenid species worldwide appear to be obligate myrmecophiles (Fiedler 1991; Fiedler 1997b). There are, however, significant differences between phylogenetic lineages and biogeographical regions with regard to the incidence of obligate myrmecophily. While in Australia and Southern Africa large fractions of the unique lycaenid faunas depend on specific host ants (Heath & Claassens 2003; Pierce 1987; Pierce et al. 2002), such intimate associations are rare in the West Palearctic region and nearly absent in the Nearctic.

As already noted by Malicky (1969, 1970), two important prerequisites of the close, yet unharmed contact with ants are the onisciform shape of the caterpillars' body and their exceptionally thick cuticle. The interactions themselves are mediated by several glandular organs (Ballmer & Pratt 1991; Cottrell 1984; Fiedler et al. 1996; Kitching 1983; Kitching & Luke 1985). The most important organ for closely ant-associated, non parasitic lycaenid caterpillars is the dorsal nectar organ (DNO, nomenclature after (Cottrell 1984)), a gland situated dorsally on the seventh abdominal segment. Tactile stimulations of the surroundings of this gland by the attendant ants (see also section 3.5.2) (Malicky 1969) elicits eventually secretion of a clear fluid (further on called "nectar"), which is readily imbibed by the attendant ants. Nectar secretions are essential to induce food recruitment behaviour in ants which have found a lycaenid caterpillar (Fiedler & Maschwitz 1989). The importance of a dorsal nectar organ for maintaining stable ant associations is further emphasized by comparative data (Fiedler 1991; Kitching & Luke 1985).

The delivery of nectar secretions is a process under the direct control of the caterpillar. The secretions are delivered only in minute amounts, and secretory behaviour is finely tuned to the particular circumstances (Agrawal & Fordyce 2000; Axén 2000; Axén & Pierce 1998; Axén et al. 1996; Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Leimar & Axén 1993). The situation is therefore completely different from the myrmecophily of aphids and other Homoptera, to which Lycaenidae are often compared. The honeydew of aphids is basically a modified waste product that is excreted in large amounts. Thus honeydew is easily available for chemical analysis and its composition is rather well known for many homopteran species (Bernays & Klein 2002; Hendrix et al. 1992; Nemec & Stary 1990; Woodring et al. in press; Yao & Akimoto 2001; Yao & Akimoto 2002). In contrast, the collection of lycaenid larval nectar secretion is a delicate process (see section 3.4), and only very few species have been examined so far (DeVries 1988; Maschwitz et al. 1975; Nomura et al. 1992; Pierce & Nash 1999). All these studies are based on small sample sizes, and usually few quantitative data are reported at all. Furthermore, apart from two studies (Maschwitz et al. 1975; Wagner 1994) all others deal with obligately myrmecophilous species. These larvae secrete comparatively large amounts of nectar, but are the extreme end of a large range of different degrees of myrmecophily. In most geographical regions and phylogenetic lineages facultatively myrmecophilous lycaenid species are far more numerous and can thus be considered as the “norm” (Fiedler 1991; Fiedler 1997a; Pierce et al. 2002). For a better understanding of the evolutionary ecology of butterfly-ant associations precise data on the quality and quantity of nectar in facultatively myrmecophilous is thus badly needed. This is particularly relevant to test the hypothesis proposed by Pierce (1987) that more generalized myrmecophiles are expected to secrete carbohydrates, whereas intimate and host specific myrmecophiles should have a more nitrogen-biased nectar.

Here for the first time a comparative analysis of the secretions of three facultatively myrmecophilous lycaenid species was undertaken. As the major study organism one of the most strongly ant-associated species in Europe, *Polyommatus coridon*, was chosen. A second species of the same genus, *P. icarus*, has a much less intimate ant association. Finally *Zizeeria knysna* was investigated, whose larvae have only occasionally been reported to be attended by ants (Fiedler 1991). The classification of the Lycaenidae is so far not ultimately resolved, but within the subtribe Polyommatiti (Lycaenidae: Lycaeninae: Polyommatini) *P. coridon* and *P. icarus* are rather closely related in the *Polyommatus* section (Wiemers 2003). *Z. knysna* belongs to the *Zizeeria* section and is therefore a far more distant relative (Eliot 1973; Scott & Wright 1990). This species selection aims towards several important questions:

1) What are the contents of the nectar secretions? In particular, is the amount of nutritious substances comparable to other ant visited nectar sources?
2) Is the allocation of carbohydrates versus amino acids different between those species? Do more closely associated species spend more nitrogen-biased secretions?

3) To what extent is the nectar composition influenced by the food, which caterpillars consume during their development?

4) Does the nectar composition reflect the phylogenetic relatedness, or is it more strongly influenced by the position of a species in the continuum between loose, facultative and intimate, obligate ant associations?

4.3 Material & Methods

4.3.1 Insect collection and rearing

Second and third instar caterpillars of *P. coridon* were collected in the field in Northern Bavaria (49°48’N 11°24’E 540m NN & 49°46’N 11°27’E 460m NN) in spring in each of the years 2001-2003. The caterpillars were placed in boxes as described below. For the second generation some additional mated females were caught at the aforementioned sites in July and August.

Females of *P. icarus* were caught at the campus of the University of Bayreuth and in the vicinity of Bayreuth in spring 2001 and 2003. *Z. knysna* females were collected on Gran Canaria, Spain (27°45N 15°34W 10-20m NN) in spring 2002 and on Fuerteventura, Spain (28°03N 14°30W 5m NN) in spring 2003. The butterflies were transferred either as a group to clear plastic fauna boxes (volume: 10l) or separately in glass vials (volume: 2l) with gauze netting as lid. The bottom of these cages was lined with cellulose tissue which was moistened daily (26°C:15°C, 75% r.h., L:D 16:8). For *P. icarus* and *Z. knysna* shoots of *Medicago sativa* were provided as egg laying substrate, for *P. coridon* shoots or potted plants of *Hippocrepis comosa* were used. Each box was supplied with a small glass vial (volume 15ml) containing water with a cellulose plug and with “artificial flowers” containing a concentrated sugar solution (67.5% w/v) (3.3.1).

Every three days the plant shoots from the cages were transferred to plastic boxes (volume 250 or 1000cm^3, dependent on group size) which were lined with moistened cellulose tissue for optimal humidity. These boxes were kept at 25°C (L:D 16:8), cleaned from the faeces of the caterpillars and old food every day and afterwards filled with new food. Upon pupation, pupae were removed and fixed with double sided glue strips (Tesa) in a new box. These boxes were placed in flight cages (*P. coridon*, *Z. knysna*: 1.95x0.88x1.15m; *P. icarus*: 1.16x0.92x0.80m) where the adults could eclose and mate (26°C:15°C, 75% r.h., L:D 16:8). For the males cellulose plugged glass vials with salt water and a small bunch of nectar plants (as available from the field in the respective season, primarily Asteraceae) was provided, as not all males accepted the artificial flowers. On several days *P. coridon* adults were placed in gauze cages outside in the sun, as they mated only reluctantly under the artificial light (8xOsram L58W, 2xL36W UV, Osram Quicktronic frequency enhancer). As soon as the first laboratory generation of the other two species mated, they could be continuously reared in the flight cages. For *P. coridon*, which generally occurs only in one generation in the field in Germany, the diapause of the eggs was experimentally broken (see section **3.3**) and a second artificial laboratory generation reared.

The caterpillars received different larval food treatments. *P. coridon* was reared on *Hippocrepis comosa*, *Securigera* (*Coronilla*) *varia* and a semi-synthetic diet (further on called “artificial food”) (see Appendix). *P. icarus* and *Z. knysna* were reared on *Medicago sativa* and artificial food. Treatments with *Lotus corniculatus* and *S. varia* for *P. icarus*, and *Amaranthus caudatus* (Amaranthaceae) and *S. varia* for *Z. knysna*, failed to generate enough larval nectar secretion for subsequent analysis. This was partly due to a severe virus infection in 2003 and apparently sub-optimal suitability of the used *A. caudatus* variety as larval food (unpublished observations).

Lasius flavus nests were collected in 2001 and 2002 at two sites in Northern Bavaria (49°46’N 11°27’E 460m NN and 49°58’N 11°39’E 480m NN). Nest boxes (0.54x0.35x0.30m) for experiments were placed in environmental chambers (26°C:15°C, 75% r.h., L:D 16:8), additional reserve nests were kept at room temperature. Colonies were provided with honey, sucrose solution with added vitamins (Multi-Sanostol, Altana) *ad libitum*, and chopped *Gryllus bimaculatus* once a week. Water was provided *ad libitum* at all times.

4.3.2 Sampling and processing of nectar secretions and hemolymph

A caterpillar was placed on a piece of moist cellulose tissue in a small Petri dish (diameter 5.5cm). The dish was secured against concussion and vibration by pieces of foam rubber which were glued to its bottom. Five to ten *Lasius flavus* ants foraging at the honeywater tubes in their nest box were collected from the nest with an exhaustor (Hölldobler & Wilson 1995) and placed next to the caterpillar. As soon as an association had formed, most caterpillars started to secrete several tiny droplets in a short time period. Secreted droplets were collected straight away with microcapillaries (0.5 µl, Hirschmann) and the number of droplets was counted until this "secretion bout" stopped. Earlier experiments with a range of lycaenid species showed that typically a high nectar secretion frequency occurs within the first 3-5min of an ant-caterpillar associations (Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler et al. 1994). The length of the fluid column obtained was immediately measured with a calibrated micrometer eyepiece (Wild M5A stereomicroscope, 25x magnification). Then the capillary was immediately dispensed into 50 µl 70%Ethanol-Millipore-Water and diligently rinsed with 70% Ethanol-Millipore-Water afterwards. Preliminary measurements showed that measurable evaporation from the highly coherent and viscous fluid column started only after one minute, so this did not constitute a problem here. The caterpillar was removed, marked with a number or sign (Staedtler Lumocolor permanent) and stored in a large Petri dish with food available on moist cellulose tissue and with closed lid. The second caterpillar was taken out of the rearing box and placed next to the ants, using the same sampling technique, and this procedure was continued with up to eight individual larvae. After some time (usually 20+ minutes) caterpillars were taken from the large storage Petri dish to be used again for sampling. Due to this procedure the caterpillars always had to re-establish the interrupted ant-association and thus secreted more droplets than they otherwise would have done. Ants were exchanged for new nest mates if motivation started to decrease, at latest after one hour.

As secretions are spend only in minute amounts (chapter 3) most samples had to be pooled from several individuals (*P. coridon* 4.2±2.1, *P. icarus* 4-10, *Z. knysna* 22.2±13.1 individuals respectively). Only in *P. coridon* four samples could be obtained from one individual caterpillar. Individuals were chosen only from the same food treatments. Mostly late fourth instars and still moving prepupae were sampled as these tend to secrete more than younger instars (Burghardt & Fiedler 1996a; Fiedler & Hagemann 1995; Fiedler & Maschwitz 1989). With the optimised harvesting method one complete *P. coridon* sample (at least >0.5 µl, in most cases 1µl, see below) could be harvested over the duration of one week (provided the right instars were available), while harvesting of the same sample size took in *P. icarus* or *Z. knysna* several weeks up to two months per sample.

Hemolymph was obtained by punctuating the dorsal vessels of late fourth instar larvae with a sterile insect pin and subsequently collecting the fluids with microcapillaries (0.5 µl, Hirschmann).

All samples were stored at –20°C until analysis. The length measurement data allowed calculation of the total secretion amount in the (convoluted) samples.

Chemical analysis was done using high-performance liquid chromatography (HPLC). The ethanolic samples were vacuum centrifuged, diluted in pure ion-free water, filtered (Spartan 3/20, 0.45µm pores, Schleicher & Schuell) and split for separate sugar and amino acid analyses. For sugar investigation Sentry Guard (3.9×150mm) and Waters carbohydrate

(4.6×250mm) columns were used with isocratic 72 % acetonitrile solvent at 35°C (Waters 510 pump, flow 1.4 ml min^{-1}). Sugar detection was performed with a refractive index detector (Waters 410). Preliminary tests showed that reliable sugar detection was achieved only when the amount of the sample was higher than 0.4µl (logistic regression $?^{2}_{(1,28)}=17.19$, $p<0.01$, $y=\exp(5.697+14.137{*}x)/(1+\exp(5.697+14.137{*}x))$). Therefore at least 0.5µl were collected, in most cases it was attempted to harvest at least 1µl in all further samples.

The amino acid samples were derivatised with borate buffered 6-aminoquinolyle-N-hydroxy-succinimidyle-carbamate. Sentry Guard (3.9 × 150mm) and Waters AccQtag columns were used with a trinary solvent system (TEA/phosphate buffer with pH 5.5, acetonitril, and water) at 37°C (Waters 600E pump, flow 1.0 ml min^{-1}). Amino acid detection was done with a fluorescent detector (Waters 470). Nine carbohydrates and 17 amino acids were used as standard. Waters Millenium 3.0 software controlled the HPLC and created the respective chromatograms.

4.3.3 Statistical analysis

Basic calculations and data sorting were done in MS Excel™. Statistical analysis was performed in Statistica 6.0™ (Statsoft 2003) and Primer v5 (Clarke & Warwick 2001). If not mentioned otherwise, values are given as mean±s.d. Not normally distributed data was either transformed to achieve normality or the appropriate non-parametric statistic was used. Bonferroni correction, if required, follows (Hochberg 1988) and is explicitly noted where applicable. Testing for differences in the coefficient of variation between groups was done as described by Sokal and Braumann (1980). The principle component analysis (PCA) was calculated with the amino acid spectrum data as proportions of total amino acid content (=100%). Two axes were extracted, rotation procedure adopted was varimax standard (Statsoft 2003). Various versions of principle component analyses performed with raw data or with subsamples or other combinations and extracting more axes delivered all the same overall picture.

4.4 Results

4.4.1 Sugar composition and concentration

Nine different sugars were identified. Five further peaks were detected, but only one of these occurred more then two times (retention time 4.92min, eleven times in 32 *P. coridon* samples).

The carbohydrate content of larval nectar secretions of *P. coridon* caterpillars reared on natural food plants (n=23) consisted mainly of sucrose. In less than half of the samples sucrose was accompanied by glucose. Arabinose, fructose, maltose, trehalose and lactose occurred only rarely and mostly in minor quantities (Fig. 4.1) (Table 4.1). Melezitose was never found in *P. coridon* samples. Total sugar concentration was 43.55±14.76g/l (respectively 47.38±19.28g/l if regarding only individuals reared on the host plant *H. comosa*, n=11). *P. coridon* nectar was neither clearly acidic nor alkaline, the pH value ranged 5.0-8.0 (measurement with pH paper pH 1-10, Merck, with the tiny dots no exact colour differentiation possible between 5.0-8.0, due to the small nectar amounts no more precise measurement method was available).

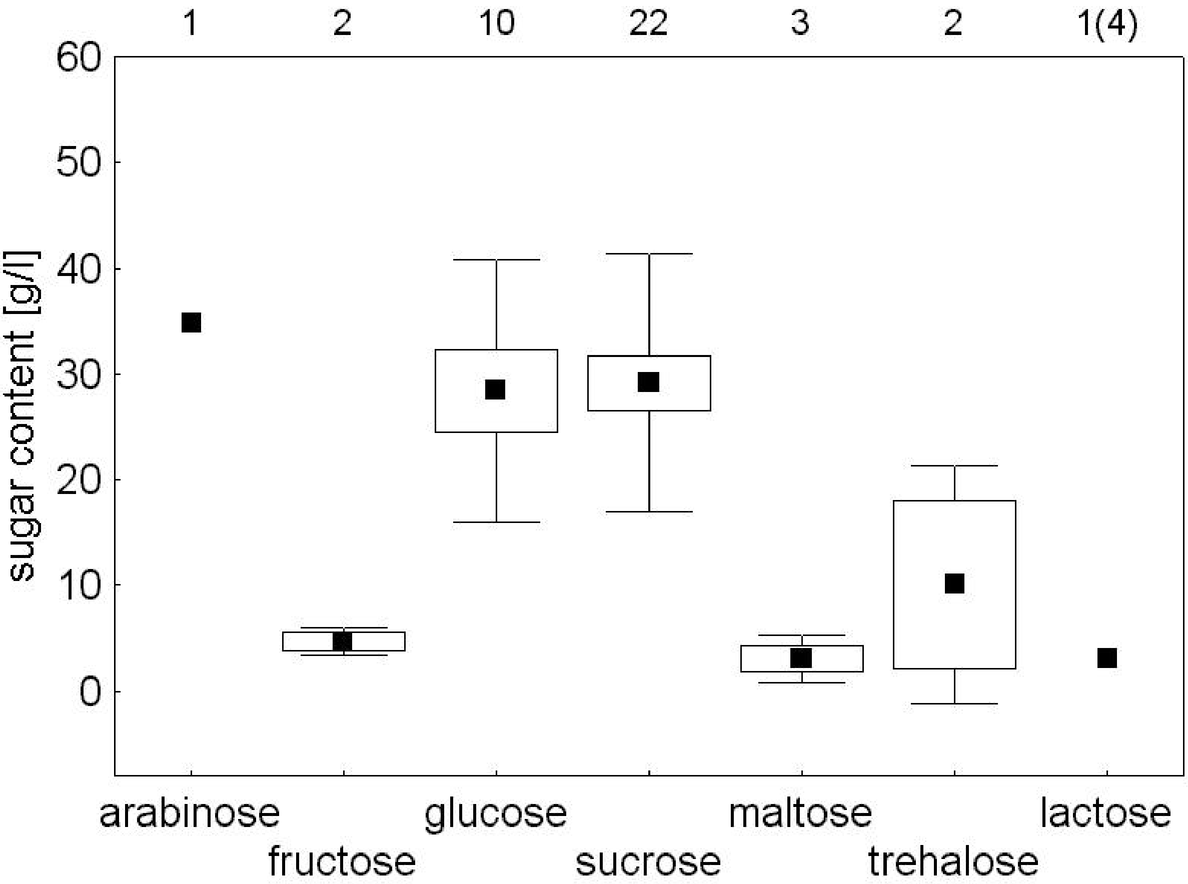


Fig. 4.1 Sugar content (box: mean±s.e. whiskers: mean±s.d.) of the identified sugars in *P. coridon* larval nectar secretions. Figures above the diagram denote the number of samples in which the respective sugar was found (total n=23 samples from plant fed caterpillars). For lactose the quantity was determinable in one case only

The larval nectar secretions of *P. icarus* (n=2, *M. sativa* fed) and *Z. knysna* (n=8, 3 reared on *M. sativa*, 5 fed with artificial food) caterpillars shared similar characteristics for sugar content and sugar composition. Main components were sucrose and melezitose, accompanied by fructose and glucose in successively lower concentrations (Fig. 4.2). Additional rare sugars were melibiose (in one *P. icarus* sample) and probably erlose (in one *P. icarus* sample and one *Z. knysna* sample, not quantified). Total concentration was 74.2g/l (median) for *P. icarus* and 68.3±22.6g/l for *Z. knysna*, respectively.

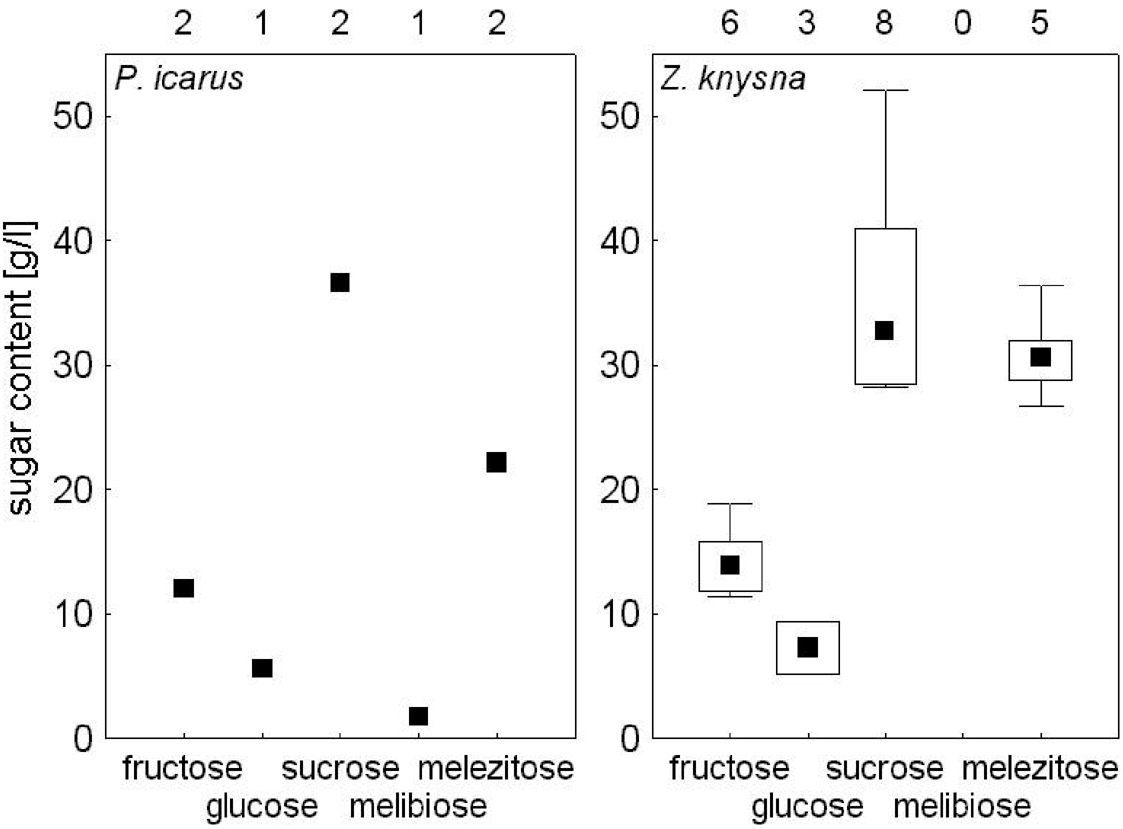


Fig. 4.2 Sugar content (black square: median, box: 25% percentiles, whiskers: min-max) of the identified sugars in *P. icarus* (left) and *Z. knysna* (right) larval nectar secretions. Figures above the diagram denote the number of samples in which the respective sugar was found (*P. icarus*: 2 samples from caterpillars fed with *M. sativa*; *Z. knysna*: 3 *M. sativa* and 5 artificial food)

Hemolymph sugar composition consisted almost exclusively of trehalose accompanied by a lower quantity of sucrose (Fig. 4.3). Each of the three other sugars (glucose, fructose and melibiose) occurred only in a single sample. Total sugar concentrations were 26.75g/l (median, n=3) for *P. coridon*, 33.92g/l (n=2) for *P. icarus*, and 13.18g/l (n=3) for *Z. knysna* hemolymph, respectively.

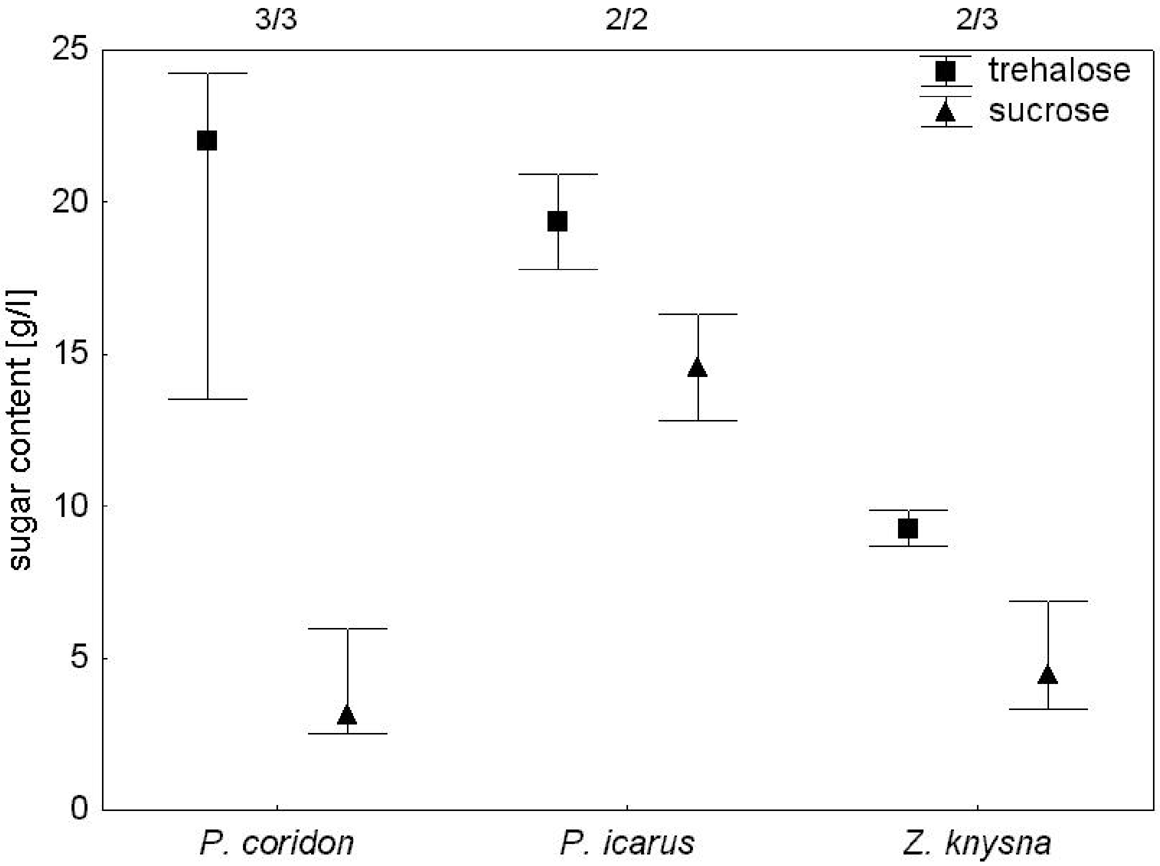


Fig. 4.3 Trehalose and sucrose content (black square: median, whiskers: min-max) in hemolymph of *P. coridon*, *P. icarus*, and *Z. knysna*, respectively. Figures above the diagram denote the number of samples in which the respective sugar was found (*P. coridon*: 3 samples from caterpillars reared on *H. comosa*, *P. icarus*: 2 samples from caterpillars reared on *M. sativa*; *Z. knysna*: 2 samples from caterpillars reared on *M. sativa* and 1 sample from caterpillars fed with artificial food)

4.4.2 Amino acid composition and concentration

A whole bouquet of up to 14 different identified amino acids was found in the larval nectar secretions of *P. coridon*. Almost half of the overall content was contributed by one single amino acid, leucine (Fig. 4.4). All others contributed much less; the next most important four amino acids were tyrosine, proline, arginine and phenylalanine. Histidine, methionine, alanine, threonine, isoleucine, and valine occurred in lower quantities, but still regularly (in more than one half of all samples). Lysine, serine and glycine were found occasionally, glutamate was rare and then discovered in highly variable quantities. The total amino acid content was 9.68±3.39g/l (n=42, all food treatments combined).

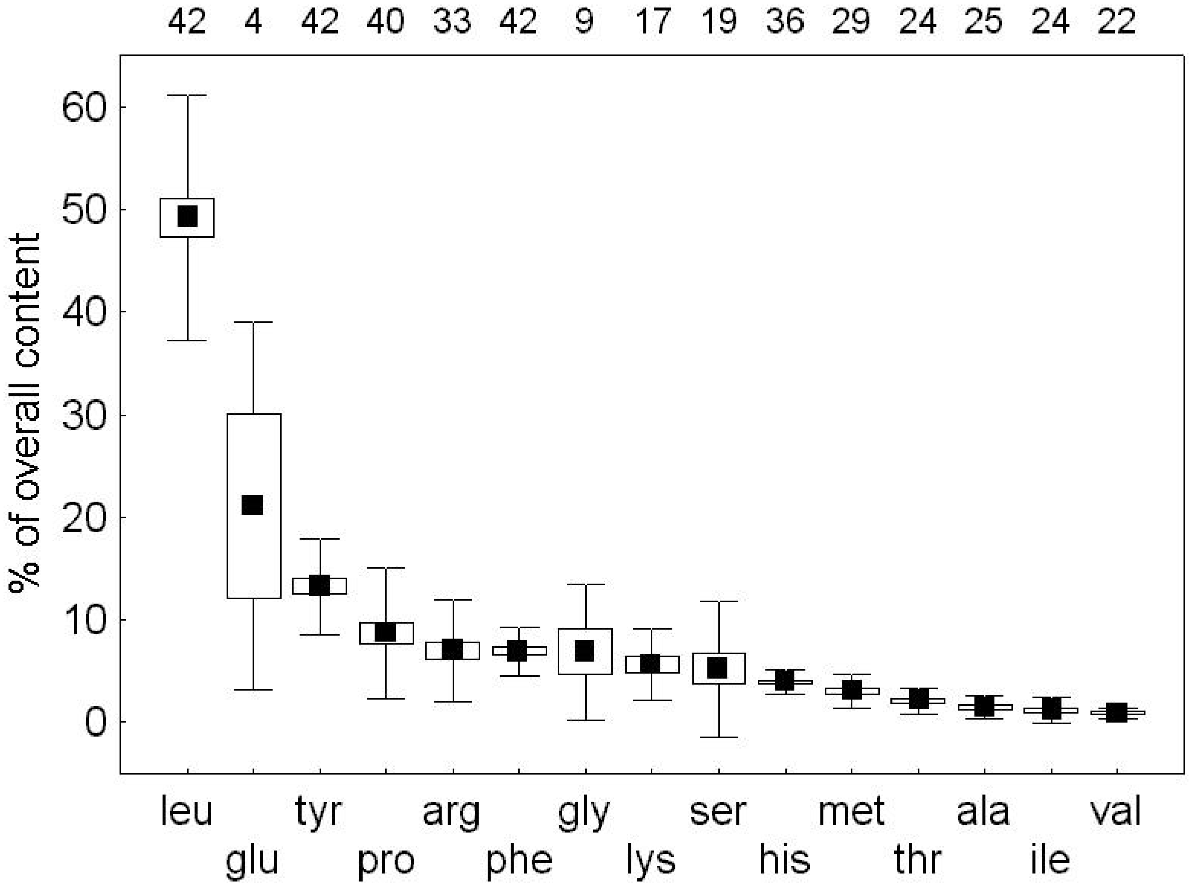


Fig. 4.4 Proportion of overall amino acid content (box: mean±s.e. whiskers: mean±s.d.) contributed by different amino acids in *P. coridon* larval nectar secretions, ranked after their mean abundance. Figures above the graph denote the number of occurrences of each amino acid in the 42 secretion samples

The larval nectar secretion of *P. icarus* contained much less amino acids. They were dominated by tyrosine, phenylalanine and arginine, in minor quantities proline, serine and alanine occurred (Fig. 4.5). Total amino acid content was only 1.2g/l (median n=2), amounting to roughly one tenth of the total amino acid content of *P. coridon* nectar. In *Z. knysna* nectar secretions alanine or proline either occurred alone or together, sometimes accompanied by threonine or arginine in low quantities (Fig. 4.6). The total amino acid content was even lower (0.30±0.17g/l, n=6), corresponding to 25% of *P. icarus* and 0.3% of *P. coridon* total amino acid content respectively.

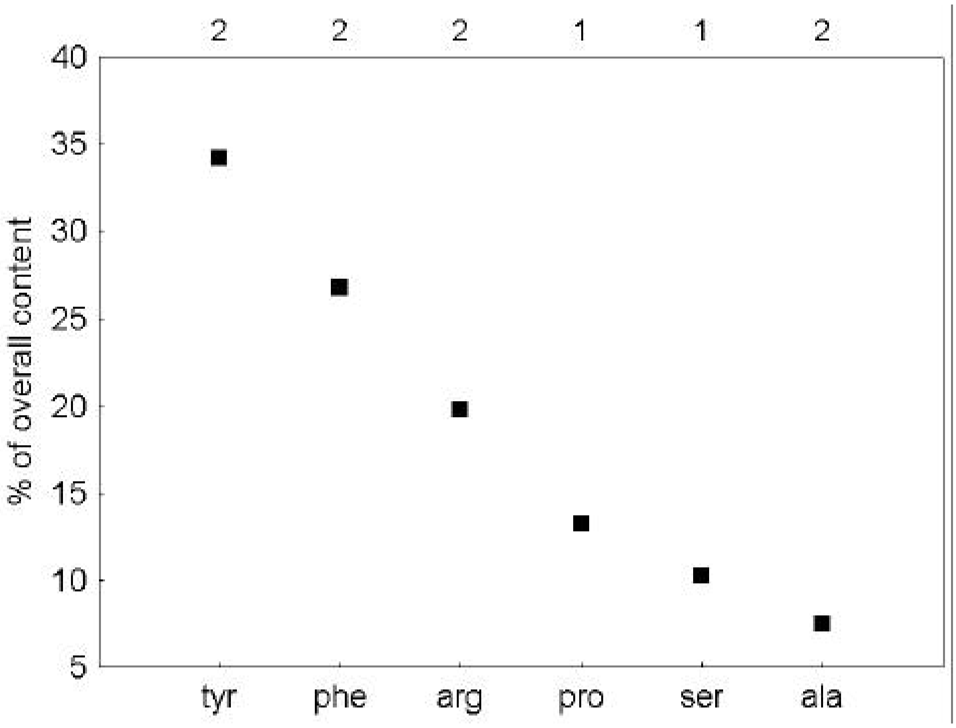


Fig. 4.5 Proportion of overall amino acid content (median) contributed by different amino acids in *P. icarus* larval nectar secretions, ranked after their mean abundance. Figures above the graph denote the number of occurrences of each amino acid in 2 secretion samples from caterpillars fed with *M. sativa*

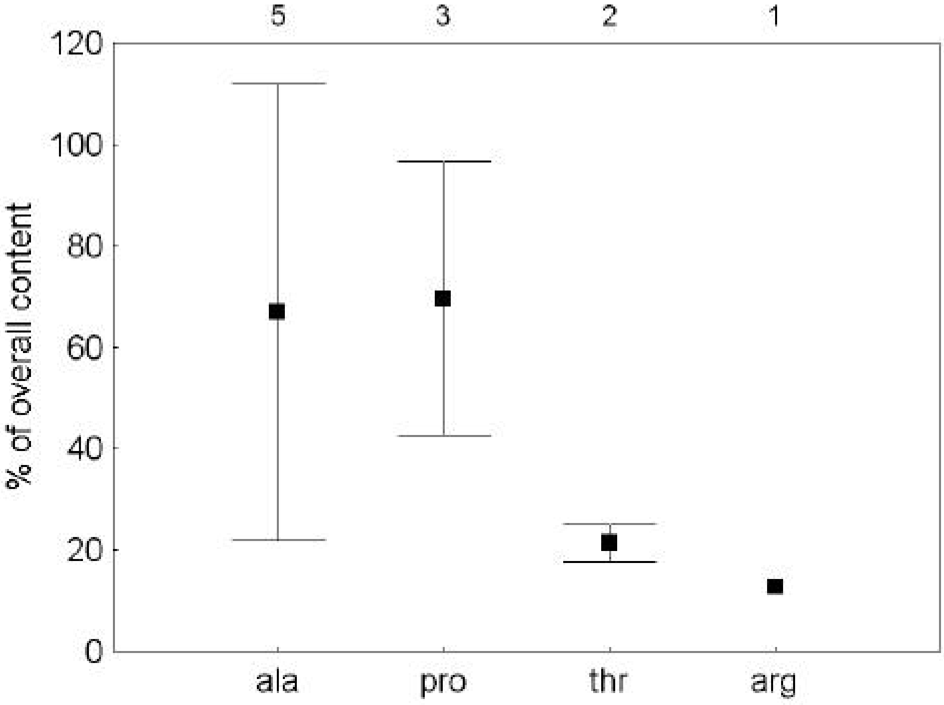


Fig. 4.6 Proportions of overall amino acid content (whiskers: mean±s.d.) contributed by different amino acids in *Z. knysna* larval nectar secretions, ranked after their mean abundance. Figures above the graph denote the number of occurrences of each amino acid in 6 secretion samples (3 from caterpillars fed with *M. sativa*, 3 with artificial food)

In hemolymph up to 16 free amino acids were found, almost all of them regularly (in >75% of all samples). Only tyrosine, glutamate and asparagine occurred irregularly (Fig. 4.7). The dominating amino acid was histidine, followed by serine and proline. All further amino acids contributed less than ten percent. Total amino acid content was high, with an average across all species and samples of 10.81±7.92g/l. Hemolymph composition was similar in the three species, see chapters 4.4.3 and 4.4.5.

The pattern of amino acid content is considerably different from the patterns found in the secretions of the three species (see also chapter 4.4.5). The mean content of the individual amino acids of secretions and of hemolymph correlated neither in *P. coridon* (r_s=-0.06, p=0.82, n=16) nor in *Z. knysna* (r_s=0.1, p=0.73, n=15). The main components of hemolymph differ from the main components found in the secretions, as indicated by the arrows in Fig. 4.7 (compare also to Fig. 4.4, Fig. 4.5, Fig. 4.6).

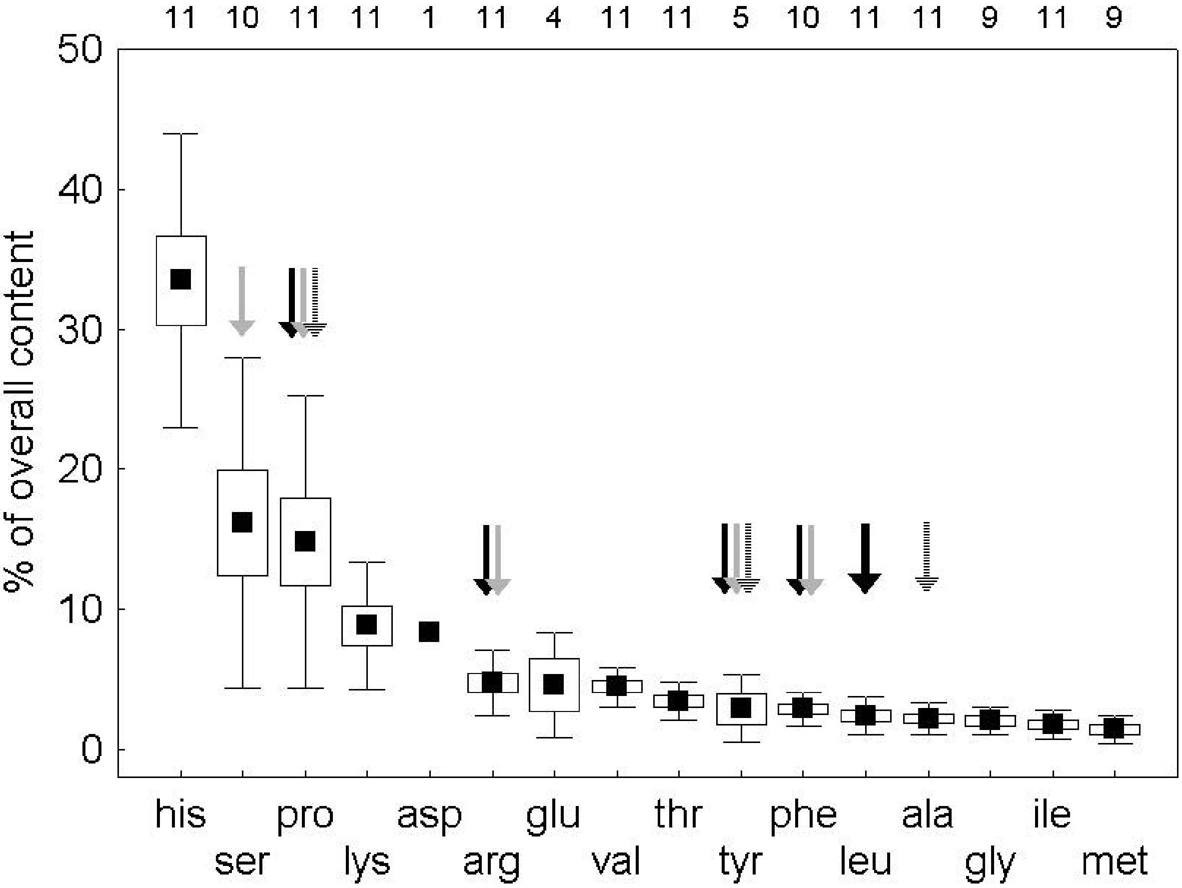


Fig. 4.7 Proportion of overall amino acid content (box: mean±s.e. whiskers: mean±s.d.) contributed by different amino acids in hemolymph, ranked after their mean abundance. Black arrows indicate the main amino acids found in *P. coridon* secretions, grey arrows the main ones in *P. icarus*, and dashed arrows the main ones in *Z. knysna* secretions, respectively. Figures above the graph denote the number of occurrences of each amino acid in 11 hemolymph samples (*P. coridon*: 3 *H. comosa* fed, 1 artificial food; *P. icarus*: 3 *M. sativa*; *Z. knysna*: 3 *M. sativa*, 1 artificial food)

Table 4.1 Larval nectar secretions and hemolymph analysed by HPLC. Symbols indicate sugar and amino concentration (mean percent of total weight of identified sugars or amino acids, respectively): ▼) <10% ●)10-50% ■)>50%. Open symbols (▽○□) were given for substances that were not present in all samples of a source. n = number of samples. Total concentrations as mean±s.d.

		sugars											amino acids																
source	n	arabinose	fructose	glucose	sucrose	maltose	trehalose	lactose	melibiose	melezitose	total [g/l]	n	aspartate	serine	glutamate	glycine	histidine	arginine	threonine	alanine	proline	tyrosine	valine	methionine	lysine	isoleucine	leucine	phenylalanine	total [g/l]
Polyommatus coridon fed with																													
H. comosa	11	○	▽	○	■	▽	○	▽	-	-	47.4±19.3	11	-	▽	-	▽	▼	▽	▽	▽	▼	●	▽	▽	▽	▽	■	▼	11.2±3.1
S. varia	12	-	-	○	□	-	-	-	-	-	40.0±8.3	21	-	▽	○	○	▽	○	▽	▽	▽	●	▽	▽	▽	▽	●	▼	8.9±3.6
artificial food	10	-	▽	○	■	-	-	-	-	-	60.4±21.8	10	-	▽	-	▽	▼	▽	▽	▽	●	●	▽	▽	▽	▼	■	▼	9.6±3.6
Polyommatus icarus fed with																													
M. sativa	2	-	●	▽	●	-	-	-	▽	●	74.2±1.9	2	-	○	-	-	-	●	-	▼	○	●	-	-	-	-	-	●	1.2±1.0
Zizeeria knysna fed with																													
M. sativa	3	-	●	○	●	-	-	-	-	●	82.6±8.8	3	-	-	-	-	-	-	○	■	□	-	-	-	-	-	-	-	0.2±0.2
artificial food	5	-	○	▽	■	-	-	-	-	○	59.7±24.7	3	-	-	-	-	-	○	○	□	□	-	-	-	-	-	-	-	0.4±0.1
hemolymph																													
Polyommatus coridon	3	-	▽	-	●	-	■	-	○	-	26.3±6.5	4	▽	●	▽	▽	●	▼	▼	▼	●	▽	▼	▽	▼	▼	▼	▽	8.6±5.4
Polyommatus icarus	2	-	-	-	●	-	■	-	-	-	33.9±0.3	3	–	●	▽	▼	●	▼	▼	▼	▼	▽	▼	▼	▼	▼	▼	▼	8.8±2.9
Zizeeria knysna	3	-	-	□	●	-	□	-	-	-	13.6±1.8	4	-	○	▽	▼	●	▼	▼	▼	●	▽	▼	▽	●	▼	▼	▼	14.6±12.0

4.4.3 Differences between the species

Nectar secretions of *Z. knysna* had a higher overall sugar content than *P. coridon* nectar secretions of plant fed caterpillars (Kruskal-Wallis ANOVA, $H_{(2,33)}=9.17$, $p=0.01$, *P. coridon* reared on plants vs. *P. icarus* vs. *Z. knysna*) (Fig. 4.8). Conversely, *P. coridon* nectar secretions had a higher content of amino acids ($H_{(2,50)}=19.88$, $p<0.001$) (Fig. 4.9), as well as a higher number of different amino acids ($H_{(2,52)}=22.76$, $p<0.001$) (Fig. 4.10). The sample size for *P. icarus* nectar secretions was too low to allow for meaningful statistical results, but the pattern follows the same trend as *Z. knysna*.

Comparison of the hemolymph showed that hemolymph from *Z. knysna* had a lower overall sugar content than *P. icarus* hemolymph ($H_{(2,8)}=6.25$, $p=0.044$). There was neither a difference detected between the species for total amino acid content ($H_{(2,11)}=0.96$, $p=0.62$), nor for the number of amino acids in hemolymph ($H_{(2,11)}=2.09$, $p=0.35$).

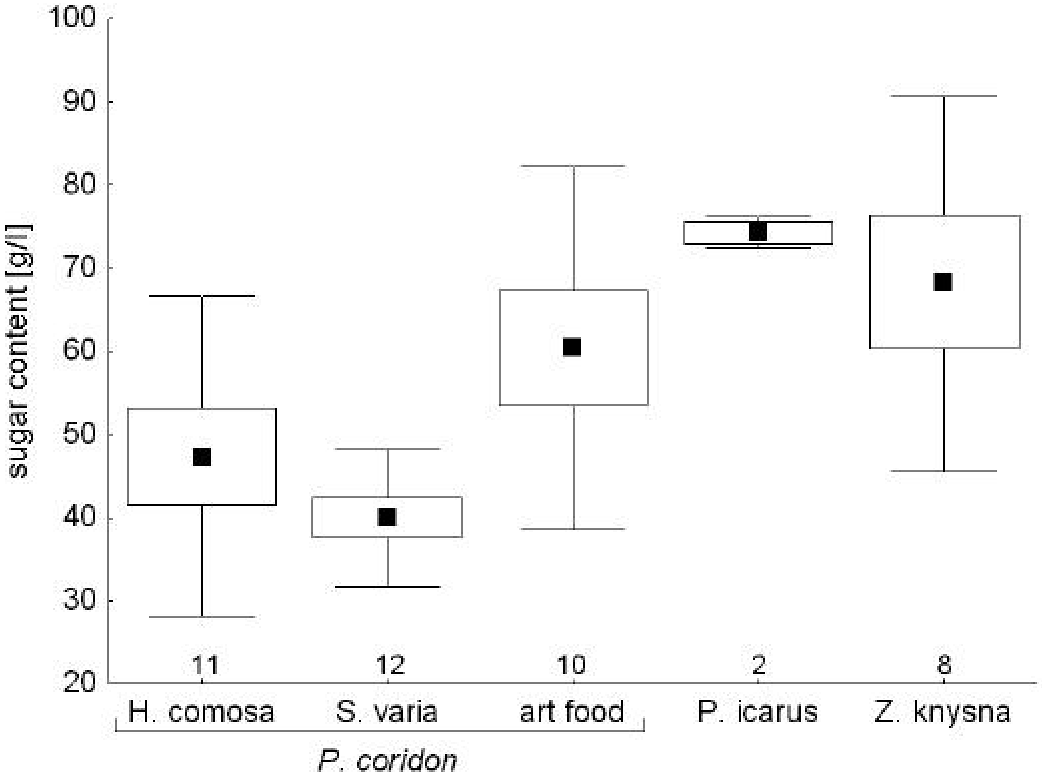


Fig. 4.8 Total sugar content (box: mean±s.e. whiskers: mean±s.d.) in *P. coridon* secretions from three different food treatments compared to *P. icarus* and *Z. knysna* secretions (number of samples noted above axis, *Z. knysna* average across food treatments *M. sativa* and art. food, statistics for food and species effects see text in chapters 4.4.3 and 4.4.4)

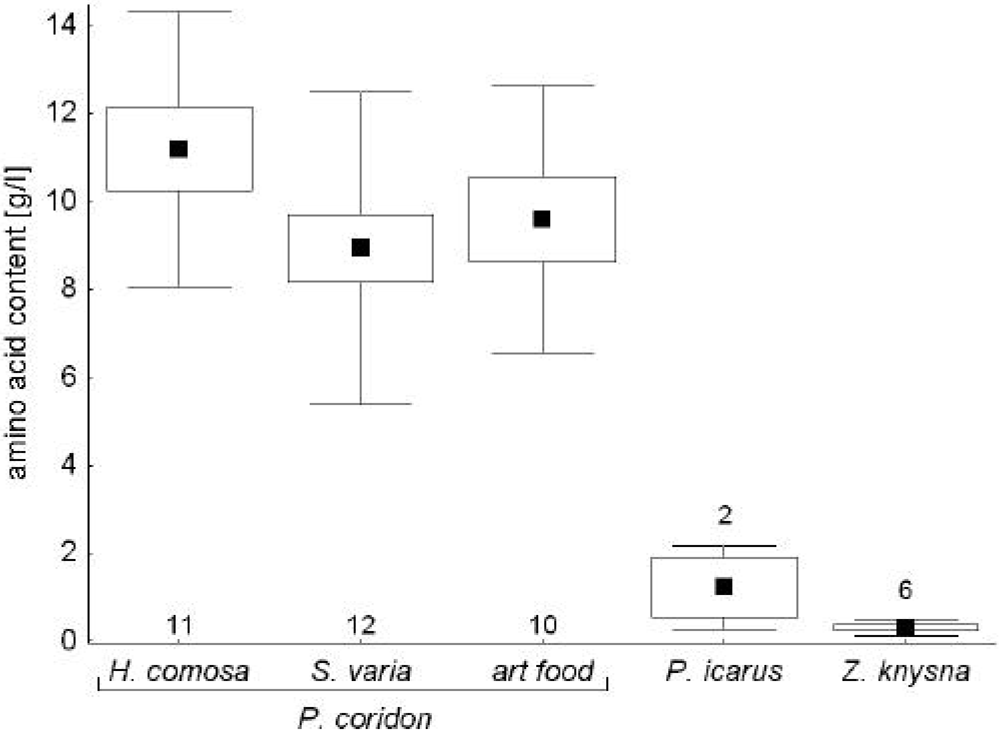


Fig. 4.9 Total amino acid content (box: mean±s.e. whiskers: mean±s.d.) in *P. coridon* secretions from three different food treatments compared to *P. icarus* and *Z. knysna* secretions (number of samples noted, *Z. knysna* average across food treatments *M. sativa* and art. food, statistics for food and species effects see text in chapters 4.4.3 and 4.4.4)

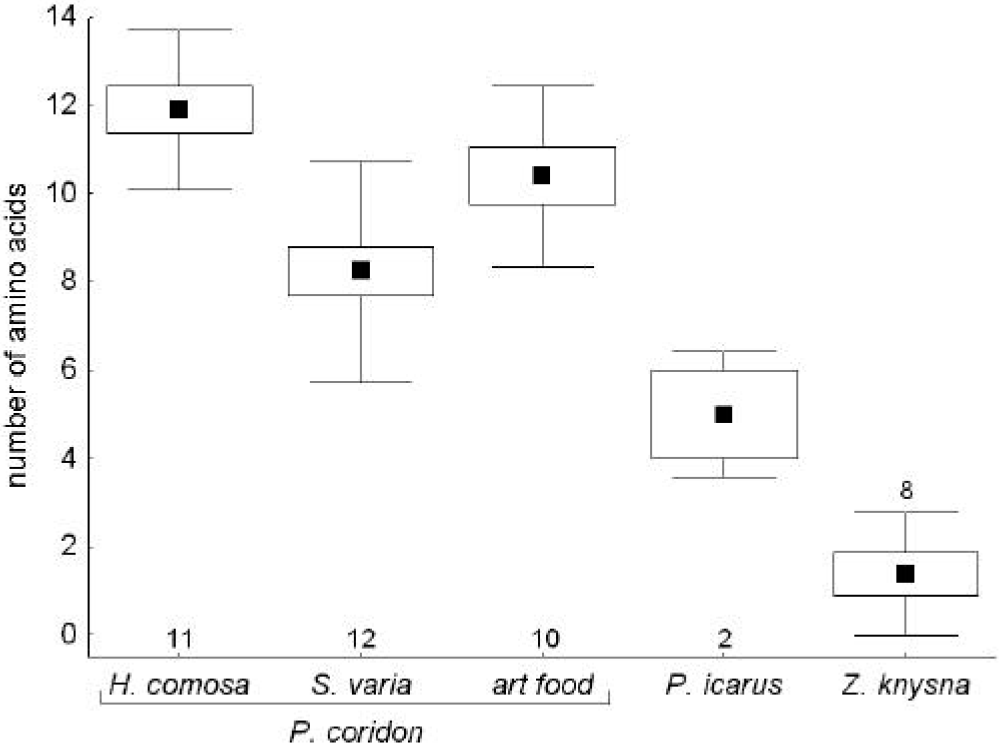


Fig. 4.10 Number of amino acids detected (box: mean±s.e. whiskers: mean±s.d.) in *P. coridon* secretions from three different food treatments compared to *P. icarus* and *Z. knysna* secretions (number of samples noted, *Z. knysna* average across food treatments *M. sativa* and art. food, statistics for food and species effects see text in chapters 4.4.3 and 4.4.4)

4.4.4 The influence of larval food

The food which *P. coridon* caterpillars had consumed during their development, clearly influenced sugar content of their secretions (Fig. 4.11). Caterpillars fed with artificial food secreted more sucrose than caterpillars fed with *H. comosa* (one-way ANOVA $F_{(2,29)}=17.78$, $p<0.001$, Scheffé: $p<0.001$). The same effect was found for *C. varia* fed caterpillars (Scheffé: $p=0.005$), although these secreted slightly less than those fed artificial food (Scheffé: $p=0.066$). Concerning total sugar content, the pattern changed (one-way ANOVA, $F_{(2,29)}=3.13$, $p=0.058$), (Scheffé art. food vs. *S. varia*: 0.062), as *H. comosa* fed individuals secreted more often additional sugars (fructose, arabinose, maltose, trehalose) than the caterpillars reared on *S. varia* or artificial food (Table 4.1). For *Z. knysna* no effects of the larval food on the sugar contents of the nectar could be detected (Mann-Whitney U-test: $U_{(1,8)}=3$, $p=0.18$), but due to the small sample size the power of the test was low. Patterns of carbohydrate contents in *Z. knysna* nectar were very similar irrespective of larval food (Fig. 4.12).

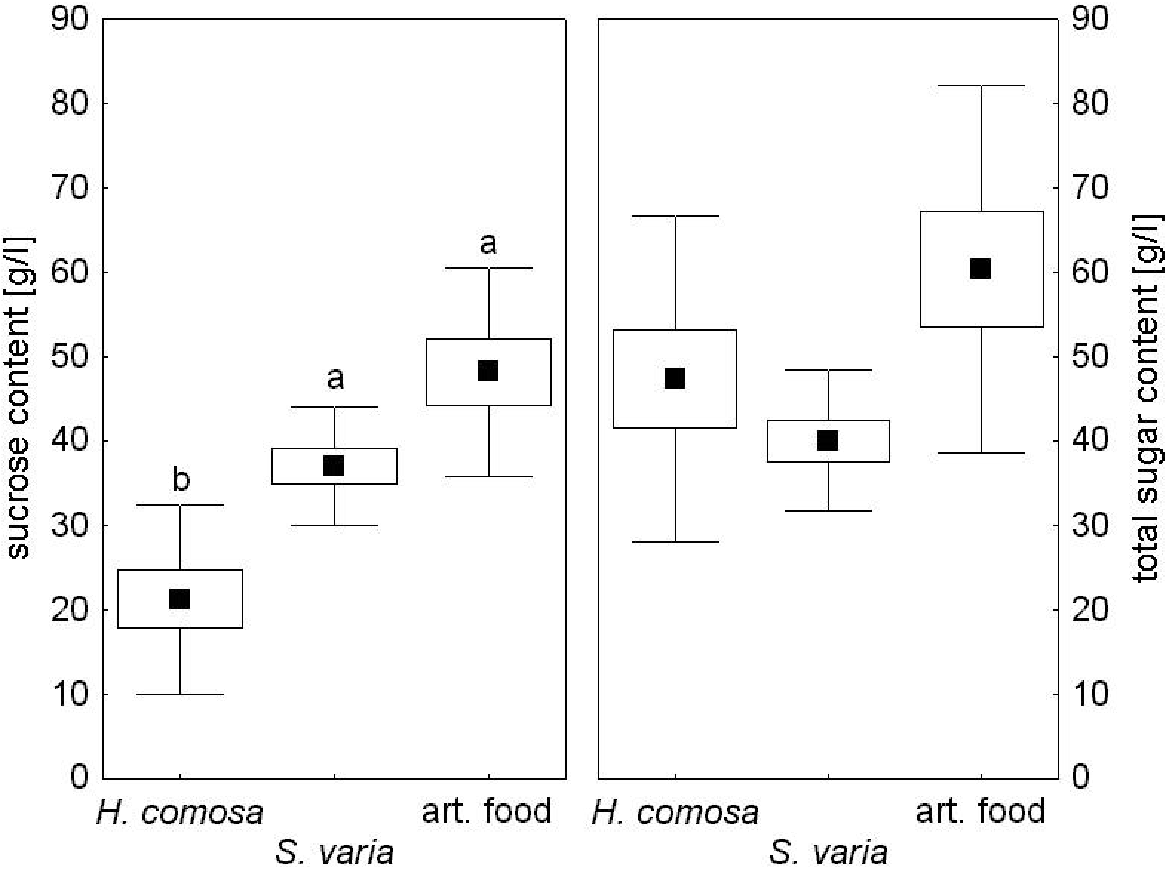


Fig. 4.11 The influence of larval food on the sucrose (left) and the total sugar content (right) of *P. coridon* larval nectar secretions (box: mean±s.e. whiskers: mean±s.d.). Influence on sucrose content: one-way ANOVA, $F_{(2,29)}=17.78$, $p<0.001$ (significant effects localized with Scheffé test), on total sugar content: $F_{(2,29)}=3.13$, $p=0.058$

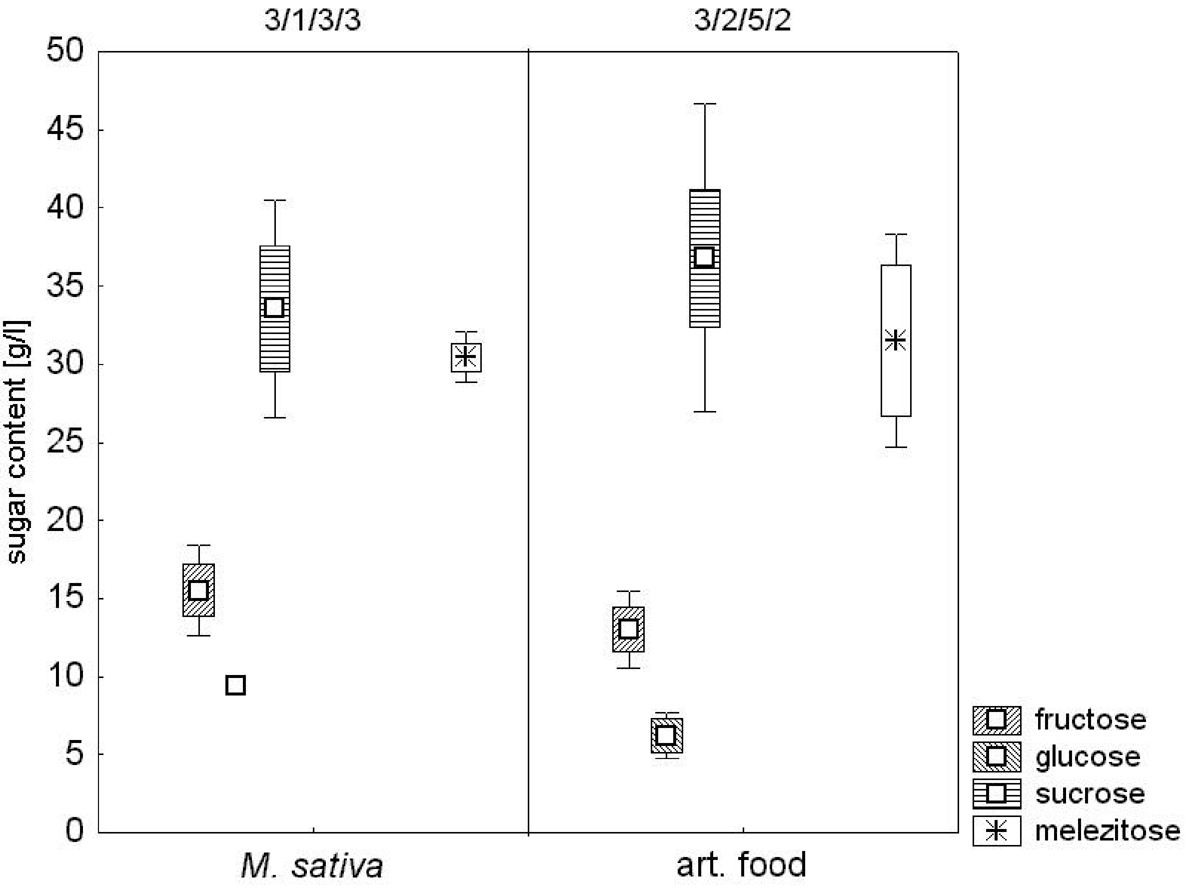


Fig. 4.12 The influence of larval food on sugar composition and content in the larval nectar secretions of *Z. knysna* (box: mean±s.e. whiskers: mean±s.d.) Figures above the graph denote the number of occurrences of each sugar in 3 secretion samples from caterpillars reared on *M. sativa*, and in 5 samples from caterpillars fed with artificial food)

The food consumed by *P. coridon* caterpillars had no effect on amino acid content (one-way ANOVA $F_{(2,39)}=1.65$, p=0.2) (Fig. 4.9), but on the number of amino acids in the secretion(one-way ANOVA $F_{(2,39)}=10.23$, $p<0.001$) (Fig. 4.10). Caterpillars reared on *H. comosa* secreted more amino acid than caterpillars reared on *S. varia* (Scheffé: $p<0.001$; Scheffé art. food vs. *S. varia*: p=0.055). The amino acid composition in secretions from *P. coridon* caterpillars fed with artificial food was significantly more homogenous than in secretions from *H. comosa* fed individuals (Fig. 4.13) (Test for differences in the coefficient of variation (Sokal & Braumann 1980) in the PCA pattern: PC1: $t_{(1,21)}=423.38$, $p<0.0002$; PC2: $t_{(1,21)}=147.99$, $p<0.01$). Feeding with *S. varia* derived significantly more variation in the amino acid profiles than feeding artificial diet and *H. comosa*: (PC1: $t_{(1,31)}=424.4$, $p<0.002$; PC2: $t_{(1,31)}=419.8$, $p<0.002$ vs. artificial food, PC1: $t_{(1,32)}=597.65$, $p<0.002$; PC2: $t_{(1,32)}=400.65$, $p<0.01$ vs. *H. comosa* respectively).

4.4.5 Comparison of the amino acid profiles

Principle components analysis of the amino acid profiles revealed a clear pattern (Fig. 4.13). The PCA explained with its two axes 39.3% of the variance. With five axes 70% of the variance could be explained, nevertheless as the graphical picture was already clear with two axes and was not further clarified, the PCA with two axes was used for subsequent interpretation. The axes could not be explained with regard to acidic or alkaline character or the essentiality of the amino acids. The first axis extracted separated mainly hemolymph and secretions (Table 4.1). Histidine and valine had positive loadings, as they occurred regularly in hemolymph, but otherwise in the secretions sometimes in *P. coridon* nectar only, therefore aligning the hemolymph samples to the 'right' in the ordination. Leucine, tyrosine and alanine were each leading components in the secretions of the three species, therefore grouping the secretions to the 'left'. The aromatic amino acids phenylalanine and tyrosine contributed markedly to the second principle axis (Table 4.1), as both were contained in secretions of *P. coridon* and *P. icarus*, but were not present in *Z. knysna* secretions. Leucine as main component of *P. coridon* secretions contributed a further high positive loading. Alanine and proline as typical components in *Z. knysna* secretions aligned these to the 'bottom'.

Table 4.1 Factor loadings of the amino acids in the principle component analysis after varimax standard rotation, load values >0.75 marked in bold

amino acid	Factor 1	Factor 2
serine	0.691	-0.036
histidine	**0.804**	-0.173
threonine	0.044	-0.512
alanine	-0.303	-0.532
proline	-0.036	-0.531
tyrosine	-0.423	**0.815**
valine	**0.854**	-0.186
lysine	0.695	-0.042
isoleucine	0.630	0.055
leucine	-0.389	0.556
phenylalanine	0.250	**0.761**

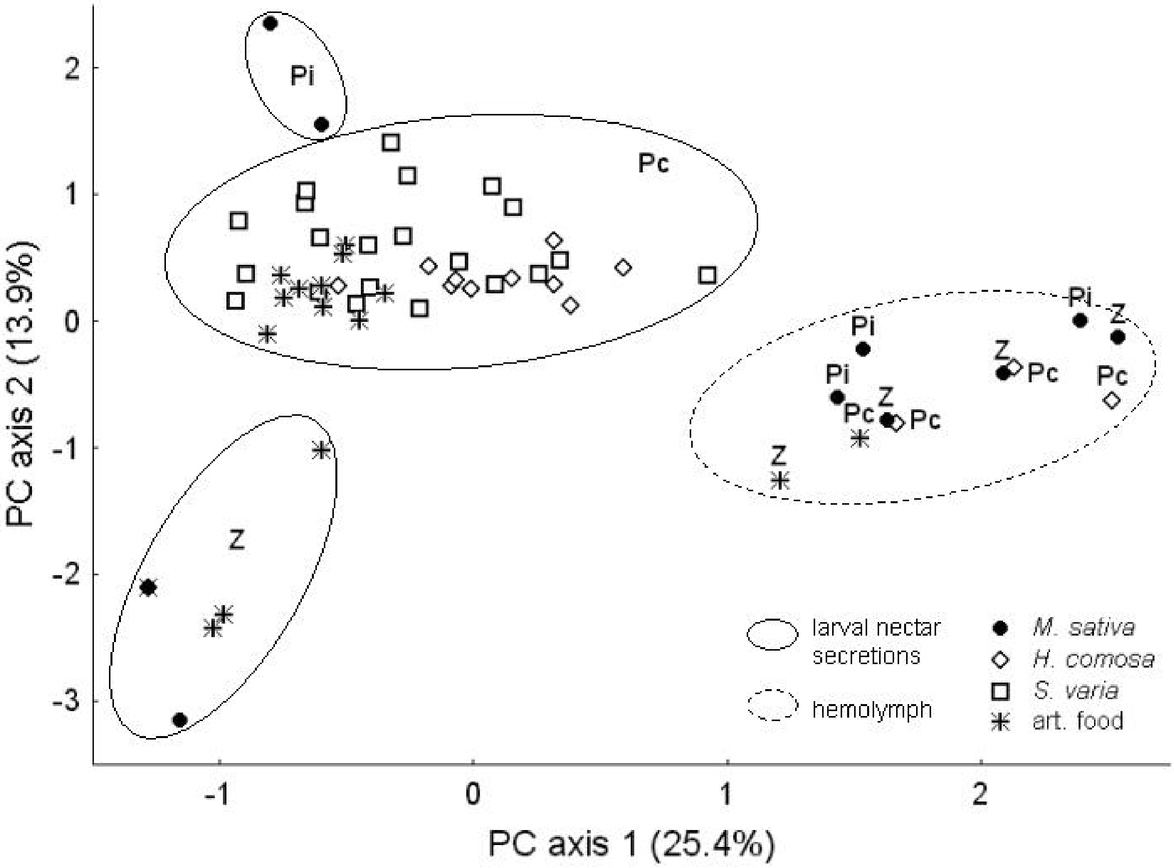


Fig. 4.13 Ordination of nectar and hemolymph samples from three facultatively myrmecophilous lycaenid species (Pc: *P. coridon*, Pi: *P. icarus*, Z: *Z. knysna*), based on a principle component analysis of amino acid proportions after varimax standard rotation. Caterpillars were reared on four different foods, as denoted in the legend. In brackets the contribution of the respective component to the explanation of observed variance.

Secretion samples harvested from *P. coridon* form one large group with no segregation due to larval nutrition (one-way ANOSIM: r_{global}=-0.012, p=0.54). *P. icarus* secretions are relatively similar to those of *P. coridon*, whereas *Z. knysna* forms a separate group (Fig. 4.13), with no segregation due to larval nutrition, too (one-way ANOSIM: r_{global}=-0.148, p=0.70). Yet the secretion amino acid profiles of all species are statistically different from another (Table 4.2).

Hemolymph has a clearly different composition from nectar secretions (one-way ANOSIM: r_{global}=0.554, p<0.001), and is remarkably similar for all three species (one-way ANOSIM: r_{global}=-0.173, p=0.93). Therefore, with regard to their amino acid composition the larval nectar secretions are not just filtered hemolymph, but have to be processed in the gland (compare also Fig. 4.7).

Table 4.2: Results of two-way ANOSIM (with food treatments nested in species), performed on Euclidian distances between nectar samples

ANOSIM two-way nested r_{global}=0.987, p<0.001				
pairwise comparisons		r	p	permutations
P. coridon	vs. *P. icarus*	0.986	<0.001	903
P. icarus	vs. *Z. knysna*	0.578	0.036	28
P. coridon	vs. *Z. knysna*	0.993	<0.001	999

4.5 Discussion

The three lycaenid species considered here show remarkable variation in nectar secretion contents. Such variation was observed with respect to carbohydrates as well as amino acids. Two factors constrained the nectar analysis. Firstly, lycaenid larvae secrete but minute amounts of nectar (chapter 3.4). Thus samples had to be pooled across individuals, and still numbers and amounts of samples remained small. Secondly, the chemical analytical method employed was unable to detect all possible nutrients in the nectar. For example, the amino acid tryptophane could not be identified by the derivatisation method chosen (G. Gottsberger, pers. comm.). Compounds like proteins, fatty acids, vitamins or other substances of low molecular weight were also not determined. However such substances may possibly occur in lycaenid nectar, as they have been found in plant nectar and homopteran honeydew (see Strong (1965)).

Contamination with invertases from e.g. microbial sources can lead to the dissociation of sucrose into glucose and fructose. This did not pose a problem here, as the samples were immediately transferred to the ethanol-water mixture. Furthermore, glucose and fructose were not found in equal amounts, which would have been the case, if the presence of these two mono-hexoses were largely due to sucrose dissociation. Interestingly, the amino acid cysteine was never found. This might be based on the fact that cysteine often occurs as the dimeric cystine, which could not be determined, as no cystine standard was available. However, Sandström and Moran (2001) also never found cysteine in honeydew, and this amino acid occurred relatively rarely in a comparison of several plant and insect nectar sources [Blüthgen, 2004 #282]. Therefore cysteine in lycaenid nectars might actually not be present (see below).

Special attention and caution is required if insect hemolymph is analysed. The results fundamentally depend on the developmental or life history stage (Buck 1953) and activity state (e.g. Božic & Woodring (1997)) of the animals used for hemolymph extraction. Furthermore, amino acids in hemolymph occur often not as single free molecules, but as di- or tripeptids, which serve as storage units (Bodnaryk 1978; Collett 1989) and are also stored in the hemocytes (Mullins 1985). Therefore the actual content of these nutrients available for predators might be much higher. Thus the results presented here for hemolymph are to be considered with appropriate caution and are valid only for late fourth instars or early prepupae, which might have started considerable biosynthesis processes related to coming metamorphosis, resulting in potentially changed hemolymph composition (Florkin & Jeuniaux 1974). Nevertheless the values found here for immature stages of lycaenid butterflies are well comparable to other literature sources (Buck 1953; Florkin & Jeuniaux 1974; Mullins 1985), unusually is only the relatively high sucrose content in the hemolymph, but this occurs if uptake of diet sugars in the gut is possible also as sucrose (Turunen 1985). The hemolymph differed from the secretions in sugar concentration and composition and in the amino acid pattern in all three species, showing that a mere filtering in the nectar gland is highly unlikely. The nectar secretions are moreover most probably products of active secretion processes in the gland.

For obligate myrmecophilous or strongly ant-associated facultatively myrmecophilous lycaenid species a higher content of nutrients than in loosely ant-associated lycaenid species should be expected, as a greater reward for the ants would serve to obtain and maintain a stable ant guard (Pierce 1987). Comparing the results obtained here for the strongly, but facultatively myrmecophilous *P. coridon* with values provided for other myrmecophilous lycaenid species in the literature (Table 4.3) (although with a slight caveat, as different analytical methods were employed) illustrates that the sugar content is lower than in obligate myrmecophilous species *Jalmenus evagoras* (Pierce 1983; Pierce & Nash 1999) and *Paralucia aurifera* (Cushman et al. 1994). It is comparable with the content reported for the parasitic *Niphanda fusca shijimia* (Nomura et al. 1992, but see Chogyoji in Wada et al. 2001) and higher than in the riodinid *Thisbe irenea* (DeVries & Baker 1989). Some additional rare sugars (lactose, maltose, trehalose, arabinose) were found compared to the results given for the facultatively myrmecophilous and very closely related *P. hispana* (Maschwitz et al. 1975). Overall in this study here a lower total sugar content was found for *P. coridon* as was reported by Maschwitz et al. (1975) for *P. hispana*, but this is almost certainly attributable to the far more inexact techniques, which had been available, when the former study was performed. The sugar content in the nectar of the less strongly myrmecophilous species *P. icarus* and *Z. knysna* is almost comparable to *J. evagoras*. The main sugar components in all the species are relatively uniform, sucrose or glucose (Table 4.3).

The finding of a complex mixture of amino acids in the nectar of the facultatively myrmecophilous *P. coridon* is comparable to the obligate myrmecophilous species. Surprisingly, the total amino acid content is the highest value reported so far for lycaenid species, only surpassed by the riodinid *Thisbe irenea*, which has an exceptionally high overall content (Table 4.3). There are marked differences between the species with regard to the main amino acid component, being serine in *J. evagoras*, glycine in *N. fusca* and glycine and glutamine in the riodinid *T. irenea* compared to leucine in *P. coridon* here. Nectar of the moderately myrmecophilous *P. icarus* is much more depauperate in total content, nevertheless exceeding the value reported for the relatively strongly associated facultative *Hemiargus isola*, whereas the content in the equivalently relatively strongly associated *Z. knysna* is comparable to the latter. In both species the main components differ from the other species. One potential explanation for these strong differences between amino acid contents in lycaenid nectar could be that such nectars are specifically tuned to match the gustatory preferences of the major ant visitors. However, only *J. evagoras* (with a few *Iridomyrmex* species) and *N. fusca* (with *Camponotus japonicus*) are host specific myrmecophilous species. Ant visitors of the facultatively myrmecophilous species are more unpredictable, even though certain ants are more commonly seen at the larvae than other. *Lasius* ants are the most frequent attendants of *P. coridon* and *P. icarus*. The ant associates of *Z. knysna* are not well known (Tolman & Lewington 1998), but are clearly different from the two *Polyommatus* species examined here. For these ants might the *Z. knysna* nectar, which is rather dilute in amino acids, nonetheless provide enough incentive to enter a strong association.

Table 4.3 Comparison of sugar and amino acid contents in nectar secretions of eight lycaenid and one riodinid species from several studies (different analytical methods), major components are defined as regularly contributing >25% of total content, - not analysed, n.g. not given 1) parasitic 2) obligate myrmecophilous 3) facultative myrmecophilous a) % w/v b) no rank order given c) single substance d) originally given as "mM" e) originally given as µM/µl f) major component, exact contribution not stated g) states six amino acids without rank order h) rank order 1.8 and 2.6 on scale of 1-16

	Lycaenidae								Riodinidae
	Niphanda fusca[1]	*Jalmenus evagoras*[2]	*Paralucia aurifera*[2]	*Hemiargus isola*[3]	*Polyommatus hispana*[3]	*Polyommatus coridon*[3]	*Polyommatus icarus*[3]	*Zizeeria knysna*[3]	*Thisbe irenea*[3]
total sugar content	0.16M+ or ca 1.3M	ca. 10%	34%	-	13-19%	4.4%[a]	7.4%[a]	6.8%[a]	<0.5%
major components	glucose	sucrose fructose[b]	glucose[c]	-	sucrose fructose	sucrose	sucrose melezitose	sucrose melezitose	n.g.
total amino acid content [mmol/l]	57.1[d]	20-40[d]	97	4.3[d]	trace	108	10	6	10.600[e]
major components	glycine	serine[f]	g)	-	methionine[c]	leucine	tyrosine phenylalanine	alanine proline	glycine glutamine[h]
study	Nomura et al. 1992, Chogyoji in Wada et al. 2001	Pierce 1983, Pierce & Nash 1999	Cushman et al. 1994	Wagner 1994	Maschwitz et al. 1975	this study	this study	this study	DeVries 1988

The nectar secretions of *P. icarus* are similar to the values reported for extrafloral and floral nectar (e.g. [Baker, 1973 #179;Baker, 1978 #178;Gottsberger, 1984 #193;Baker, 1986 #196;Gottsberger, 1989 #194;Gottsberger, 1990 #195;Engel, 2001 #291;Blüthgen, 2004 #282] with regard to the amino acid content. But the sugar contents of *P. icarus* secretions and *Z. knysna* secretions, too, are clearly on the lowest end of the range of nectar sugar concentrations. *Z. knysna* nectar is also comparatively low in amino acid content. *P. coridon* nectar is something different, as it is depauperate in sugar content, but it is inherently more rich in amino acids than all floral or extrafloral sources, apart from exceptions like Flagellariaceae and Smilacaceae in an Australian rainforest [Blüthgen, 2004 #282].

In this regard a comparison to honeydew from aphids is interesting. *P. coridon* nectar secretions contain less total sugar than honeydew of most of the myrmecophilous aphids, but markedly higher total content of amino acids. *P. icarus* and *Z. knysna* nectar secretions are comparable to most honeydews regarding total sugar content, but are inferior in amino acid content (Bernays & Klein 2002; Engel et al. 2001; Febvay et al. 1999; Fischer et al. 2002; Völkl et al. 1999; Woodring et al. in press; Yao & Akimoto 2001; Yao & Akimoto 2002).

The occurrence of melezitose in *P. icarus* and *Z. knysna* secretions is conspicuous. Melezitose occurs relatively regularly in homopteran honeydew (e.g. [Michel, 1942 #364;Bacon, 1957 #388;Nemec, 1990 #404;Hendrix, 1992 #165;Wilkinson, 1997 #283;Byrne, 2003 #307;Blüthgen, 2004 #282]. It was speculated that this unique trisaccharide might fertilise soil and enhance nitrogen fixation, therefore benefiting the plant and ultimately the aphid (Owen 1978; Owen & Wiegert 1976), but this could not be corroborated (Petelle 1980). One likely function is the reduction of osmotic pressure in the aphid's gut (Fisher et al. 1984; Kennedy & Fosbrooke 1972; Rhodes et al. 1997; Wilkinson et al. 1997 and references therein). However, the nectar of lycaenid species is a product of a specialized gland rather than the excretion of more or less superfluous waste contents of ingested nutrition as in aphids. This gland possesses its own chitinized reservoir (Malicky 1969), thus osmotic problems as in the aphid gut are not to be expected. A further function might be to reduce the suitability of honeydew as food source or kairomone for parasitoids (Wäckers 2000). This is also not likely in the case of facultatively myrmecophilous lycaenid species, as their nectar is presented in extremely low amounts. Furthermore only few parasitoids drink larval nectar as a means of host feeding (Schurian et al. 1993), and this does not prevent parasitation of the visited caterpillar (Fiedler et al. 1995). Certain ant species appear to have a preference for melezitose (Cornelius et al. 1996; Kiss 1981; Ricks & Vinson 1970; Tinti & Nofre 2001; Völkl et al. 1999), but not all ant species show this preference, at least not as strong as *Lasius niger* (Blüthgen & Fiedler 2004; Bristow & Yanity 1999; Cornelius et al. 1996) (see chapter 5). The suitability and significance of melezitose for ant nutrition remains unclear (compare Boevé & Wäckers 2003; Wäckers 2000; Wäckers 2001; Zoebelein 1956). This trisaccharide might serve as an advertisement substance, indicating sources of valuable sugars to the ants. But then the question remains why, in particular, *P. coridon* as the most strongly ant-associated of the three examined lycaenid species, and with *Lasius niger* as one of the most important visitors, does not secrete melezitose with its nectar at all.

The main sugar component for all three lycaenid species was sucrose. This disaccharide provides a high amount of energy and is almost universally preferred as food source in the animal kingdom (Adler 1989; Blem et al. 2000; Glaser et al. 2000; Herrera et al. 2000), so that its prevalence is not surprising. Ants prefer also sucrose over monosaccharides (Blüthgen & Fiedler 2004; Boevé & Wäckers 2003; Cornelius et al. 1996; Koptur 1979; Ricks & Vinson 1970; Tinti & Nofre 2001; Vander Meer et al. 1995; Vander Meer & Merdinger 1990; Völkl et al. 1999), but sucrose is not necessarily the dominant component in Homopteran honeydew [Nemec, 1990 #404;Hendrix, 1992 #165;Völkl, 1999 #5;Yao, 2001 #357;Engel, 2001 #291;Fischer, 2002 #292;Blüthgen, 2004 #282;Woodring, in press #536]. With regards to monosaccharides, most ant species do prefer glucose over fructose (Boevé & Wäckers 2003; Koptur 1979; Tinti & Nofre 2001; Vander Meer et al. 1995), but see (Cornelius et al. 1996). This is mirrored in the occurrence of glucose as next important sugar in the *P. coridon* nectar secretions, but the situation in both *P. icarus* and *Z. knysna* is at odds with these recorded preferences. Yet, the equally high energy content in these hexose sugars and the high total sugar content might override any lesser preference for one of the individual components of the whole sugar mixture in the nectar secretion. In the lycaenid species examined here, the sugars arabinose, lactose, maltose, melibiose, trehalose and probably erlose were infrequently found. These sugars occur as well in homopteran honeydew [Völkl, 1999 #5;Fischer, 2001 #18;Engel, 2001 #291;Yao, 2001 #357;Blüthgen, 2004 #282].

The leading amino acid in the nectar secretions of *P. coridon* was leucine. This branched chain amino acid is one of the essential amino acids for insects (Beattie 1985; Träger 1953; Urich 1994) and, at least in mammals, an important regulator of protein turnover (Bender 1985). The remaining mixture contained various non-essential and almost all essential amino acids. Missing of the latter was cysteine, which might be attributed to the inability to detect the dimere cystine (see above). But it might be absent indeed. Most animals are limited in the sulphur containing amino acids methionine and cysteine, whereby methionine is less limiting and sometimes used to synthesize additional cysteine (Bender 1985). So possibly cysteine is just too valuable for *P. coridon* and therefore not secreted. *P. icarus* presented a less rich amino acid mixture in a considerably smaller total concentration. The leading amino acids were the aromatic tyrosine and phenylalanine. Phenylalanine is essential, whereas tyrosine is considered semi-essential, as not all insects require it fundamentally. These are precursors of neurotransmitters (Bender 1985; Träger 1953; Urich 1994) and are important for melanizing processes (Buck 1953; Träger 1953), which play also a significant role in insect immune response (Gillespie et al. 1997; Leclerc & Reichhart 2004). Generally the aromatic amino acids are precursors for several pigments (e.g. (Koch et al. 1998; Nijhout 1991; Urich 1994). Phenylalanine and tyrosine are furthermore required for the formation of the pupae and the sclerotization of insect cuticles (Andersen 1973). In *Z. knysna* relatively small amounts of alanine and/or proline occurred, both being non-essential, although the latter is semi-essential for some insects. Proline is used as a fuel for muscle metabolism in insects (Urich 1994; Wacht et al. 2000), and is therefore valuable not only for anabolic processes. Interestingly Blüthgen et. al. (2004) found alanine, proline and threonine as main amino acids in all nectar and honeydew sources analysed in an Australian rainforest. *Z. knysna* (which essentially has an African distribution) might thus mirror in its nectar a general preference scheme expressed by subtropical and tropical ant species. Overall, the leading amino acids in the nectars of all three lycaenid species examined here are different from the ones reported for obligately myrmecophilous species (Table 4.3), but nevertheless are valuable nutrients in their own right.

Glycine, which is a major component in nectar of *N. fusca* and *T. irenea* (Table 4.3), enhances gustatory stimulation (Tateda & Hidaka 1966; Wada et al. 2001) and seems to play a role in insect learning (Kim & Smith 2000), but constituted here only a minor component in *P. coridon* nectar secretions. Both the non-essential amino acids glycine and serine, which is a major component in *J. evagoras* nectar (Table 4.3), are precursors of formic acid (Urich 1994), the most important defence substance of the Formicinae (Hölldobler & Wilson 1990). This might explain their occurrence as main components in the secretions of some obligately myrmecophilous species. However, *J. evagoras* is visited by certain species of the Dolichoderinae genus *Iridomyrmex*. The defensive substances of the dolichoderines are completely different (Hölldobler & Wilson 1990), and formic acid is not known to occur. In the facultatively myrmecophilous lycaenid species considered in this study serine and glycine seem to be not very important, in contradiction to the notion of Yao and Akimoto (2002) who stated that these amino acids may play a universally important role in myrmecophilous insect-ant interactions. *P. coridon* and *P. icarus* both even “sacrifice” essential amino acids to their ant partners. Ultimately, preferences of particular ant species for these secretions, and thus the success of lycaenid larvae in initiating a lasting association, has to be tested experimentally for each combination of partner species (see also chapter 5). Generally preferences for amino acids vary tremendously across ant species (Blüthgen & Fiedler 2004; Kay 2002; Lanza & Krauss 1984; Pierce 1989). In contrast, sugar preferences of ants are much more predictable and usually follow the rank order sucrose>glucose>fructose (but see the variable role of melezitose, above). Therefore, an optimal composition of the nectar secretion may not exist, in the sense that such a secretion would fit the gustatory preferences and physiological needs of all ant visitors. This makes it impossible for facultatively associated lycaenid species with their unpredictable ant associates to present a secretion equally attractive to all possible visiting ant species. Rather in such generalized interactions the nectar currency is selected to be a compromise between the physiological potential of the caterpillars and the preferences of the more important ant visitors.

At least in *P. coridon*, the pattern of sugars in the larval nectar secretion is further modified by the larval food, concerning the type of occurring carbohydrates as well as their contents. Reared on the natural host plant *H. comosa*, on which western European *P. coridon* caterpillars feed exclusively under natural conditions (Pfeuffer 2000; SBN 1987; Tolman & Lewington 1998), a lower sucrose content was detected, while on *S. varia* and pronouncedly on artificial food more sucrose was secreted. The nectars of plant-fed larvae did not differ in total content however. When raised on artificial food total sugar content in nectar was even higher, albeit this effect did not quite reach statistical significance. The artificial food is especially rich in nutrients, and the included antibiotics might further enhance the health status of the caterpillars therefore allowing a more valuable secretion. The quality of larval nutrition was already shown to be an important predictor of the ability to maintain myrmecophily (Baylis & Pierce 1991; Burghardt & Fiedler 1996a; Fiedler 1990). Furthermore, both *P. coridon* and *Z. knysna* caterpillars produced voluminous frass when fed with artificial food, indicating a surplus of water compared to plant fed individuals. The water content of food is an important component of caterpillar performance (Baylis & Pierce 1993; Bowers et al. 1991; Slansky 1993) and water availability might be important for the maintenance of myrmecophilous interactions (Burghardt & Fiedler 1996a) as well. Reared on the alternative food plant *S. varia*, the secretions of the *P. coridon* caterpillars were far more variable in the amino acid composition and contained a slightly lower number of amino acids. Caterpillars reared on *H. comosa* exhibited less variance in amino acid composition. But fed with the artificial food, which is a very homogeneous substrate compared to the plants which were reared under outdoor conditions, secretions very homogenous in amino acid composition were produced. A more uniform food thus generates less variable amino acid patterns. This demonstrates another effect the larval food exerts on the nectar secretions. Unfortunately not enough nectar samples from caterpillars reared on different foods could be obtained from more polyphagous lycaenid species to further elaborate the topic of food influence. One might expect more pronounced food effects on nectar quality in *P. icarus*, whose larvae feed on a range of plants on the family Fabaceae (Ebert & Rennwald 1991; Martín Cano 1984), and even more so in *Z. knysna*, which utilises host plants from a variety of unrelated families. In homopterans an effect of different host plants or artificial diets on honeydew composition and concentration of sugars (Byrne et al. 2003; Febvay et al. 1999; Fischer & Shingleton 2001; Hendrix et al. 1992) and amino acids as well (Febvay et al. 1999) could be demonstrated. Differences between the sexes in the investment in the secretions might also be expected, an issue testable with strongly, but facultatively ant-associated species like *P. coridon* or especially *P. bellargus*, where sex differences in development have been shown (Fiedler & Saam 1994). Thus the field is open for several rewarding research topics.

Comparing the three lycaenid species one might have expected a higher similarity between the nectar secretions of the two more closely related *Polyommatus* species, while *Zizeeria knysna* is phylogenetically more distant. This is indeed the case for the amino acid patterns, as can be seen in the ordination analysis (Fig. 4.13). With regard to the sugar pattern however, the less strongly associated species *P. icarus* and *Z. knysna* are more similar to each other, especially concerning the regular occurrence of complementary sugars apart from sucrose. The secretion of *P. coridon*, on the other hand, is clearly more similar to the ones reported so far from obligately myrmecophilous species. Thus the intimacy of ant-association overrides the potential of physiological similarities rooted in relatedness. Thus the chemical composition of the larval nectar secretions appears to be related to the variance in the strength of myrmecophilous interactions.

Whether these differences in the nectar composition translate into functional differences in the lycaenid-ant interactions will be examined experimentally in chapter 5 with regard to the gustatory preferences of ants. The nutritional benefits of these different secretions are investigated in chapter 6.

4.6 Acknowledgements

We are grateful to Hans Malchus for technical assistance. The district government of Oberfranken kindly issued permits to collect individuals in the field for starting of laboratory populations. Dr. Klaus G. Schurian kindly provided *Zizeeria knysna* larvae and pupae from the Canary Islands, and Annick Servant helped rearing the *P. icarus* and *Z. knysna* caterpillars.

4.7 References

Ackery, P. R., de Jong, R. & Vane-Wright, R. I. 1999: The butterflies: Hedyloidea, Hesperioidea and Papilionoidea. In: Lepidoptera, Moths and Butterflies, Vol. 1 (Kristensen, N. P. ed). W deGruyter, Berlin, New York. pp. 263-300.

Adler, P. H. 1989: Sugar feeding of the adult corn earworm (Lepidoptera: Noctuidae) in the laboratory. J Econ Entomol **82**, 1344-1349.

Agrawal, A. A. & Fordyce, J. A. 2000: Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B **267**, 1857-1861.

Andersen, S. O. 1973: Comparison between the sclerotization of adult and larval cuticle in *Schistocerca gregaria*. J Insect Physiol **19**, 1603-1614.

Axén, A. H. 2000: Variation in behavior of lycaenid larvae when attended by different ant species. Evol Ecol **14**, 611-625.

Axén, A. H. & Pierce, N. E. 1998: Aggregation as a cost-reducing strategy for lycaenid larvae. Behav Ecol **9**, 109-115.

Axén, H. A., Leimar, O. & Hoffman, V. 1996: Signalling in a mutualistic interaction. Anim Behav **52**, 321-333.

Bacon, J. S. D. & Dickinson, B. 1957: The origin of melezitose: a biochemical relationship between the lime tree (*Tilia* spp.) and an aphis (*Eucallipterus tiliae* L.). Biochem J **66**, 289-297.

Baker, H. G. & Baker, I. 1973: Amino acids in nectar and their evolutionary significance. Nature **241**, 543-545.

-. 1986: The occurrence and significance of amino acids in floral nectar. Pl Syst Evol **151**, 175-186.

Baker, H. G., Opler, P. A. & Baker, I. 1978: A comparison of the amino acid complements of floral and extrafloral nectars. Bot Gaz **139**, 322-332.

Ballmer, G. R. & Pratt, G. F. 1991: Quantification of ant attendance (myrmecophily) of lycaenid larvae. J. Res Lepid **30**, 95-112.

Baylis, M. & Pierce, N. E. 1991: The effect of host-plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*. Ecol Entomol **16**, 1-9.

-. 1993: The effects of ant mutualism on the foraging and diet of lycaenid caterpillars. In: Caterpillars (Stamp, N. E. & Casey, T. M. eds). Chapman & Hall, New York. pp. 404-421.

Beattie, A. J. 1985: The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, Cambridge.

Bender, D. A. 1985: Amino acid metabolism. John Wiley & Sons, Chichester.

Bernays, E. A. & Klein, B. A. 2002: Quantifying the symbiont contribution to essential amino acids in aphids: the importance of tryptophan for *Uroleucon ambrosiae*. Physiol Entomol **27**, 275-284.

Blem, C. R., Blem, L., Felix, J. & van Gelder, J. 2000: Rufous hummingbirds sucrose preference: precision of selection varies with concentration. Condor **102**, 235-238.

Blüthgen, N. & Fiedler, K. 2004: Preferences for sugars and amino acids in a diverse nectar-feeding ant community. J Anim Ecol **73**, 155-166.

Blüthgen, N., Gottsberger, G. & Fiedler, K. 2004: Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Aust Ecol. **29** in press.

Bodnaryk, R. P. 1978: Structure and function of insect peptides. Adv Insect Physiol **13**, 69-132.

Boevé, J.-L. & Wäckers, F. L. 2003: Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* workers. Oecologia **136**, 508-514.

Bowers, M. D., Stamp, N. E. & Fajer, E. D. 1991: Factors affecting calculation of nutritional indices for foliage-fed insects: an experimental approach. Ent exp & appl **61**, 101-116.

Božic, J. & Woodring, J. 1997: Effect of activity on the haemolymph sugar titre in honey bees. J Apic Res **36**, 33-39.

Bristow, C. M. & Yanity, E. 1999: Seasonal response of workers of the Allegheny Mound Ant, *Formica exsectoides* (Hymenoptera: Formicidae) to artificial honeydews of varying nutrtitional content. Great Lakes Entomol **32**, 15-27.

Buck, J. B. 1953: Physical properties and chemical composition of insect blood. In: Insect Physiology (Roeder, K. D. ed). John Wiley & Sons

Chapman & Hall, New York.

Burghardt, F. & Fiedler, K. 1996a: The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). Ecol Entomol **21**, 1-8.

-. 1996b: Myrmecophilous behaviours in caterpillars of the butterfly, *Polyommatus icarus* (Rottemburg, 1775): temporal patterns and age dependency (Lepidoptera: Lycaenidae). Nota lepid **19**, 212-229.

Byrne, D. N., Hendrix, D. L. & Livy, H. W. I. 2003: Presence of trehalulose and other oligosaccharids in Hemiptera honeydew, particularly Aleyrodidae. Physiol Entomol **28**, 144-149.

Clarke, K. R. & Warwick, R. M. 2001: Change in marine communities: an approach to statistical analysis and interpretation. Primer-E, Plymouth.

Collett, J. I. 1989: Characterization of the peptidases of *Calliphora*: many features allow the utilization of small peptides as an amino acid reservoir. Insect Biochem **19**, 535-547.

Cornelius, M. L., Grace, J. K. & Yates, J. R. I. 1996: Acceptability of different sugars and oils to three tropical ant species. Anz Schädlingskde, Pflanzenschutz, Umweltschutz **69**, 41-43.

Cottrell, G. B. 1984: Aphytophagy in butterflies: its relationship to myrmecophily. Zool J Linnean Soc **79**, 1-57.

Cushman, J. H., Rashbrook, V. K. & Beattie, A. J. 1994: Assessing benefits to both participants in a lycaenid-ant association. Ecology **75**, 1031-1041.

Deml, R. & Dettner, K. 1995: Effect of emperor moth larval secretions, hemolymph, and components on microorganisms and predators. Ent exp & appl **76**, 287-293.

-. 2003: Comparative morphology and secretion chemistry of the scoli in caterpillars of *Hyalophora cecropia*. Naturwissenschaften **90**, 460-463.

DeVries, P. J. 1988: The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants. Zool J Linnean Soc **94**, 379-393.

DeVries, P. J. & Baker, I. 1989: Butterfly exploitation of an ant-plant mutualism: adding insult to herbivory. J New York Entomol Soc **97**, 332-340.

Dyer, L. A. 1997: Effectiveness of caterpillars defenses against three species of invertebrate predators. J Res Lepid **34**, 48-68.

Ebert, G. & Rennwald, E. 1991: Die Schmetterlinge Baden-Württembergs. Eugen Ulmer Verlag, Stuttgart.

Eliot, J. N. 1973: The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. Bull Brit Mus Nat Hist (Entomol) **28**, 371-505.

Engel, V., Fischer, M. K., Wäckers, F. L. & Völkl, W. 2001: Interactions between extrafloral nectaries, aphids and ants: are there competition effects between plant and homopteran sugar sources? Oecologia **129**, 577-584.

Febvay, G., Rahbe, Y., Rynkiewicz, M., Guillaud, J. & Bonnot, G. 1999: Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, *Acyrtosiphon pisum*, reared on different diets. J exp Biol **202**, 2639-2652.

Fiedler, K. 1990: Effects of larval diet on myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenide). Oecologia **83**, 284-287.

-. 1991: Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionidae). Bonner Zoologische Monographien **31**.

-. 1997a: Geographical patterns in life-history trait of Lycaenidae butterflies - ecological and evolutionary implications. Zoology **100**, 336-347.

-. 1997b: Life-history patterns of myrmecophilous butterflies and other insects: their implications on tropical species diversity. In: Proceedings of the International Symposium on Biodiversity and Systematics in Tropical Ecosystems (Ulrich, H. ed). Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

Fiedler, K. & Hagemann, D. [1995]: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K., Hölldobler, B. & Seufert, P. 1996: Butterflies and ants: the communicative domain. Experientia **52**, 14-24.

Fiedler, K. & Hummel, V. 1995: Myrmecophily in the brown argus butterfly, *Aricia agestis* (Lepidoptera: Lycaenidae): Effects of larval age, ant number and persistence of contact with ants. Zoology **99**, 128-137.

Fiedler, K. & Maschwitz, U. 1989: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. Ethology **80**, 71-80.

Fiedler, K. & Saam, C. 1994: Does ant-attendance influence development in 5 European Lycaenide butterfly species ? (Lepidoptera). Nota lepid **17**, 5-24.

Fiedler, K., Schurian, K G. & Hahn, M. 1994: The life history and myrmecophily of *Polyommatus candalus* (Herrich-Schäffer) from Turkey (lep., Lycaenidae). Linn Belg **14**, 315-332.

Fiedler, K., Seufert, P., Pierce, N. E., Pearson, J. G. & Baumgarten, H.-T. [1995]: Exploitation of lycaenid-ant mutualisms by braconid parasitoids. J Res Lepid **31**, 153-168.

Fischer, M. K. & Shingleton, A. W. 2001: Host plants and ants influence the honeydew sugar composition of aphids. Funct Ecol **15**, 544-550.

Fischer, M. K., Völkl, W., Schopf, R. & Hoffmann, K. H. 2002: Age-specific patterns in honeydew production and honeydew composition in the aphid *Metopeurum fuscoviride*: implications for ant-attendance. J Insect Physiol **48**, 319-326.

Fisher, D. B., Wright, P. J. & Mittler, T. E. 1984: Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharids. J Insect Physiol **30**, 387-393.

Florkin, M. & Jeuniaux, C. 1974: Hemolymph: composition. In: The physiology of Insecta, Vol. V (Rockstein, M. ed). Academic Press, New York. pp. 255-307.

Gillespie, J. P., Kanost, M. R. & Trenczek, T. 1997: Biological mediators of insect immunity. Annu Rev Entomol **42**, 611-643.

Glaser, D., Wanner, M., Tinti, J. M. & Nofre, C. 2000: Gustatory responses of pigs to various natural and artificial compounds known to be sweet in man. Food Chem **68**, 375-385.

Gottsberger, G., Arnold, T. & Linskens, H. F. 1989: Are amino acid and sugar concentration correlated in floral nectar? Proc K Ned Akad Wet Ser C **92**, 461-464.

-. 1990: Variation in floral nectar amino acids with aging of flowers, pollen contamination, and flower damage. Isr J Bot **39**, 167-176.

Gottsberger, G., Schrauwen, J. & Linskens, H. F. 1984: Amino acids and sugars in nectar, and their putative evolutionary significance. Pl Syst Evol **145**, 55-77.

Heath, A. & Claassens, A. J. M. 2003: Ant-association among Southern African Lycaenidae. J Lepid Soc **57**, 1-16.

Hendrix, D. L., Wei, Y.-A. & Leggett, J. E. 1992: Homopteran honeydew sugar composition is determined by both the insect and plant species. Comp Biochem Physiol B **101**, 1/2.

Herrera, L. G., Leblanc, D. & Nassar, J. 2000: Sugar discrimination and gustatory thresholds in captive-born frugivorous Old World Bats. Mammalia **64**, 135-143.

Hochberg, Y. 1988: A sharper bonferroni procedure for multiple tests of significance. Biometrika **75**, 800-802.

Hölldobler, B. & Wilson, E. O. 1990: The ants. Harvard University Press / Springer, Heidelberg.

-. 1995: Ameisen - Die Entdeckung einer faszinierenden Welt. Birkhäuser, Basel.

Kay, A. 2002: Applying optimal foraging theory to assess nutrient availability ratios for ants. Ecology **83**, 1935-1944.

Kennedy, J. S. & Fosbrooke, J. H. M. 1972: The plant in the life of an aphid. In: Insect plant relations (van Emden, H. F. ed). Blackwell Science.

Kim, Y. S. & Smith, B. H. 2000: Effect of an amino acid on feeding preferences and learning behavior in the honey bee, *Apis mellifera*. J Insect Physiol **46**, 793-801.

Kiss, A. 1981: Melezitose, aphids and ants. Oikos **37**, 382.

Kitching, R. L. 1983: Myrmecophilous organs of the larvae and pupae of the lycaenid butterfly *Jalmenus evagoras*. J Nat Hist **17**, 471-481.

Kitching, R. L. & Luke, B. 1985: The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist **19**, 259-276.

Koch, P. B., Keys, D. N., Rocheleau, T., Aronstein, K., Blackburn, M., Carroll, S. B. & Ffrench-Constant, R. H. 1998: Regulation of dopa decarboxylase expression during colour formation in wild-type and melanic tiger swallowtail butterflies. Development **125**, 2303-2313.

Koptur, S. 1979: Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. Amer J Bot **66**, 1016-1020.

Lanza, J. & Krauss, B. R. 1984: Detection of amino acids in artificial nectars by two tropical ants, *Leptothorax* and *Monomorium*. Oecologia **63**, 423-425.

Leclerc, V. & Reichhart, J.-M. 2004: The immune response of *Drosophila melanogaster*. Immunol Rev **198**, 59-71.

Leimar, O. & Axén, A. H. 1993: Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav **46**, 1177-1182.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

Martín Cano, J. 1984: Biología comparada de Lampides boeticus (L.), *Syntarucus pirithous* (L.) y *Polyommatus icarus* (Rot.) (Lep., Lycaenidae). Graellsia **40**, 163-193.

Maschwitz, U., Wüst, M. & Schurian, K. 1975: Bläulingsraupen als Zuckerlieferanten. Oecologia **18**, 17-21.

Michel, E. 1942: Beiträge zur Kenntnis von *Lachnus* (*Pterochlorus*) *roboris* L., einer wichtigen Honigtauerzeugerin an der Eiche. Z Angew Entomol **29**, 243-2814.

Montllor, C. B. & Bernays, E. A. 1993: Invertebrate predators and caterpillar foraging. In: Caterpillars (Stamp, N. E. & Casey, T. M. eds). Chapman & Hall, New York. pp. 170-202.

Mullins, D. E. 1985: Chemistry and physiology of the hemolymph. In: Comprehensive Insect physiology, biochemistry and pharmacology, Vol. 3 (Kerkut, G. A. & Gilbert, L. A. eds). Pergamon Press, Oxford. pp. 355-400.

Nemec, V. & Stary, P. 1990: Sugars in honeydew. Biologia Bratislava **45**, 259-264.

Nijhout, H. F. 1991: The development and evolution of butterfly wing patterns. Smithsonian Institution Press, Washington, London.

Nomura, K., Hirukawa, N., Yamaoka, R. & Imafuku, M. 1992: Problems on the symbiosis between the lycaenid butterfly larva, *Niphanda fusca shijimia* and the ant *Camponotus* (*Camponotus*) *japonicus*. Tyo to Ga **43**, 138-143.

Owen, D. F. 1978: Why do aphids synthesize melezitose? Oikos **31**, 264-267.

Owen, D. F. & Wiegert, R. G. 1976: Do consumers maximize plant fitness? Oikos **27**, 488-492.

Petelle, M. 1980: Aphids and melezitose: a test of Owen's 1978 hypothesis. Oikos **35**, 127-128.

Pfeuffer, E. 2000: Zur Ökologie der Präimaginalstadien des Himmelblauen Bläulings (*Lysandra bellargus* ROTTEMBURG 1775) und des Silbergrünen Bläulings (*Lysandra coridon* PODA 1761), unter besonderer Berücksichtigung der Myrmekophilie. Ber Naturwiss Ver Schwaben **104**, 72-98.

Pierce, N. E. 1983: The ecology and evolution of the symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University.

-. 1987: The evolution and biogeography of associations between lycaenid butterflies and ants. Oxford Surveys in Evolutionary Biology **4**, 89-116.

-. 1989: Butterfly-ant mutualisms. In: Towards a more exact ecology (Grubb, P. J. & Whittaker, J. eds). Blackwell Scientific, Oxford. pp. 299-324.

Pierce, N. E., Braby, M. F., Huxley, C. R., Lohman, D. J., Mathew, J., Rand, D. B. & Travassos, M. A. 2002: The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol **47**, 733-771.

Pierce, N. E. & Nash, D. R. 1999: The Imperial Blue, *Jalmenus evagoras* (Lycaenide). In: Biology of Australian Butterflies, Vol. 6 (Kitching, R. L., Scheermeyer, E., Jones, R. E. & Pierce, N. E. eds). CSIRO Publishing, Melbourne. pp. 279-317.

Rhodes, J. D., Croghan, P. C. & Dixon, A. F. G. 1997: Dietary sucrose and oligosaccharide synthesis in relation to osmoregulation in the pea aphid, *Acyrthosiphon pisum*. Physiol Entomol **22**, 373-379.

Ricks, B. L. & Vinson, S. B. 1970: Feeding acceptability of certain insects and various water-soluble compounds to two varieties of the imported Fire ant. J Econ Entomol **63**, 145-148.

Sandström, J. P. & Moran, N. A. 2001: Amino acid budgets in three aphid species using the same host plant. Physiol Entomol **26**, 202-211.

SBN. 1987: Tagfalter und ihre Lebensräume. Schweizerischer Bund für Naturschutz. Fotorotar AG Egg/ZH, Basel.

Schurian, K. G., Fiedler, K. & Maschwitz, U. 1993: Parasitoids exploit secretions of myrmecophilous lycaenid butterfly caterpillars (Lycaenidae). J Lepid Soc **47**, 150-154.

Scott, J. A. & Wright, D. M. 1990: Butterfly phylogeny and fossils. In: Butterflies of Europe Vol 2: Introduction to lepidopterology (Kudrna, O. ed). Aula Verlag, Wiesbaden.

Slansky, F. 1993: Nutritional ecology: the fundamental quest for nutrients. In: Caterpillars: Ecological and Evolutionary Constraints on foraging (Stamp, N. E. & Casey, T. M. eds). Chapman & Hall, New York.

Sokal, R. R. & Braumann, C. A. 1980: Significance tests for coefficients of variation and variability profiles. Syst Zool **29**, 50-66.

Statsoft. 2003: Statistica 6.0. Statistica Inc, Tulsa.

Strong, F. E. 1965: Detection of lipids in the honeydew of an aphid. Nature **205**, 1242.

Tateda, H. & Hidaka, I. 1966: Taste responses to sweet substances in rat. Mem Fac Sci Kyushu Univ - Series E **4**, 137-149.

Tinti, J.-M. & Nofre, C. 2001: Responses of the ant *Lasius niger* to various compounds perceived as sweet in humans: a structure-activity relationship study. Chem Senses **26**, 231-237.

Tolman, T. & Lewington, R. 1998: Die Tagfalter Europas und Nordwestafrikas. Franckh-Kosmos, Stuttgart.

Träger, W. 1953: Nutrition. In: Insect Physiology (Roeder, K. D. ed). John Wiley & Sons

Chapman & Hall, New York.

Turunen, S. 1985: Absorption. In: Comprehensive Insect physiology, biochemistry and pharmacology, Vol. 4 (Kerkut, G. A. & Gilbert, L. A. eds). Pergamon Press, Oxford. pp. 165-491.

Urich, K. 1994: Comparative Animal Biochemistry. Springer, Berlin.

Vander Meer, R. K., Lofgren, C. S. & Seawright, J. A. 1995: Specificity of the red imported fire ant (Hymenoptera: Formicidae) phagostimulant response to carbohydrates. Fla Entomol **78**, 144-154.

Vander Meer, R. K. & Merdinger, E. 1990: Fire Ant phagostimulants: laboratory and practical studies. Farmacia **38**, 93-102.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M. W. & Hoffmann, K. H. 1999: Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia **118**, 483-491.

Wacht, S., Lunau, K. & Hansen, K. 2000: Chemosensory control of pollen ingestion in the hoverfly *Eristalis tenax* by labellar taste hairs. J Comp Physiol A **186**, 193-203.

Wäckers, F. L. 2000: Do oligosaccharids reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect synthetized honeydew sugars. Oikos **90**, 197-201.

-. 2001: A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. J Insect Physiol **47**, 1077-1084.

Wada, A., Isobe, Y., Yamaguchi, S., Yamaoka, R. & Ozaki, M. 2001: Taste-enhancing effects of glycine on the sweetness of glucose: a gustatory aspect of symbiosis between the ant, *Camponotus japonicus*, and the larvae of the lycaenid butterfly, *Niphanda fusca*. Chem Senses **26**, 983-992.

Wagner, D. 1994: Species-specific effects of tending ants on the life history of *Hemiargus isola*, a North American lycaenid butterfly. Ph.D. thesis, Princeton University, Princeton.

Wiemers, M. 2003: Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*) - a molecular phylogenetic approach. Ph.D. thesis, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn.

Wilkinson, T. L., Ashford, D. A., Pritchard, J. & Douglas, A. E. 1997: Honeydew sugars and osmoregulation in the Pea Aphid *Acyrthosiphon pisum*. J exp Biol **200**, 2137-2143.

Woodring, J., Wiedemann, R., Fischer, M. K., Hoffmann, K. H. & Völkl, W. in press: Honeydew amino acids in relation to sugars and their role in the establishment of ant-attendance hierarchy in eight species of aphids feeding on tansy (*Tanacetum vulgare*). Physiol Entomol.

Yao, I. & Akimoto, S. 2001: Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Oecologia **128**, 36-43.

-. 2002: Flexibility in the composition and concentration of amino acids in honeydew of the drepanosiphid aphid *Tuberculatus quercicola*. Ecol Entomol **27**, 745-752.

Zoebelein, G. 1956: Der Honigtau als Nahrung der Insekten. Z Angew Entomol **39**, 129-167.

5 A taste for blues? – Bioassays with ants from different subfamilies on components and analoga of lycaenid caterpillar secretions

H. Daniels* & K. Fiedler

Department of Animal Ecology I, University of Bayreuth, D-95440 Bayreuth, Germany

* *corresponding author*

5.1 Abstract

Preferences of three ant species from the subfamilies Formicinae (*Lasius niger*), Myrmicinae (*Myrmica rubra*) and Dolichoderinae (*Tapinoma melanocephalum*) for different sugars and amino acids occurring in nectar secretions of facultatively myrmecophilous lycaenid butterfly larvae were tested in simultaneous choice tests with 0.1mol/l sucrose as standard. Furthermore preferences for different kinds of mixtures and for complete nectar analoga were examined.

Among carbohydrates, the disaccharide sucrose was preferred over the monosaccharide glucose. In *T. melanocephalum* fructose was qualitatively less attractive than glucose. Melezitose as trisaccharide (in approximately *Polyommatus icarus* nectar concentration) was never significantly preferred over sucrose by any of the three species.

Some single amino acids in sucrose solutions were preferred over pure sucrose. *L. niger* preferred leucine, *M. rubra* phenylalanine and tyrosine. In general, raising the concentration did not enhance preferences and even reduced it in some cases, e.g. leucine in *L. niger*.

Mixtures of four or eleven amino acids (partly comparable to *Polyommatus coridon* nectar) were preferred to sucrose by *L. niger* and *T. melanocephalum*. *L. niger* preferred a more balanced mixture over an energetically similar, but less balanced mixture.

Complete *P. coridon* and *P. icarus* nectar analoga were significantly preferred by *T. melanocephalum*. *M. rubra* showed the same tendency, but preferences were not statistically significant.

The conditionality of ant responses to nectar compounds and possible influences on the test results are discussed. Facultatively myrmecophilous lycaenid caterpillars cannot produce an ‘optimal’ nectar secretion, due to the tremendous variability in gustatory preferences as exhibited by the broad range of largely unpredictable ant visitors. They should therefore either decide on a balanced mixture, containing possible ‘key compounds’ in moderate concentrations, or if this investment does not pay, should secrete sugar rich nectars.

5.2 Introduction

The ants with their about 11000 known species are the leading terrestrial invertebrate predators (Hölldobler & Wilson 1990). They must be furthermore granted leadership in total abundance (estimated 10^{16} individuals) and also in biomass (Hölldobler 1996a; Hölldobler 1996b). Ants occupy virtually all terrestrial ecological niches, be it as predators, scavengers, fungus garden growers, seed harvesters or as harvester of different kinds of “nectar”. Nectar consists mainly of an aqueous solution of a range of sugars at different concentrations. The often high contents of (different) sugars are frequently accompanied by amino acids and occasionally other compounds occur (Strong 1965).

As liquid carbohydrate sources ants may use extrafloral nectar, floral nectar (infrequently), or engage in trophobiotic interactions with other insects, mainly homopterans (Bristow 1991; Buckley 1987; Stadler et al. 2003). Ant attendants of trophobionts are mainly from the families of Formicinae and Dolichoderinae, but to a lesser degree also members of Myrmicinae, Ponerinae and a few other subfamilies are involved (DeVries 1991; Eastwood & Fraser 1999; Fiedler 1991; Fiedler 2001). A special case of trophobiotic interactions are the caterpillars of many species in the butterfly family Lycaenidae (Pierce et al. 2002). During their development more than half of all lycaenid species associate with ants at least partly in some instars (Fiedler 1991; Pierce et al. 2002). Myrmecophilous caterpillars show morphological and behavioural specializations (Cottrell 1984; Malicky 1969; Malicky 1970), which enable survival upon meeting the (normally predatory) ants and allow them to engage in long-lasting or permanent interactions. An important role plays the dorsal nectar gland (Fiedler 1991; Fiedler & Maschwitz 1989), which secretes a liquid, which is harvested by the ants. The secretory behaviour is highly controlled by the caterpillar (Agrawal & Fordyce 2000; Axén & Pierce 1998; Fiedler & Hagemann 1995; Leimar & Axén 1993) which renders harvesting of these secretions of these secretions a long-lasting endeavour (chapter 3.4). The secretions from the dorsal nectar organ are further imminently essential for the establishment of an at least partly stable association and further recruitment of ants, as only caterpillars with functioning nectar gland can achieve this (Fiedler & Maschwitz 1989). The intensity and specificity of interactions between lycaenid larvae and ants shows considerable variability, ranging from facultative to obligate myrmecophily. Caterpillars of different butterfly species are differently favoured by different ant species (Malicky 1969). Obligately myrmecophilous species are tended by specific ant species, e.g *Iridomyrmex* sp. tending *Jalmenus* sp. (Pierce & Nash 1999) or obligate partners of *Oecophylla smaragdina* (Seufert & Fiedler 1996a). The specificity of these obligate interactions is on the one hand mediated by the chemical communication of the caterpillars, and on the other hand fundamentally by the oviposition behaviour of the female, which selects the hostplants according to the presence of the right ant species (Pierce & Elgar 1985; Seufert & Fiedler 1996a; Seufert & Fiedler 1996b; Smiley et al. 1988; van Dyck et al. 2000, but see Thomas & Elmes 2001, Fraser et al. 2002).

In contrast, facultative ant associations are not characterized by species specificity ((Fiedler 1991; Malicky 1969; Peterson 1993; Schmidt & Rice 2002; Seufert & Fiedler 1996a), a possible exception might be *Plebejus argus* (Jordano & Thomas 1992; Seymour et al. 2003)). Nevertheless certain ant species are more often found tending these lycaenids than other ant species. These differences might possibly be attributed to the contents of the nectar secretions of the respective caterpillars (Pierce 1987). Starting from the first comparative study of the composition of nectar secretions of facultatively myrmecophilous lycaenid species (chapter 4), the role of gustatory response to different compounds and especially to mixtures and whole nectar analoga was thus tested with ant species, which participate in trophobiosis.

Three species from three different subfamilies of the Formicidae were examined. As representative for the Formicinae *Lasius niger* was chosen. This very common ant species is also quantitatively one of the most important visitors of the lycaenids *Polyommatus coridon* and *P. icarus* in Central Europe (Fiedler 1991). *Myrmica rubra* was selected as representative of the Myrmicinae, several related species tend both *Polyommatus* species, too (Fiedler 1991). As a Dolichoderinae, *Tapinoma melanocephalum* was chosen, which is known to tend larvae of *Zizeeria knysna* and other (sub-)tropical lycaenid species (Fiedler 1991; Fiedler 2001).

The following questions were adressed:

Do these ants exhibit preferences for any single sugars or for any single amino acids which are components of the nectar secretions?

Are nutrient mixtures preferred over single nectar components?

Do the ants' choices merely reflect the energetic value of a nectar source? Or do qualitative aspects, like the presence of certain substances or the presence of a balanced mixture, play a role?

Do the ants discriminate between the complete lycaenid nectar mimics?

Which nectar components should a facultatively myrmecophilous lycaenid caterpillar secrete to meet the preferences of potential ant visitors?

5.3 Material & Methods

Lasius niger was tested at 15 natural colonies, located in plant beds (1.2x2.4m) at the roof of the "NW I" university building. The other two species were tested in the laboratory. Eight *Tapinoma melanocephalum* nests were received from the Umweltbundesamt (Berlin). Colony sizes were about 200-300 workers with several queens. As nests the ants received 5.0x3.8x2.5cm transparent plastic boxes with plaster of Paris bottom which were placed in larger arenas of 20x10x6.5cm. *Myrmica rubra* nests (n=12) were excavated in North-East Bavaria (49°57'N 11°23'E). Colony sizes were 150-300 workers and several queens. As nests these ants were provided with 20x20x6.5cm transparent plastic boxes with a bottom made from plaster of Paris. A small quantity of earth, wood and clay flowerpot pieces could serve as shelters. All laboratory colonies were kept in environmental chambers at 26°C:15°C (L:D 16:8) and 70-75% r.h. They were fed with honey in water, sucrose solution with added vitamins (Multi-Sanostol, Altana) *ad libitum*, and chopped *Gryllus bimaculatus* once a week. Water was provided *ad libitum* at all times. At least 24h prior to experiments all food stuff was removed.

For all test substances standard "Safe-lock" 1.5ml microcaps were used. These were filled with one milliliter of the particular substance. A cotton wick (untreated, 50x1.65mm, Webotex) was placed in the substance, and soaked for one hour. Afterwards it was drawn out with forceps, so that one centimetre of the wick was outside the lid. The microcaps were recapped which did not disrupt fluid flow in the wicks. A similar setup was established earlier by Lanza and co-workers (Lanza 1988; Lanza 1991; Lanza et al. 1993).

For experiments wooden blocks (42x42x17mm, untreated *Pica abies*) were used. A square arrangement of four holes (1cm diameter) was drilled in central position. After weighing to the nearest 0.01mg (Sartorius MC 210P) the fully equipped microcaps were placed into the four positions of each block in random order, wicks orientated towards the corners. The blocks were placed near *L. niger* nests (after providing for 15min sucrose solution (67.5% w/v) to ensure activity at the test site and selecting ten nests with high activity) or directly into each laboratory nest box, respectively. Experiments outside (with *L. niger*) were conducted at sunny weather between 09:00-11:00 and 14:00-17:00 hours (CEST), to avoid any lowered activity around midday (Ayre 1959). Most experiments were performed from 25. April to 13. June 2003, plus ten additional tests in August and early September. Climate data were measured at a meteorological station in the Botanical Garden in Bayreuth, at about 300m linear distance from the test site.

In bioassays with *L. niger* there were always one water control, a sucrose control (35g/l or 0.102mol/l) and two test substances available. The concentration of 35g/l sucrose was chosen, as in preliminary analysis of *P. coridon* nectar secretions this value was determined as the sucrose content. The concentration is furthermore almost exactly 0.1mol/l and therefore comparable to other bioassays in the literature more easily. In all cases there were similar equipped blocks with the same substances placed as evaporation controls near the test site, but unaccessible to any insects. For the laboratory tests one sucrose control (concentration as above, 35g/l) and three test substances were offered simultaneously. A water control was not necessary in the laboratory tests, as water was provided *ad libitum* at all times. The test substances were dissolved always in sucrose solution (35g/l or 0.102mol/l, Millipore water), as preliminary tests showed very low acceptance of pure amino acid solutions in all three species. Exceptions from this rule were the nectar analoga (Table 5.1) and tests with pure sugars. Test substances were from Merck (Darmstadt, Germany), Fluka (Sigma-Aldrich Chemie, Steinheim, Germany) and Roth (Karlsruhe, Germany), all in p.a. purity.

Pure sugars were tested at 35g/l or 70g/l. Melezitose was tested at 23.3g/l, two thirds of sucrose concentration, thus both solutions had similar energetic profitability (of monosaccharide units). Its occurrence in lycaenid nectar at the time of the sugar preference tests was only pure speculation, but later it was found in *P. icarus* nectar in almost this concentration (22.1g/l).

Single amino acids were tested at concentrations based on preliminary analyses of *P. coridon* secretions in *L. niger*. Some amino acids were also tested pairwise in equal concentrations of either 4.2g/l (preliminary leucine content in *P. coridon* nectar) or 10g/l (Table 5.1). Tyrosine was the only amino acid which did not solute completely. Realised solubility of tyrosine in the sugar solutions and mixtures was unknown, but presumably lower than nominal value given in the tables and figures, as solubility in water is 0.3g/l (Roth EU Sicherheitsdatenblatt).

Mixtures Mix2, Mix3, and Mix4 contained the two, three or four most important amino acids in *P. coridon* nectar (Table 5.1). Mixture Mix11 contained eleven of the main amino acids. Mixture Mix4e contained the four most important amino acids, but leucine concentration was enhanced as to match total amino acid concentration of mixture Mix11. To test if melezitose enhances preference for sucrose/amino acid mixtures it was added to mixture Mix4, deriving Mixture Mix4z. As concentration one fourth (8.75g/l) of the sucrose content (35g/l) was chosen, as its occurrence in lycaenid nectar at this time was only pure speculation (the actual occurrence of melezitose in *P. icarus* and *Z. knysna* nectar was only later corroborated).

Preferences for amino acids of *M. rubra* and *T. melanocephalum* were tested mostly in equal concentrations of 10g/l. Alanine, proline, threonine were the main amino acids in all nectar sources analysed in an Australian rainforest [Blüthgen, 2004 #282], so these three amino acids were especially tested with the tropical species *T. melanocephalum*.

The nectar secretion analoga (cor, ica, kny) were based on the final results from a comparative analysis of nectar secretions from three facultatively myrmecophilous species (chapter 4). At the end of the testing season of *L. niger* these data were not yet available, and thus the preferences for the analoga could not be tested in this ant species.

After placing the blocks in the foraging area of an ant colony, every 15min (*L. niger*) or 5min (laboratory), all ants actually sucking at a wick were counted. The experiments lasted usually one hour (They were immediately interrupted, if one nest had consumed one substance completely). All samples were retrieved and the microcaps weighed again. All ant individuals observed at a given resource were summed and the weight difference minus evaporation was calculated. The following formula (Vander Meer et al. 1995) was used for statistical analysis of both visitation and consumption (in the laboratory bioassays visitation or consumption of water was scored as zero).

$$\frac{\text{visitation sum substance x} \quad - \quad \text{visitation sum water control}}{\text{visitation sum sucrose control} \quad - \quad \text{visitation sum water control}}$$

This calculation allowed to score ant responses to all test substances as a dimensionless value relative to the sucrose standard which by definition always is equal to one. On the one hand this procedure neutralized water requirements in the natural situation, and on the other hand it controlled for different activities or sizes of the colonies and for activity differences between experimental days. Note that negative values for consumption can result in the *L. niger* tests, if the visitation on or consumption of water was greater than the visitation on or consumption of the sugar control, then the colony was omitted from analysis. Negative values are also possible if the test substance was even less attractive than water, respective colonies were retained in the analysis.

Basic calculations and data sorting were done in MS Excel™. For other statistical analyses with Statistica 6.0™ (Statsoft 2003) was used. Sequential Bonferroni correction was employed separately for visitation and consumption data (for the philosophy of this decision consider Cabin & Mitchell 2000; Cottenie & de Meester 2003; Hurlbert 1984; Kroodsma et al. 2001; Moran 2003; Oksanen 2001). The procedure followed Hochberg (1988). Repeated measures ANOVA could not be employed, as not in every trial identical colonies could be tested. Moreover, data could not be transformed to achieve normality and homogenous variance. Therefore Friedman ANOVAs were employed to check for globally significant effects and the Wilcoxon-Wilcox test for the localisation of significant effects was calculated after Sachs (1999).

Table 5.1 Composition of nutrient solutions used for bioassays on the attractiveness of single amino acids, or amino acid mixtures, to ants. Given are concentrations in g/l, last two rows mol/l. Single test concentrations and mixtures Mix2-4,11 based on preliminary results of *P. coridon* nectar. Note, that sucrose (35g/l) was always present. Artificial nectars according to chemical analyses (chapter 4): cor: *P. coridon* nectar main components, ica: *P. icarus*, kny: *Z. knysna* nectar. a) tyrosine maximum solubility in water is 0.3 g/l (Roth EU Sicherheitsdatenblatt), realised solubility in the respective solutions unknown

substances	single tests	Mix2	Mix3	Mix4	Mix4e	Mix4z	Mix11	cor	ica	kny
sucrose	35	35	35	35	35	35	35	29.16	36.7	35.6
glucose								28.36	5.46	7.26
fructose									11.9	14.24
melezitose						8.75			22.14	30.9
alanine	0.09 or 10						0.09	0.17	0.086	2.835
arginine	0.62 or 4.2 or 10						0.62	0.778	0.193	
glycine	0.07									
histidine	0.29						0.29	0.384		
isoleucine	0.09						0.09	0.161		
leucine	4.2 or 10	4.2	4.2	4.2	5.73	4.2	4.2	4.52		
methionine	0.23						0.23	0.339		
phenylalanine	0.65 or 10			0.65	0.65	0.65	0.65	0.645	0.321	
proline	0.94 or 4.2 or 10		0.94	0.94	0.94	0.94	0.94	0.755	0.251	6.05
serine	0.24 or 10							0.623	0.195	
threonine	0.15 or 10						0.15	0.271		
tyrosine $^{a)}$	1.2 or 10	1.2	1.3	1.2	1.2	1.2	1.2	1.176	0.395	
valine	0.06						0.06	0.121		
ca. mol/l $^{a)}$ amino	acids	0.039	0.047	0.051	0.063	0.051	0.063	0.072	0.100	0.085
ca. mol/l $^{a)}$ total	mixture	0.141	0.149	0.153	0.165	0.172	0.165	0.315	0.257	0.369

5.4 Results

Evaporation from the control solutions in the natural test situation was strongly correlated to air temperature (air temp. at 2m, r=0.69, p<0.001, n=32 tests). It was therefore important to correct for evaporation losses before resource consumption could be calculated. Visitation and actual consumption of the test substances was highly correlated for *L. niger* in the first test period (r=0.92, p<0.001, n=22 tests). If the ten tests of the second test period were included, the visitation and consumption were less highly correlated (r=0.72, p<0.001, n=32 tests), as the colonies showed higher variability in responses. In the laboratory tests in the environmental chambers the correlation between visitation and consumption was much weaker, but still significant (*M. rubra*: $r_S=0.57$, $p<0.001$, n=14 tests). Here evaporation controls were placed in empty boxes, but evaporation in the empty boxes was higher than in the boxes occupied by colonies, where the ants regulated moisture to a higher degree. In several cases colonies had to be excluded from analysis, as in the subsequent calculations negative consumption of the sucrose control resulted.

Examining sugar preferences, *L. niger* preferred sucrose to glucose (concentrations as in preliminary *P. coridon* nectar analyses). *L. niger* did not prefer melezitose to sucrose offered in similar energetic profitability (of monosaccharide units), but quite different molarity (35g/l disaccharide (0.1mol/l) vs. 23.3g/l trisaccharide (0.046mol/l)) (Fig. 5.1). Mean visitation and consumption was less than 80% of the sucrose standard, but with rather high variance between the ant colonies. Melibiose (35g/l) and xylose (35g/l) (occurrence in lycaenid nectars at the time of tests was speculative) were both highly significantly discriminated against by *L. niger* (both sugars visitation and consumption: Friedman ANOVA $p<0.001$, Wilcoxon-Wilcox test $p<0.001$).

M. rubra discriminated (consumption) against glucose, and not significant (consumption, $p<0.1$) against fructose, even though the monosaccharides were offered in almost fourfold molarity. The visitation at the monosaccharides was not different to the visitation at the standard sucrose solution. *M. rubra* discriminated (consumption) against melezitose (Fig. 5.1). The differential responses to carbohydrates in *M. rubra* could primarily be detected with consumption rather than visitation.

Sugar preferences of *T. melanocephalum* could be tested only with 3 nests, therefore no statistics are shown. Qualitatively glucose is preferred only, if presented in energetic equivalent to sucrose (standard). Fructose in lower concentration was even inferior to sucrose and glucose at the same mass concentration. *T. melanocephalum* visited and consumed melezitose less than sucrose (standard).

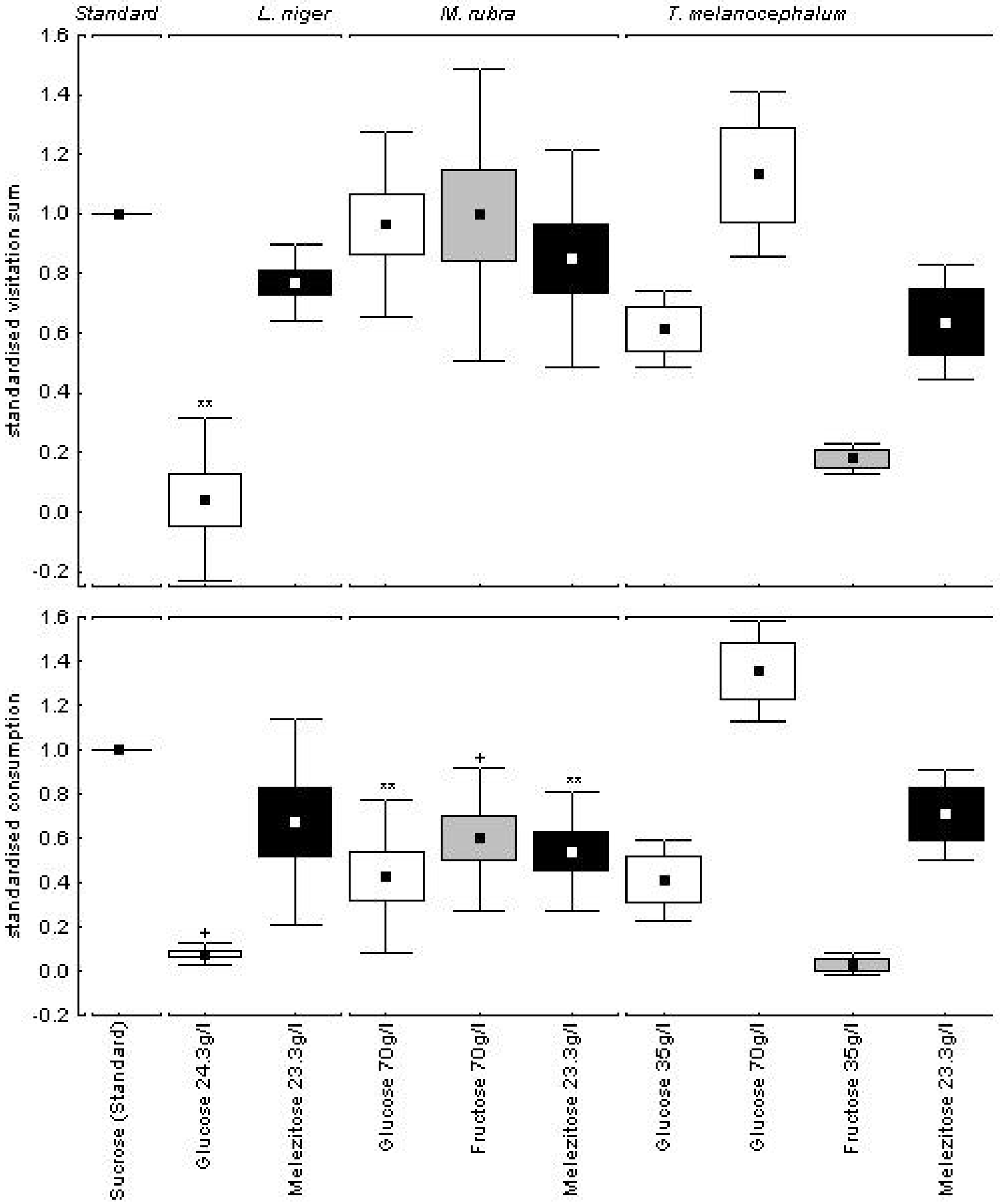


Fig. 5.1 Preferences for different sugars exhibited in choice situations by three ant species (box: mean±s.e., whiskers: mean±s.d.). Upper graph: standardised visitation sum, lower graph: standardised consumption. Number of colonies tested: *L. niger*: 10, *M. rubra*: 10, *T. melanocephalum*: 4. +) difference to sucrose Wilcoxon-Wilcox test, p<0.1 **) p<0.01. [Results combined from the following choice situations: *L. niger* glucose vs. melibiose (discriminated against, not shown) & melezitose vs. xylose (discriminated against, not shown); *M. rubra* as shown; *T. melanocephalum*: glucose 35g/l vs. fructose 35g/l vs. melibiose (n.s. discriminated against, not shown) & glucose 70g/l vs. melezitose 23.3g/l vs. xylose 35g/l (n.s. discriminated against, not shown)]

Considering amino acids, *L. niger* preferred only leucine (at a concentration of 4.2g/l) significantly compared to the sucrose standard (Table 5.2). At higher concentration, however, this preference completely vanished, as leucine was less visited and consumed (Fig. 5.2, Fig. 5.3). Phenylalanine tended to be more attractive at higher concentration, albeit the ant colonies showed a high variability in their response to this amino acid (n. s.).

Table 5.2 Preferences of *Lasius niger* (10 colonies) for sucrose solutions containing several single amino acids and mixtures (figures behind single substances are g/l). Indicated are results from Friedman ANOVAs and subsequent Wilcoxon-Wilcox test. o no significant preference, < significant preference of column to row, > preference of row to column. If significant differences were observed, the first symbol refers to ant visitation, the second symbol to consumption. (>) or (<) not significant after Bonferroni correction. a)realised solubility in the respective solutions unknown b)Mix 4 in this case contained 43.75g/l sucrose, to control for the energetic effect of added melezitose

sucrose 35+	leucine 4.2	leucine 10	Mix3	Mix4	Mix11	standard (sucrose 35)
arginine 0.62	o					o
arginine 4.2	**</<**					o
glycine 0.07	o					o
leucine 4.2				o	o	**>/>**
leucine 10						o
phenylalanine 0.65	**</<**					o
phenylalanine 4.2	o					o
phenylalanine 10		o				o
proline 0.94	o					o
proline 4.2	**</<**					o
proline 10		o				o
serine 0.24	o					o
tyrosine 1.2 a)	o					o
tyrosine 10 a)		o				o/(<)
Mix2				o		o
Mix3				o		o
Mix4	o		o		o	**>/>**
Mix4e					**</o**	o
Mix4z				o b)		o/(<)
Mix11	o					**>/>**

Mixture Mix11 (preliminary *P. coridon* nectar analogon) and Mixture Mix4 were both significantly preferred to the sucrose control. Adding of melezitose (Mix4z: Mix4+8.75g/l, 0.172mol/l) did not lead to a preference of this solution to mixture Mix4 (in this test further 8.75g/l sucrose was added to level out differences in total sugar content on the level of mass concentration, 0.179mol/l), even though the mixture Mix4z had a slightly higher energy content. *L. niger* preferred to visit the mixture with a high content of different amino acids (Mix11) to a mixture of similar overall amino acid content, but less balanced composition (Mix4e, leucine in higher content), although the actual consumption was not different. Serine and mixture Mix4z had a relatively high visitation compared to the actual consumption of these.

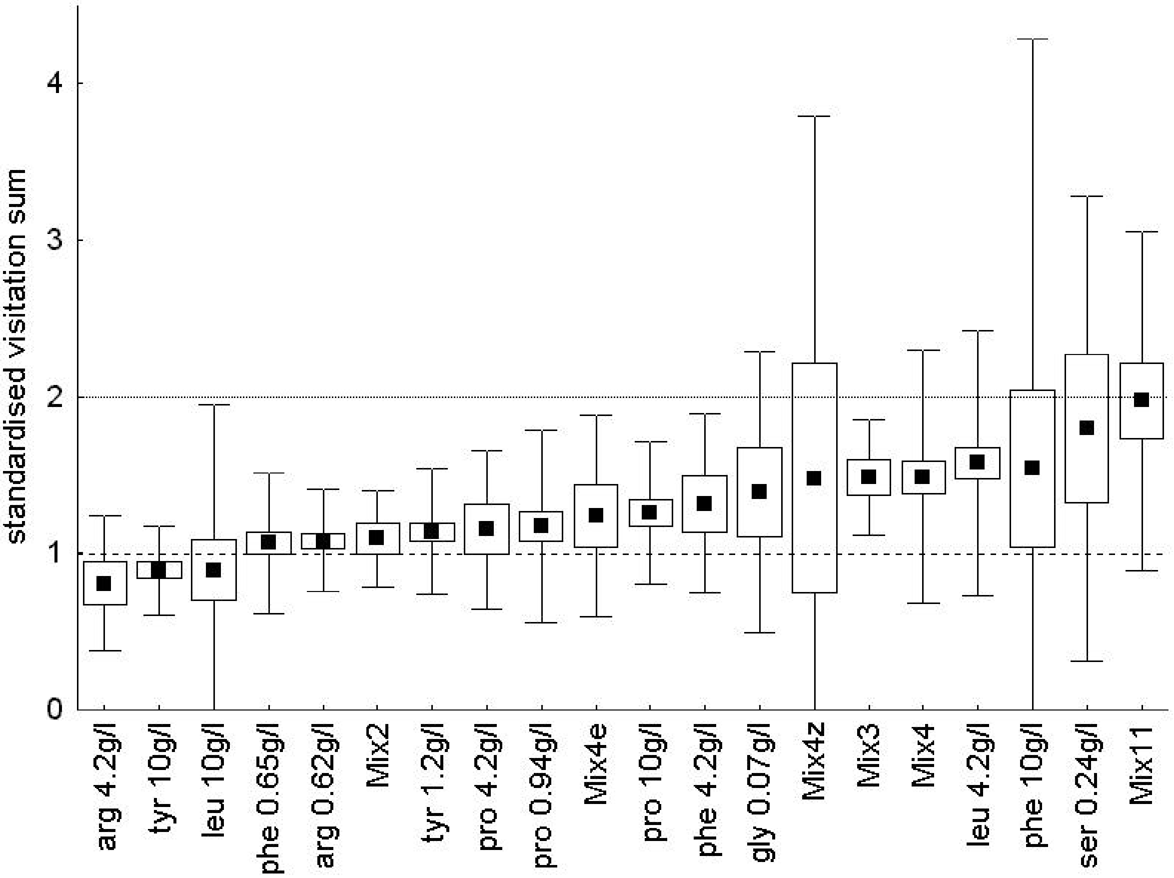


Fig. 5.2 Preferences for different amino acids and mixtures exhibited by *Lasius niger* in choice situations. Given is the standardised visitation sum (box: mean±s.e., whiskers: mean±s.d.). Dashed line: visitation of sucrose standard, dotted line: doubled visitation compared to sucrose standard. Number of test colonies: 10

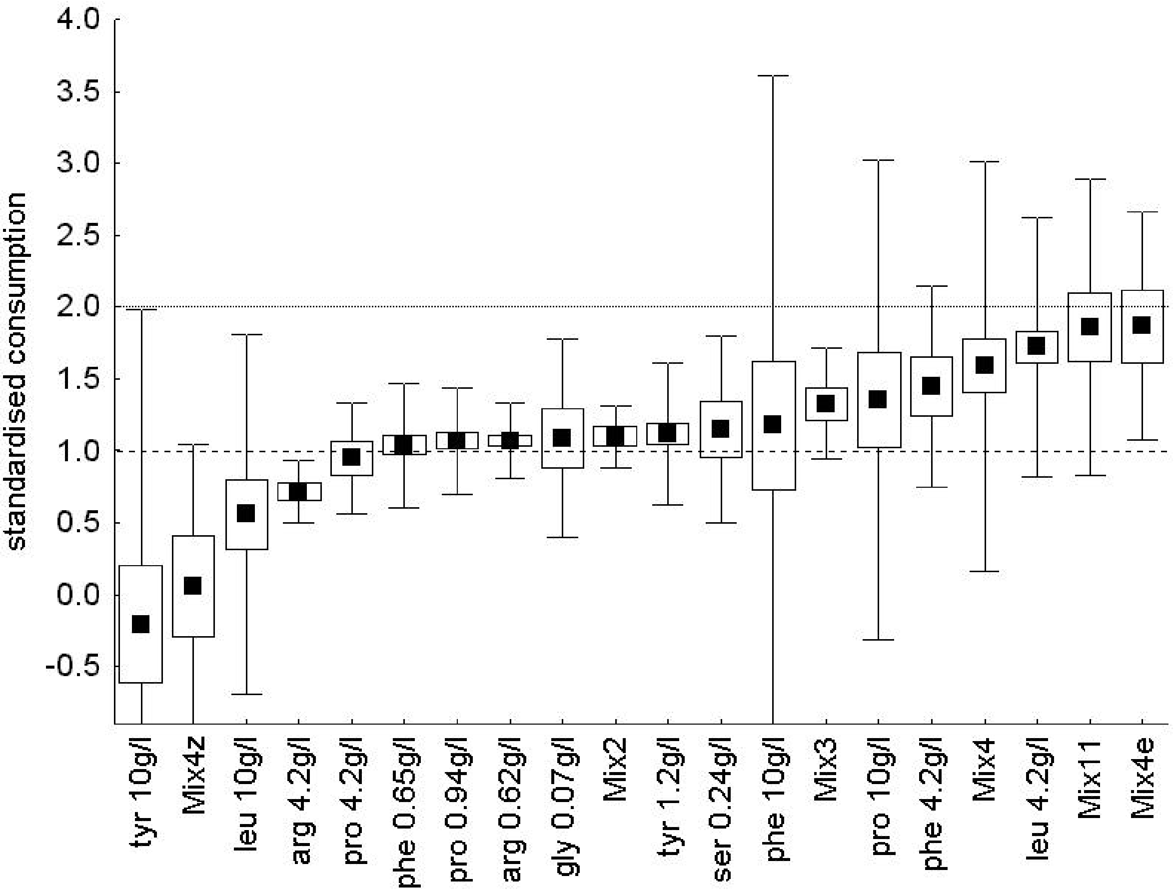


Fig. 5.3 Preferences for different amino acids and mixtures exhibited by *Lasius niger* in choice situations. Given is the standardised consumption (box: mean±s.e., whiskers: mean±s.d.). Dashed line: visitation of sucrose standard, dotted line: doubled visitation compared to sucrose standard. Number of test colonies: 10

M. rubra preferred phenylalanine (at concentrations of 0.65g/l and 10g/l) and tyrosine (at a concentration of 10g/l) significantly to the sucrose standard (Table 5.3, Fig. 5.4). These ants tended to prefer arginine in low concentration (0.65g/l) to sucrose (no longer significant after Bonferroni correction), while they discriminated against arginine in a high concentration (10g/l). The amino acids phenylalanine, proline, serine and tyrosine were all preferred to arginine in high concentrations (all 10g/l). Leucine was preferred to serine in high concentrations. The preference of a lower phenylalanine concentration (0.65g/l) to proline (0.94g/l) was also exhibited in high concentrations. *M. rubra* ants showed no clear preferences for any of the mixtures (Mix4 no longer significant after Bonferroni correction) and did not distinguish between the energetically equivalent, but dissimilarly rich mixtures Mix4e and Mix11. None of the complete nectar analoga was significantly preferred (Table 5.3, Fig. 5.7), and the test colonies showed very variable responses towards these (Fig. 5.4, Fig. 5.5).

Table 5.3 Preferences of *Myrmica rubra* (9-11 colonies) for several single amino acids and mixtures (figures behind single substances are g/l). Indicated are the results of Friedman ANOVAs and subsequent Wilcoxon-Wilcox test. o no significant preference, < significant preference of column to row, > preference of row to column. If significant differences were observed, the first symbol refers to ant visitation, the second symbol to consumption. (>) or (<) not significant after Bonferroni correction. a)realised solubility in the respective solutions unknown

sucrose 35+	arginine 10	phenyl-alanine 0.65	phenyl-alanine 10	leucine 4.2	leucine 10	Mix3	Mix4	Mix11	standard (sucrose 35)
alanine 10									o
arginine 0.62				o					(>)/o
arginine 10					**</<**				**</<**
glycine 10	o				o				o
leucine 4.2		o							o
leucine 10			o						o
phenylalanine 0.65				o					**o/>**
phenylalanine 10	**>/>**				o				**o/>**
proline 0.94		**</<**		**</<**					o
proline 10	**>/>**		o/<		o				o
serine 10	**>/>**				**o/<**				o
threonine 10									o
tyrosine 1.2 a)				o					o
tyrosine 10 a)	**>/>**				o				**o/>**
Mix2						o	o		o
Mix3							o	o	o
Mix4						o		o	(>)/o
Mix4e							o	o	o
Mix11						o	o		o
cor									o
ica									(>)/o
kny									o

T. melanocephalum preferred neither alanine, proline nor threonine (major amino acids in *Z. knysna* nectar secretion) significantly to sucrose (Table 5.4). There was a tendency to discriminate against serine and to prefer phenylalanine (data not shown, as tested on 3-4 nests only). There was a tendency to favour the rich mixture Mix11 to sucrose (no longer significant after Bonferroni correction). Between the energetically equivalent, but dissimilarly rich mixtures Mix11 and Mix4e *T. melanocephalum* did not show any discrimination. The *P. coridon* nectar analogon was clearly preferred to sucrose, the preference for the *P. icarus* nectar analogon was slightly less pronounced. There was no significant preference detectable between the three nectar analoga (Table 5.4, Fig. 5.6, Fig. 5.7).

Table 5.4 Preferences of *Tapinoma melanocephalum* (7 colonies) for several single amino acids and mixtures (figures behind single substances are g/l). Reported are the results Friedman ANOVAs and subsequent Wilcoxon-Wilcox test. o no significant preference, < significant preference of column to row, > preference of row to column. If significant differences were observed, the first symbol refers to ant visitation, the second symbol to consumption. (>) or (<) no longer significant after Bonferroni correction)

sucrose 35+	alanine 10	proline 10	threonine 10	Mix4	Mix4e	Mix11	cor	ica	kny	stan-dard
alanine 10		o	o							o
proline 10			o							o
threonine 10										o
Mix4					o	o				o
Mix4e						o				o
Mix11										o/(>)
cor								o	o	**>/>**
ica									o	**(>)/>**
kny										o

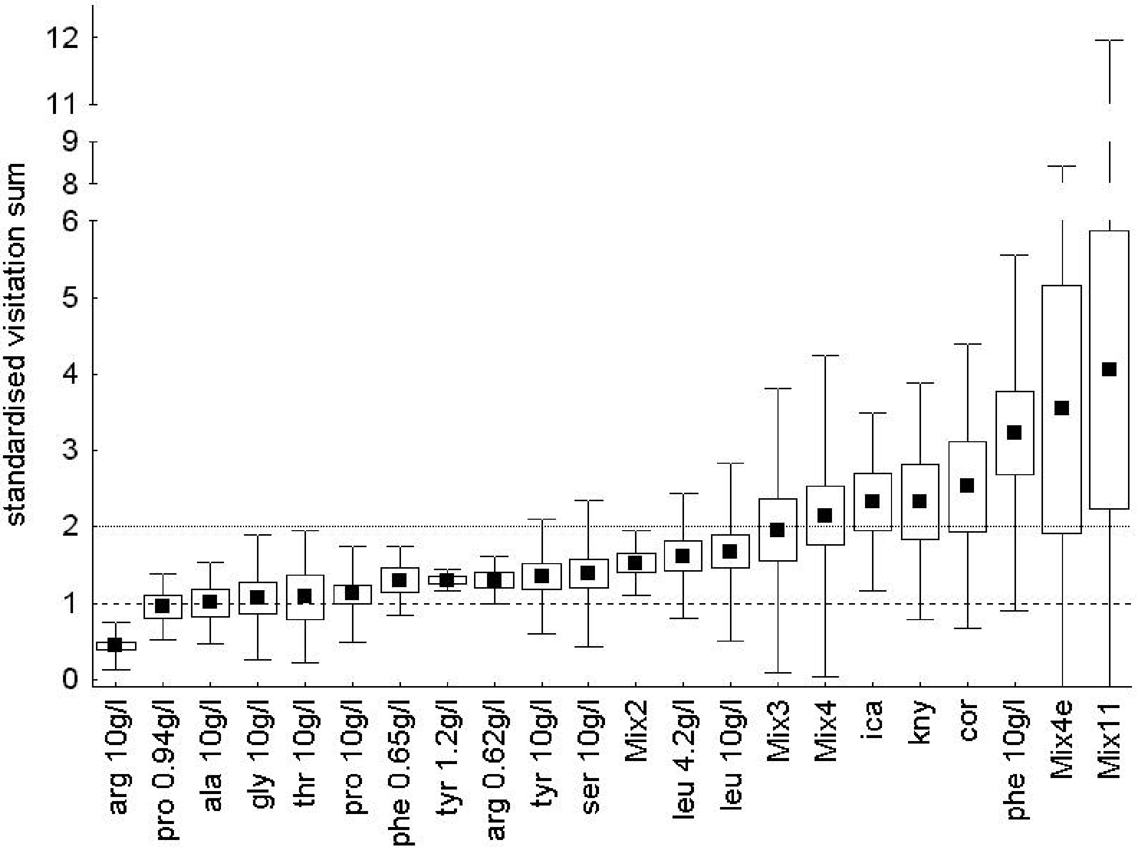


Fig. 5.4 Preferences for different individual amino acids and mixtures exhibited by *Myrmica rubra* in choice situations. Given is the standardised visitation sum (box: mean±s.e., whiskers: mean±s.d.). Composition of test solutions in Table 5.1; cor, ica, kny: complete nectar analoga of *P. coridon*, *P. icarus* and *Z. knysna*, respectively. Dashed line: visitation of sucrose standard, dotted line: doubled visitation compared to sucrose standard. Number of test colonies: 9-11

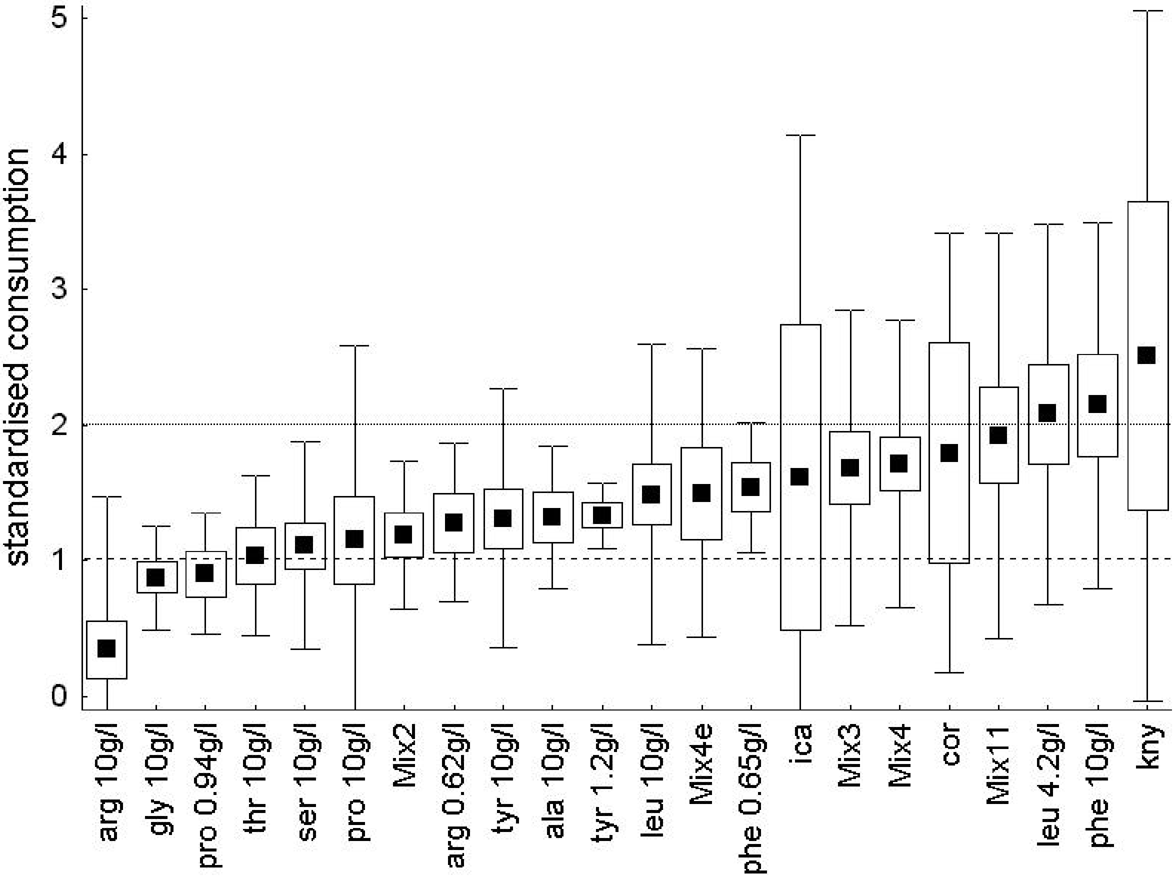


Fig. 5.5 Preferences for different individual amino acids and mixtures exhibited by *Myrmica rubra* in choice situations. Given is the standardised consumption (box: mean±s.e., whiskers: mean±s.d.). Composition of test solutions in Table 5.1; cor, ica, kny: complete nectar analoga of *P. coridon*, *P. icarus* and *Z. knysna*, respectively. Dashed line: visitation at sucrose standard, dotted line: doubled visitation compared to sucrose standard. Number of test colonies: 9-11

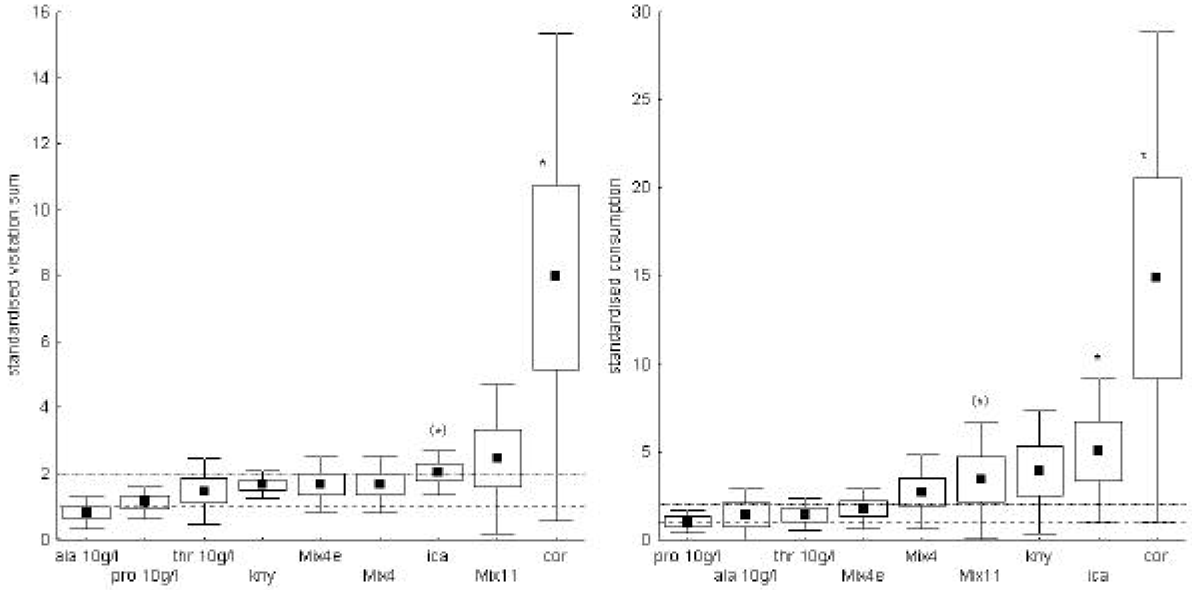


Fig. 5.6 Preferences for different amino acids, mixtures and complete lycaenid nectar analoga exhibited by *Tapinoma melanocephalum* in choice situations (ala vs. pro vs. thr, Mix4 vs. Mix4e vs. Mix11, nectar analoga cor vs. ica vs. kny (compositions in Table 5.1)) Box: mean±s.e., whiskers: mean±s.d. Dashed line: visitation/consumption of sucrose standard, dotted line: doubled visitation/consumption compared to standard. Left graph showing standardised visitation sum, right graph standardised consumption, note the different scales. *: difference to sucrose (Wilcoxon-Wilcox test, p<0.05), (*): difference no longer significant after Bonferroni correction. Dashed line: visitation at sucrose standard, dotted line: doubled visitation compared to sucrose standard. Number colonies: 7

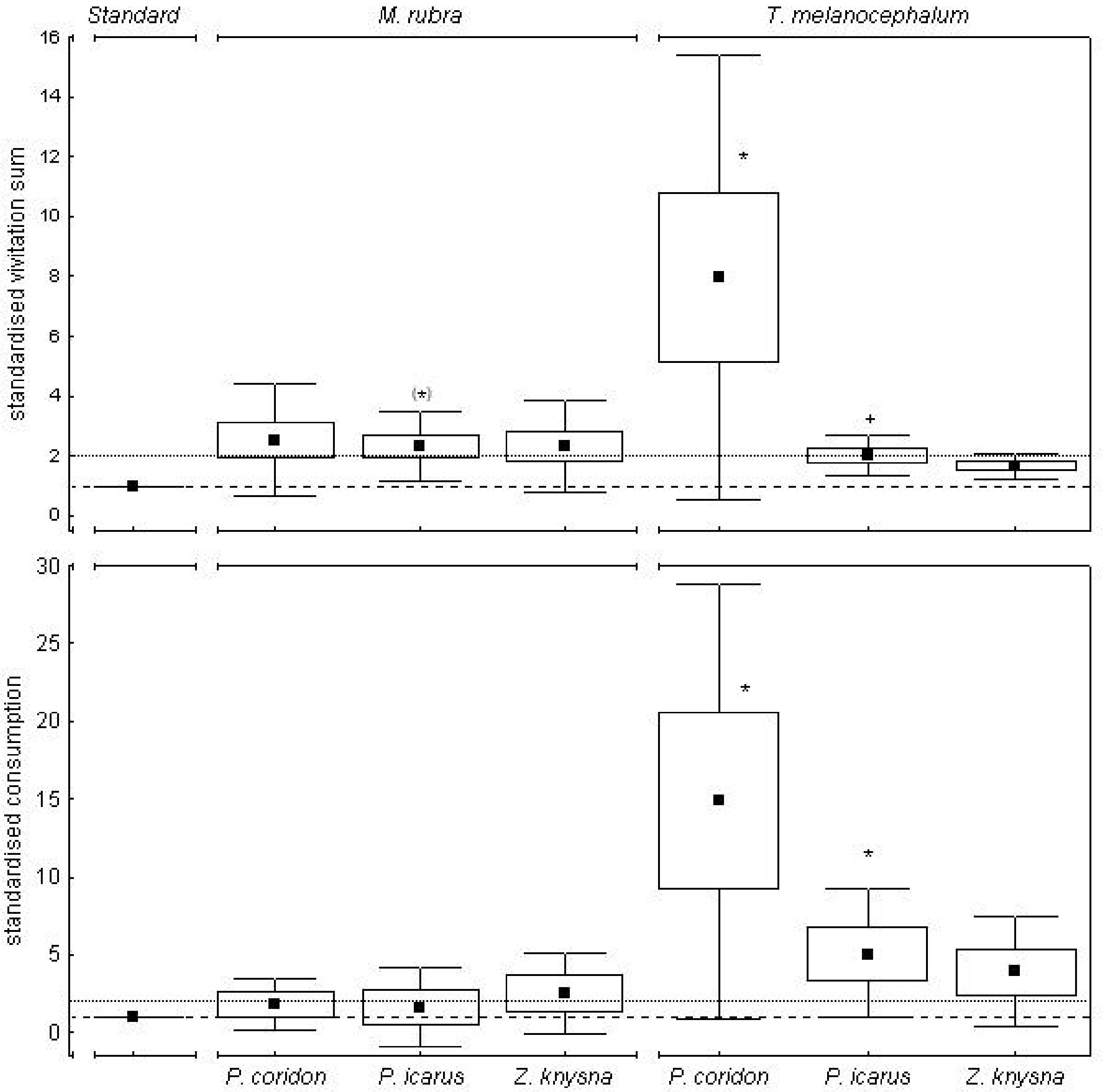


Fig. 5.7 Comparison of preferences for different lycaenid nectar analoga exhibited by two ant species (box: mean±s.e. whiskers: mean±s.d.), upper graph showing standardised visitation sum, lower graph standardised consumption, note the different scales. *: difference to sucrose Wilcoxon-Wilcox test, $p<0.05$, +: $p<0.1$, (*): no longer significant after Bonferroni correction Dashed line: visitation at sucrose standard, dotted line: doubled visitation compared to sucrose standard. Number of colonies tested, *M. rubra*: 10/9, *T. melanocephalum*: 7

5.5 Discussion

The three ant species, representatives of different subfamilies, showed preference patterns which shared some similarities, but on the other hand also revealed differences. Both visitation at and consumption of the test substances were recorded. Counting of ants actually sucking at the wick allowed a more reliable tally than e.g. counting ants with filled gasters, especially in small ant species like *T. melanocephalum*. Observation of visitation behaviour is inherently influenced by chance, as necessarily only snapshots of responses at certain time points are recorded. In contrast, recording the consumption integrates the preference shown over the whole duration of the test. Thus measuring the consumption might yield a more reliable picture (Lanza et al. 1993), but note that in this study evaporation was not controlled for). In the laboratory measurements evaporation of the test solutions in the controls was higher than in the ant nests, thus leading to an underestimation of the consumption. Some cases and tests had to be discarded for analysis, as negative consumption values resulted. Nevertheless for most substances visitation and consumption were in good accordance to each other, for *L. niger* both measures were highly correlated. In *M. rubra* consumption tended to show preferences more clearly than visitation, corroborating Lanza et al. (1993). Just after the test series had been finished, a new arrangement of controls in relation to actual test replicates was published (Prince et al. 2004). Employing this new procedure in future tests might further increase reliability.

The calculation after Vander Meer et al. (1995) (see also Vander Meer & Merdinger 1990) allowed to compare all choice situations to the same standard (sucrose) and eliminated any water requirements in the natural situation. Furthermore it allowed to control for differences in the activity levels of the test colonies and also for activity differences between different days. In most studies about preferences of ants on honeydew or extrafloral nectar these important influences are not controlled for, e.g. (Bristow & Yanity 1999; Cornelius et al. 1996; Völkl et al. 1999).

L. niger and *M. rubra* showed a preference for the disaccharide sucrose to the monosaccharide glucose. *M. rubra* showed furthermore a slight preference of sucrose to fructose, too, even though both monosaccharides were even tested in higher molarity. This higher molarity still holds if sucrose would be dissociated (204mol/l), which ants can manage with invertase from their mandibular glands (Ricks & Vinson 1972).

L. niger was already reported to prefer sucrose to glucose and the latter to fructose (Tinti & Nofre 2001). *M. rubra* exhibits a similar preference sequence (Boevé & Wäckers 2003). Qualitatively, *T. melanocephalum* (not statistically tested) showed an increasing preference with higher concentration (glucose), as would be expected. The sugar preferences of this ant are contrarily reported in earlier studies (no preference (Koptur & Truong 1998), sucrose>glucose, fructose>glucose (Blüthgen & Fiedler 2004)). Here fructose was in tendency less attractive then glucose, although this might be due to variability of the few colonies tested in these trials. Generally ants do prefer the disaccharide sucrose to the monosaccharides glucose and fructose, both if tested in same mass concentration and if tested in same molarity. In contrast, discrimination between glucose and fructose, if present, is highly species-specific (Cornelius et al. 1996; Koptur 1979; Ricks & Vinson 1970; Vander Meer et al. 1995; Vander Meer & Merdinger 1990). The sensory threshold for sucrose in ants is markedly lower than for the monosaccharides (Schmidt 1938). Sucrose was the main sugar component in the nectar secretions of all three facultatively myrmecophilous lycænid species tested in comparison, so all these species might expect some success in attracting the interest of ants to the nectar secretions (chapter 4).

The sugars xylose, melibiose were highly unattractive for *L. niger*. Although these sugars can be found infrequently in homopteran honeydew [Völkl, 1999 #5;Engel, 2001 #291;Blüthgen, 2004 #282], most ant species show a very low acceptance of these sugars (Blüthgen & Fiedler 2004; Boevé & Wäckers 2003; Ricks & Vinson 1970; Tinti & Nofre 2001; Vander Meer et al. 1995; Vander Meer & Merdinger 1990; Völkl et al. 1999). For lycaenid caterpillars secretion of these sugars would not pay, and indeed, melibiose was found only once in a nectar sample (*P. icarus*) (chapter 4). Further sugars amongst others in honeydew are e.g. lactose, maltose, erlose and raffinose [Nemec, 1990 #404;Engel, 2001 #291;Fischer, 2001 #18;Byrne, 2003 #307;Blüthgen, 2004 #282;Woodring, in press #536]. Ant responses to these sugars tend to be different. Whereas preference of maltose depends on the species investigated (Blüthgen & Fiedler 2004; Boevé & Wäckers 2003; Cornelius et al. 1996; Ricks & Vinson 1970; Vander Meer et al. 1995; Vander Meer & Merdinger 1990; Völkl et al. 1999), raffinose is attractive for *L. niger* and *Solenopsis invicta* (Schmidt 1938; Vander Meer et al. 1995; Völkl et al. 1999) but equally for many species not [Blüthgen, 2004 #282]. Lactose is inattractive for *L. niger* and *M. rubra* (Boevé & Wäckers 2003; Tinti & Nofre 2001), while erlose has be shown to be attractive for *M. rubra* (Boevé & Wäckers 2003), both sugars are seldomly found in lycaenid nectar (chapter 4).

The trisaccharide melezitose was not preferred to sucrose in *L. niger*, and even discriminated significantly against by *M. rubra*. However, profitability of the solutions was similar only in energetic profitability of monosaccharide units, but not for the molarity of the oligosaccharides. For *L. niger*, though, melezitose has a double sweetness effectiveness compared to sucrose according to Tinti & Nofre (2001), and as such the sweetness was comparable in this study. As a preference of *M. rubra* for melezitose was stated in the literature ((Boevé & Wäckers 2003), but see Fig.2 there) and since a high preference of *L. niger* for melezitose was repeatedly emphasized ((Duckett 1974), cited e.g. in (Kiss 1981; Wäckers 2000); and tested in equal molarity (Tinti & Nofre 2001); equal weight/volume (Völkl et al. 1999)), one might have expected to see a preference, even if a sucrose solution is energetically more profitable. In homopterans melezitose seems to serve osmotic regulation (Fisher et al. 1984; Kennedy & Fosbrooke 1972; Rhodes et al. 1997; Wilkinson et al. 1997, and references therein) or parasitoid deterrence (Wäckers 2000). Certain ants might have secondarily evolved a preference for this substance, using it as an indicator for a profitable nutrient source, especially as the gustatory suitability is unclear (compare (Boevé & Wäckers 2003; Wäckers 2000; Wäckers 2001; Zoebelein 1956). Nevertheless, a general preference of ants for melezitose does apparently not exist (found: (Cornelius et al. 1996; Ricks & Vinson 1970; Tinti & Nofre 2001; Völkl et al. 1999), not found: (Blüthgen & Fiedler 2004; Bristow & Yanity 1999; Cornelius et al. 1996)). Thus, while a preference for the disaccharide sucrose is generally pronounced in ants, the relevance of the trisaccharide melezitose to ants remains more conditional.

Caterpillars of facultatively myrmecophilous species should therefore secrete nectar with sucrose as main sugar, probably melezitose as a further component, while monosaccharides are less important. A similar pattern was found in the nectars of the three facultatively myrmecophilous lycaenid species, the most strongly ant-associated species however, did not secrete melezitose (chapter 4).

Considering single amino acids presented in a sucrose solution (35g/l), *L. niger* showed a clear preference for leucine in the natural concentration observed in *P. coridon* nectar, although this vanished at higher concentration. Generally, it appears that for several amino acids (leucine, tyrosine, arginine Fig. 5.3) *L. niger* has a tendency to increasingly avoid these at higher concentrations. At the same time, however variability between the colonies in the strength of their response increased. Raising the amino acid concentration therefore did not generally enhance visitation or consumption in *L. niger*. An exception might be phenylalanine, but colonies exhibited a high variability in response at the high concentration.

Interestingly, serine wicks were strongly visited (Fig. 5.2), but consumption was only marginally higher than sucrose (Fig. 5.3). Serine is the main amino acid in nectar secretions of the Australian lycaenid *Jalmenus evagoras* (Pierce 1983 ; Pierce 1989; Pierce & Nash 1999). This amino acid can act as precursor in the biosynthesis of formic acid, a common defence substance of ants in the subfamily Formicinae (Hölldobler & Wilson 1990). Alternatively it might be attractive as it is a hemolymph component of the caterpillars (chapter 4), especially as trehalose seems not to be detectable by ants (Schmidt 1938; Tinti & Nofre 2001; Vander Meer et al. 1995; Völkl et al. 1999). The experiments presented here suggest that serine attracts formicine workers (e.g. *L. niger*), but does not enhance nectar consumption considerably. The observed preference for leucine in *L. niger* is especially interesting, a similar preference was also found in the unrelated *Solenopsis* sp. (subfamily Myrmicinae) (Ricks & Vinson 1970). Leucine is the main amino acid component in *P. coridon* nectar (chapter 4), and *L. niger* and closely related congeneric ants are the main tenders of *P. coridon* larvae (Fiedler 1991). Hence, at least in the species combination *P. coridon* / *L. niger* the major amino acid component in the nectar secretion coincides with the feeding preference of the attendant ant.

Preferred amino acids of *M. rubra* were phenylalanine (even at high concentration) and tyrosine (in high concentration). Arginine in low concentration was accepted (n.s. preferred), but was discriminated against at higher concentration. A similar preference of phenylalanine in high concentration is also exhibited e.g. by honeybees (Inouye & Waller 1984). Phenylalanine and tyrosine are main amino acid components of *P. icarus* nectar secretion and are among the five main components in *P. coridon* nectar secretion (chapter 4). No clear preference of single amino acids could be found in *T. melanocephalum*, but due to the limited number of ant colonies available for tests, not too much emphasis should be given to this observation.

Mixtures of four or more amino acids in sucrose (Mix4, Mix11) were preferred over pure sucrose solution by *L. niger* and *T. melanocephalum*, but in *M. rubra* this was not statistically significantly (Mix4 n.s. after Bonferroni correction). Adding of melezitose to a mixture of four amino acids (Mix4z) only slightly (and not significantly) enhanced preference of *L. niger* exhibited as visitation. Thus in this test melezitose did not act particularly as advertisement substance (see above). Moreover relative consumption of the melezitose spiked nectar Mix4z was even lower than that of pure sucrose solution (n.s. after Bonferroni correction), whereas visitation was increased by ca. 50%, with a high variance between ant colonies. This might indicate that, as with serine, certain nectar compounds are able to increase attractiveness of a resource behaviourally, even though such compounds are not necessarily profitable nutrients. Mixtures of only two or three amino acids in sucrose solution were not significantly more attractive. Especially interesting is the discovery that the four main amino acids of *P. coridon* nectar (viz. leucine, tyrosine, proline, phenylalanine) were already sufficient to render a mixture attractive. Adding further amino acids might improve gustatory sensation or such compounds might act as signal substances for other ant species. *L. niger*, on the other hand, preferred the more balanced mixture Mix11 to the equally concentrated Mix4e, which was composed of only four amino acids, where leucine was enhanced. This might have been due to an over-optimal leucine concentration, but a more likely explanation is that *L. niger* workers in this case opted for the more balanced diet.

Comparing the complete lycaenid nectar analoga (viz. imitating the whole array of sugars and amino acids as measured in the natural secretions), no significant preferences over pure sucrose (n.s. after Bonferroni correction) could be found in *M. rubra*. This ant species tended to weakly prefer the *P. icarus* and *Z. knysna* analoga. This might indicate a difference to the other two ant species. *T. melanocephalum* showed a marked preference for the *P. coridon* analogon and a statistically significant preference for the *P. icarus* analogon.

Thus *L. niger* (Formicinae) and *T. melanocephalum* (Dolichoderinae) prefer mixtures of amino acids over sucrose solutions, similar as in previous studies ((Koptur & Truong 1998) (*T. melanocephalum*), (Lanza 1988; Lanza 1991) (Myrmicinae); (Blüthgen & Fiedler 2004) (*T. melanocephalum*, several subfamilies)), whereas certain single amino acids like tyrosine and histidine were discriminated against (Lanza & Krauss 1984). Mixture preferences were not significant for *M. rubra* in the experiments presented here, but this might also be a laboratory artefact, as preferences in the laboratory (Lanza 1991) and in the natural situation (Lanza 1988; Lanza et al. 1993) can differ. Probably with a larger sample size (i.e. colonies) the slight differences observed might have reached significance. A preference of a mixture of amino acids and sugars is in the Lepidoptera sometimes exhibited only by the females (Alm et al. 1990; Erhardt & Rusterholz 1998, but see Erhardt 1991; Erhardt 1992; Romeis & Wäckers 2000). Similarly preferences for amino acid mixtures are widespread among nectarivores (e.g. honey bees Alm et al. (1990)), but are not universal and are for example lacking in some solitary bees (Roubik et al. 1995). Like honeybees, ant workers forage for proteins, which are fed to the larvae (Brian 1973; Macom & Porter 1995) and the queen, whereas the workers themselves use the sugar as fuel (Brian & Abbott 1977; Cassill & Tschinkel 1999; Sorensen et al. 1983; Vinson 1968; Wheeler 1994). Therefore the demand for certain substances and exhibited preferences depend on the nutritional and reproductive state of the colony and its actual requirements (Cassill & Tschinkel 1999). In the natural situation supply with different nutrients is unpredictable and highly variable, therefore the exhibited choices should also vary accordingly (Beattie 1985; Cassill & Tschinkel 1999; Kay 2002; Sudd & Sudd 1985). Blüthgen & Fiedler (2004) showed that feeding ants with particular amino acids *ad libitum* for two days decreased preference for these same amino acids in subsequent tests. Nectar preferences exhibited by ants vary also with the season (Bristow & Yanity 1999; Skinner 1980; Stradling 1978; Sudd & Sudd 1985). Normal diet composition influences the preferences of ant species (Kay 2002), and sensory capabilities differ between ant species (Nonacs & Dill 1991; Schmidt 1938). The range of concentrations tested also has an influence, as shown for some amino acids here. This might be expected as sensory systems exhibit different perception accuracy at different concentrations (e.g. (Blem et al. 2000). The viscosity of the test solutions also affects preferences (Cassill & Tschinkel 1999; Josens et al. 1998), since viscosity also influences the sweetness, at least in *Homo sapiens* (Portmann et al. 1992). Very generally, different ant species even within same genus exhibit different preferences (Lanza et al. 1993). Even (laboratory) colonies of the same species can show high variability (Lanza 1991; Pierce 1989). Due to this tremendous variability and plasticity, so far no 'universal' ant bait could be found (Knight & Rust 1991).

Although the leucine preference of *L. niger* translated to a preference of mixtures containing leucine and thus also the mixture Mix11 (preliminary *P. coridon* nectar mimic), ant responses to mixtures are generally not predictable from preferences of single components (Lanza et al. 1993). For an understanding of structuring of interactions and the stability of ant mutualisms with lycaenids, homopterans or plants it would be desirable to predict foraging decisions based on a knowledge of nectar compositions. Thus far, however the idiosyncratic (and variable) responses of ants to nectar compounds largely preclude such generalizations. Some ‘key compounds’ among amino acids might exist, e.g. leucine for *L. niger* and *Solenopsis* (Ricks & Vinson 1970), phenylalanine for *M. rubra* and *Oecophylla smaragdina* (Blüthgen & Fiedler 2004) or glycine for *Camponotus japonicus* (Wada et al. 2001). Glycine enhanced sweetness for *Camponotus japonicus*, an effect which was also shown for *Apis mellifera* (Kim & Smith 2000) and *Rattus* (Tateda & Hidaka 1966), but could not be detected here with three species of ants. However, the glycine concentration used here was very low (preliminary *P. coridon* nectar concentration), and not all ant species prefer glycine (Blüthgen & Fiedler 2004; Lanza & Krauss 1984).

Therefore from the perspective of a facultatively myrmecophilous caterpillar, which cannot predict which ant species it will meet and possibly interact with, it could be rewarding to produce some ‘key compounds’ which increase attractiveness for certain ant groups. But such a caterpillar will not be able to produce the ‘optimal’ nectar secretion which would enable it to bind all possible visiting ant species. Therefore selection should favour the delivery of a balanced mixture, that also contains possible ‘key compounds’. The nectar produced by *Polyommatus coridon* matches these requirements. The positive aspects of an amino acid mixture will likely conceal any repellent function singular components might have for some ant species. Nevertheless, as long as a caterpillar does not specifically interact with just a limited number of ant species with similar preferences, the ‘key compounds’ should also not be concentrated too high. If for a facultatively myrmecophilous caterpillar it does not pay to secrete a whole array of amino acids, as necessary for a balanced mixture, it should opt towards a high concentration of (disaccharide) sugars, as done by *Polyommatus icarus* and *Zizeeria knysna*.

5.6 Acknowledgements

Dr. Peter Hartmann helped acquiring the *Myrmica rubra* nests in his own garden. The Umweltbundesamt (Berlin), namely Gabriele Schrader, kindly provided the *Tapinoma melanocephalum* nests. The meteorological station in the Botanical Garden of the University of Bayreuth, namely Dr. Johannes Lüers, provided climate data for the period during which the bio-assays with *L. niger* were conducted. We thank Franziska Kuhlmann and Jörg Hager for technical assistance in conducting several of the numerous bioassays.

5.7 References

Agrawal, A. A. & Fordyce, J. A. 2000: Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B **267**, 1857-1861.

Alm, J., Ohnmeiss, T. E., Lanza, J. & Vriesenga, L. 1990: Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia **84**, 53-57.

Axén, A. H. & Pierce, N. E. 1998: Aggregation as a cost-reducing strategy for lycaenid larvae. Behav Ecol **9**, 109-115.

Ayre, G. L. 1959: Food habits of *Formica subnitens* CREIGHTON (Hymenoptera: Formicidae) at Westbank, British Columbia. Insectes Sociaux **6**, 105-114.

Beattie, A. J. 1985: The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, Cambridge.

Blem, C. R., Blem, L., Felix, J. & van Gelder, J. 2000: Rufous hummingbirds sucrose preference: precision of selection varies with concentration. Condor **102**, 235-238.

Blüthgen, N. & Fiedler, K. 2004: Preferences for sugars and amino acids in a diverse nectar-feeding ant community. J Anim Ecol **73**, 155-166.

Blüthgen, N., Gottsberger, G. & Fiedler, K. 2004: Sugar and amino acid composition of ant-attended nectar and honeydew sources from an Australian rainforest. Aust Ecol **29**, in press.

Boevé, J.-L. & Wäckers, F. L. 2003: Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* workers. Oecologia **136**, 508-514.

Brian, M. V. 1973: Feeding and growth in the ant *Myrmica*. J Anim Ecol **42**, 37-53.

Brian, M. V. & Abbott, A. 1977: The control of food flow in a society of the ant *Myrmica rubra* L. Anim Behav **25**, 1047-1055.

Bristow, C. M. 1991: Why are so few aphids ant-tended. In: Ant-plant interactions (Huxley, C. R. & Cutler, D. F. eds). Oxford University Press, Oxford. pp. 104-119.

Bristow, C. M. & Yanity, E. 1999: Seasonal response of workers of the Allegheny Mound Ant, *Formica exsectoides* (Hymenoptera: Formicidae) to artificial honeydews of varying nutritional content. Great Lakes Entomol **32**, 15-27.

Buckley, R. 1987: Interactions involving plants, Homoptera, and ants. Ann Rev Ecol Syst **18**, 111-135.

Byrne, D. N., Hendrix, D. L. & Livy, H. W. I. 2003: Presence of trehalulose and other oligosaccharids in Hemiptera honeydew, particularly Aleyrodidae. Physiol Entomol **28**, 144-149.

Cabin, R. J. & Mitchell, R. J. 2000: To Bonferroni or not to Bonferroni: When and how are the questions. ESA Bulletin **81**, 246-248.

Cassill, D. L. & Tschinkel, W. R. 1999: Regulation of diet in the Fire Ant, *Solenopsis invicta*. J Insect Behav **12**, 307-328.

Cornelius, M. L., Grace, J. K. & Yates, J. R. I. 1996: Acceptability of different sugars and oils to three tropical ant species. Anz Schädlingskde, Pflanzenschutz, Umweltschutz **69**, 41-43.

Cottenie, K. & de Meester, L. 2003: Comment to Oksanen (2001): reconciling Oksanen (2001) and Hurlbert (1984). Oikos **100**, 394-396.

Cottrell, G. B. 1984: Aphytophagy in butterflies: its relationship to myrmecophily. Zool J Linnean Soc **79**, 1-57.

DeVries, P. J. 1991: Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. Biol J Linnean Soc **43**, 179-195.

Duckett, D. P. 1974: Further studies of ant-aphid interactions, University of London, London.

Eastwood, R. & Fraser, A. M. 1999: Associations between lycaenid butterflies and ants in Australia. Aust Ecol **24**, 503-537.

Engel, V., Fischer, M. K., Wäckers, F. L. & Völkl, W. 2001: Interactions between extrafloral nectaries, aphids and ants: are there competition effects between plant and homopteran sugar sources? Oecologia **129**, 577-584.

Erhardt, A. 1991: Nectar sugar and amino acid preferences of *Battus philenor* (Lepidoptera, Papilionidae). Ecol Entomol **16**, 425-434.

-. 1992: Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). Oecologia **90**, 581-585.

Erhardt, A. & Rusterholz, H.-P. 1998: Do peacock butterflies (*Inachis io* L.) detect and prefer nectar amino acids and other nitrogenous compounds. Oecologia **117**, 536-542.

Fiedler, K. 1991: Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionidae). Bonner Zoologische Monographien **31**.

-. 2001: Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. Divers Distrib **7**, 45-60.

Fiedler, K. & Hagemann, D. [1995]: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K. & Maschwitz, U. 1989: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. Ethology **80**, 71-80.

Fischer, M. K. & Shingleton, A. W. 2001: Host plants and ants influence the honeydew sugar composition of aphids. Funct Ecol **15**, 544-550.

Fisher, D. B., Wright, P. J. & Mittler, T. E. 1984: Osmoregulation by the aphid *Myzus persicae*: a physiological role for honeydew oligosaccharids. J Insect Physiol **30**, 387-393.

Fraser, A. M., Tregenza, T., Wedell, N., Elgar, M. A. & Pierce, N. E. 2002: Oviposition tests of ant preference in a myrmecophilous butterfly. J Evol Biol **15**, 861-870.

Hochberg, Y. 1988: A sharper bonferroni procedure for multiple tests of significance. Biometrika **75**, 800-802.

Hölldobler, B. 1996a: Signale und Informationsverarbeitung in Ameisenpopulationen. Verhandlungen der Gesellschaft Deutscher Naturforscher und Ärzte **119 Versammlung**, 313-330.

-. 1996b: Verständigung im Ameisenstaat. Verh Dtsch Zool Ges **89.2**, 7-24.

Hölldobler, B. & Wilson, E. O. 1990: The ants. Harvard University Press / Springer, Heidelberg.

Hurlbert, S. H. 1984: Pseudoreplication and the design of ecological experiments. Ecol Monographs **54**, 187-211.

Inouye, D. W. & Waller, G. D. 1984: Responses of honey bees (*Apis mellifera*) to amino acid solutions mimicking floral nectars. Ecology **65**, 618-625.

Jordano, D. & Thomas, C. D. 1992: Specificity of an ant-lycaenid interaction. Oecologia **91**, 431-438.

Josens, R. B., Farina, W. M. & Roces, F. 1998: Nectar feeding by the ant *Camponotus mus*: intake rate and crop filling as a function of sucrose concentration. J Insect Physiol **44**, 579-585.

Kay, A. 2002: Applying optimal foraging theory to assess nutrient availability ratios for ants. Ecology **83**, 1935-1944.

Kennedy, J. S. & Fosbrooke, J. H. M. 1972: The plant in the life of an aphid. In: Insect plant relations (van Emden, H. F. ed). Blackwell Science.

Kim, Y. S. & Smith, B. H. 2000: Effect of an amino acid on feeding preferences and learning behavior in the honey bee, *Apis mellifera*. J Insect Physiol **46**, 793-801.

Kiss, A. 1981: Melezitose, aphids and ants. Oikos **37**, 382.

Knight, R. L. & Rust, M. K. 1991: Efficacy of formulated baits for control of the Argentine Ant (Hymenoptera: Formicidae). J Econ Entomol **84**, 510-514.

Koptur, S. 1979: Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. Amer J Bot **66**, 1016-1020.

Koptur, S. & Truong, N. 1998: Facultative ant-plant interactions: nectar sugar preferences of introduced pest ant species in South Florida. Biotropica **30**, 179-189.

Kroodsma, D. E., Byers, B. E., Goodale, E., Johnson, S. & Liu, W.-C. 2001: Pseudoreplication in playback experiments, revisited a decade later. Anim Behav **61**, 1029-1033.

Lanza, J. 1988: Ant preferences for *Passiflora* nectar mimics that contain amino acids. Biotropica **20**, 341-344.

-. 1991: Response of fire ants (Formicidae: *Solenopsis invicta* and *S. geminata*) to artificial nectars with amino acids. Ecol Entomol **16**, 203-210.

Lanza, J. & Krauss, B. R. 1984: Detection of amino acids in artificial nectars by two tropical ants, *Leptothorax* and *Monomorium*. Oecologia **63**, 423-425.

Lanza, J., Vargo, E. L., Pulim, S. & Chang, Y. Z. 1993: Preferences of the Fire Ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. Entomol Soc Am **22**, 411-417.

Leimar, O. & Axén, A. H. 1993: Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav **46**, 1177-1182.

Macom, T. E. & Porter, S. D. 1995: Food and energy requirements of laboratory fire ant colonies (Hymenoptera: Formicidae). Environ Entomol **24**, 387-391.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

-. 1970: New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymneoptera). J Lep Soc **24**, 190-202.

Moran, M. D. 2003: Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos **100**, 403-405.

Nemec, V. & Stary, P. 1990: Sugars in honeydew. Biologia Bratislava **45**, 259-264.

Nonacs, P. & Dill, L. M. 1991: Mortality risk versus food quality trade-offs in ants: patch use over time. Ecol Entomol **16**, 73-80.

Oksanen, L. 2001: Logic of experiments in ecology: is pseudoreplication a pseudoissue? Oikos **94**.

Peterson, M. A. 1993: The nature of ant attendance and the survival of larval *Icaricia acmon* (Lycaenidae). J Lepid Soc **47**, 8-16.

Pierce, N. E. 1983: The ecology and evolution of the symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University.

-. 1987: The evolution and biogeography of associations between lycaenid butterflies and ants. Oxford Surveys in Evolutionary Biology **4**, 89-116.

-. 1989: Butterfly-ant mutualisms. In: Towards a more exact ecology (Grubb, P. J. & Whittaker, J. eds). Blackwell Scientific, Oxford. pp. 299-324.

Pierce, N. E., Braby, M. F., Huxley, C. R., Lohman, D. J., Mathew, J., Rand, D. B. & Travassos, M. A. 2002: The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol **47**, 733-771.

Pierce, N. E. & Elgar, M. A. 1985: The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. Behav Ecol Sociobiol **16**, 209-222.

Pierce, N. E. & Nash, D. R. 1999: The Imperial Blue, *Jalmenus evagoras* (Lycaenide). In: Biology of Australian Butterflies, Vol. 6 (Kitching, R. L., Scheermeyer, E., Jones, R. E. & Pierce, N. E. eds). CSIRO Publishing, Melbourne. pp. 279-317.

Portmann, M.-O., Serghat, S. & Mathlouthi, M. 1992: Study of some factors affecting intensity/time characteristics of sweetness. Food Chemistry **44**, 83-92.

Prince, J. S., LeBlanc, W. G. & Maciá, S. 2004: Design and analysis of multiple choice feeding preference data. Oecologia **138**, 1-4.

Rhodes, J. D., Croghan, P. C. & Dixon, A. F. G. 1997: Dietary sucrose and oligosaccharide synthesis in relation to osmoregulation in the pea aphid, *Acyrthosiphon pisum*. Physiol Entomol **22**, 373-379.

Ricks, B. L. & Vinson, S. B. 1970: Feeding acceptability of certain insects and various water-soluble compounds to two varieties of the imported Fire ant. J Econ Entomol **63**, 145-148.

-. 1972: Digestive enzymes of the imported Red Fire Ant, *Solenopsis richteri* (Hymenoptera: Formicidae). Ent exp & appl **15**, 329-334.

Romeis, J. & Wäckers, F. L. 2000: Feeding responses by female *Pieris brassicae* butterflies to carbohydrates and amino acids. Physiol Entomol **25**, 247-253.

Roubik, D. W., Yanega, D., Aluja, M., Buchmann, S. L. & Inouye, D. W. 1995: On optimal nectar foraging by some tropical bees. Apidologie **26**, 197-211.

Sachs, L. 1999: Angewandte Statistik. Springer, Heidelberg.

Schmidt, A. 1938: Geschmacksphysiologische Untersuchungen an Ameisen. Z Vergl Physiol **25**, 351-378.

Schmidt, D. J. & Rice, S. J. 2002: Association of ants with juvenile *Ogyris amaryllis amaryllis* (Hewitson) (Lepidoptera: Lycaenidae) in south-eastern Queensland. Aust J Entomol **41**, 164-169.

Seufert, P. & Fiedler, K. 1996a: The influence of ants on the patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a south-east Asian tropical rain forest. Oecologia **106**, 127-136.

-. 1996b: Life history diversity and local coexistence of three closely related lycaenid butterflies (Lepidoptera: Lycaenidae) in Malaysian rainforests. Zool Anz **234**, 229-239.

Seymour, A. S., Gutiérrez, D. & Jordano, D. 2003: Dispersal of the lycaenid *Plebejus argus* in response to patches of its mutualist ant *Lasius niger*. Oikos **103**, 162-174.

Skinner, G. J. 1980: The feeding habits of the wood-ant *Formica rufa* (Hymenoptera: Formicidae), in limestone woodland in north-west england. J Anim Ecol **49**, 417-433.

Smiley, J. T., Atsatt, P. R. & Pierce, N. E. 1988: Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. Oecologia **76**, 416-422.

Sorensen, A. A., Busch, T. M. & Vinson, S. B. 1983: Behaviour of workers subcastes in the Fire Ant, *Solenopsis invicta*, in response to proteinaceous food. Physiol Entomol **8**, 83-92.

Stadler, B., Kindlmann, P., Smilauer, P. & Fiedler, K. 2003: A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance. Oecologia **135**, 422-430.

Statsoft. 2003: Statistica 6.0. Statistica Inc, Tulsa.

Stradling, D. J. 1978: Food and feeding habits of ants. In: Production ecology of ants and termites (Brian, M. V. ed). Cambrigde University Press, Cambridge.

Strong, F. E. 1965: Detection of lipids in the honeydew of an aphid. Nature **205**, 1242.

Sudd, J. H. & Sudd, M. E. 1985: Seasonal change in the response of wood ants (*Formica lugubris*) to sucrose baits. Ecol Entomol **10**, 89-97.

Tateda, H. & Hidaka, I. 1966: Taste responses to sweet substances in rat. Mem Fac Sci Kyushu Univ - Series E **4**, 137-149.

Thomas, J. A. & Elmes, G. W. 2001: Food-plant niche selection rather than the presence of ant nests explains oviposition patterns in the myrmecophilous butterfly genus *Maculinea*. Proc R Soc Lond B **268**, 471-477.

Tinti, J.-M. & Nofre, C. 2001: Responses of the ant *Lasius niger* to various compounds perceived as sweet in humans: a structure-activity relationship study. Chem Senses **26**, 231-237.

van Dyck, H., Oostermeijer, J. G. B., Talloen, W., Feenstra, V., van der Hidde, A. & Wynhoff, I. 2000: Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? Proc R Soc Lond B **267**, 861-866.

Vander Meer, R. K., Lofgren, C. S. & Seawright, J. A. 1995: Specificity of the red imported fire ant (Hymenoptera: Formicidae) phagostimulant response to carbohydrates. Fla Entomol **78**, 144-154.

Vander Meer, R. K. & Merdinger, E. 1990: Fire Ant phagostimulants: laboratory and practical studies. Farmacia **38**, 93-102.

Vinson, S. B. 1968: The distribution of an oil, carbohydrate and protein food source to members of the Imported Fire Ant colony. J Econ Entomol **61**, 712-714.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M. W. & Hoffmann, K. H. 1999: Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia **118**, 483-491.

Wäckers, F. L. 2000: Do oligosaccharids reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect synthetized honeydew sugars. Oikos **90**, 197-201.

-. 2001: A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. J Insect Physiol **47**, 1077-1084.

Wada, A., Isobe, Y., Yamaguchi, S., Yamaoka, R. & Ozaki, M. 2001: Taste-enhancing effects of glycine on the sweetness of glucose: a gustatory aspect of symbiosis between the ant, *Camponotus japonicus*, and the larvae of the lycaenid butterfly, *Niphanda fusca*. Chem Senses **26**, 983-992.

Wheeler, D. E. 1994: Nourishment in ants: patterns in individuals and societies. In: Nourishment and Evolution in Insect Society (Hunt, J. H. & Nalepa, C. A. eds). Westview Press, Oxford & IBH Publishing Co Pvt Ltd, Boulder, New Delhi.

Wilkinson, T. L., Ashford, D. A., Pritchard, J. & Douglas, A. E. 1997: Honeydew sugars and osmoregulation in the Pea Aphid *Acyrthosiphon pisum*. J exp Biol **200**, 2137-2143.

Woodring, J., Wiedemann, R., Fischer, M. K., Hoffmann, K. H. & Völkl, W. in press: Honeydew amino acids in relation to sugars and their role in the establishment of ant-attendance hierarchy in eight species of aphids feeding on tansy (*Tanacetum vulgare*). Physiol Entomol.

Zoebelein, G. 1956: Der Honigtau als Nahrung der Insekten. Z Angew Entomol **39**, 129-167.

6 Benefits derived from larval nectar secretions of facultatively ant-associated lycaenid butterflies – does tending pay for ants?

H. Daniels* & K. Fiedler

Department of Animal Ecology I, University of Bayreuth, D-95440 Bayreuth, Germany

* *corresponding author*

6.1 Abstract

The energetic value of the nectar secretions from three facultatively myrmecophilous lycaenid species was calculated based on carbohydrate and amino acid profiles of their nectar secretions and improved measurements of secretion droplet sizes.

Polyommatus coridon nectar secretions have an energetic value of 1.0J/µl, *P. icarus* secretions 1.3J/µl and *Z. knysna* secretions 1.2J/µl, respectively.

Using own measurements of droplet sizes and published values of secretion rates the lifetime energetic value of secretions of one individual caterpillar tended from the third instar until pupation was estimated. One *P. coridon* caterpillar tended throughout would thus deliver 5.45-24.44J, one *P. icarus* caterpillar 1.92-14.23J and one *Z. knysna* caterpillar 0.72-2.76J, respectively.

Dissection and gravimetric analysis of caterpillars provided an estimate of the energetic value of caterpillar biomass for two of the species in different instars. Early *P. coridon* third instar have a caloric equivalent of 17.6J, moving prepupae of 656J. *Z. knysna* second instars have a caloric equivalent of 0.95J, third instars of 3.2J, and moving prepupae of 83.1J.

Thus preying on the caterpillars rather than harvesting their secretions would be of greater energetic benefit. These findings are compared with earlier models which are shown to have overestimated secretion quantity 2-4fold and energy benefit 4-28fold.

Based on studies of ant energetics possible benefits and cost for ant colonies and evolutionary implications for the stability of facultative ant-associations, which bear low costs for the lycaenid caterpillars, are discussed.

6.2 Introduction

Interspecific mutualisms are considered to be of universal importance and are extensively studied (Hoeksema & Bruna 2000). Although defined already in the 19th century (van Beneden 1875), mutualisms were for a long time considered as rare (e.g. Williams (1966)) or were at least ignored next to the big themes in ecology, viz. competition and predation (Boucher 1982). Structuring of communities was mainly ascribed to these antagonistic forces. But in the last decades mutualism more and more moved into the focus of the scientific community again. Mutualisms are now considered to be ubiquitous both evolutionary and geographically (Boucher 1982; Boucher 1985; Herre et al. 1999), and references therein), and of importance for the structure of ecological communities ((Hacker & Gaines 1997; Stachowicz 2001). Yet proving their existence is not that easy (Boucher 1982), especially if the currency for benefits and costs is different between the participating species (Addicott 1986).

Equally vexing is the question how (potentially) beneficial interactions between totally different species can evolve. Such mutualisms may often even involve organisms that usually would be expected to be antagonistic to each other. One example for the latter are the associations between many ant species and lycaenid butterfly larvae (DeVries 1991a; Fiedler 1991; Fiedler 2001; Pierce 1987; Pierce et al. 2002). Here, a butterfly larva, which is a ‘prototype’ of ant prey, cooperates with its potential predator organism. Solutions and theoretical frameworks describing how mutualisms might be possible were previously derived from game theory, especially the Prisoner’s dilemma and its iterated version (IPD) (Axelrod & Hamilton 1981; Trivers 1971, but see (Bendor & Swistak 1995; Boyd & Lorberbaum 1987), reviewed e.g. (Brembs 1996; Dugatkin 1997)). These models considered mainly the question: when does it pay to defect. Furthermore, they are limited to competition within the own class, any payments are fixed (Doebeli & Knowlton 1998), and at least originally active partner choice was not included (Bull & Rice 1991). Doebeli & Knowlton (1998) constructed the first PD model explicitly for mutualisms, and if competition occurs within different trader classes, mutualisms evolve instead of disruption of associations. The model introduces effects of space and shows therefore similarities to one other big class of models, the biological markets (Noe & Hammerstein 1994; Noe & Hammerstein 1995). Indeed, I would expect a convergence of these different model classes in the near future. Market models view mutualisms as interactions between different classes of traders which offer commodities, which are difficult or impossible to obtain for the other respective class. Specialization of the class on the own, easily obtained commodity pays (Schwartz & Hoeksema 1998), and eventually the commodities are traded according to the rules of supply and demand in analogy to human economics. Indeed, lycaenid-ant interactions are more complex than IPDs (Brembs 1996), and Hoeksema & Bruna (2000) even propose that the question is not whether an interaction is functioning as biological market, but rather the extent to which it is doing so.

To assess and prove that an interaction is a mutualism, benefits for both sides must be shown. Ant-lycaenid interactions are typically viewed as protection mutualism (Bronstein 2001), where the service of protection by the ants is rewarded with nutrition from the lycaenid caterpillars. Lycaenid-ant interactions actually can be neutral, commensalic, mutualistic, competitive, or even parasitic, although parasitic relationships are exceptionally rare (37 confirmed cases worldwide (Fiedler 1998), in a family of >4500 species (Ackery et al. 1999)). Nevertheless, most butterfly-ant associations are considered to be mutualistic (Fiedler 1991; Pierce 1987). The most important organ in this respect in the Lycaeninae is the dorsal nectar organ (Fiedler 1991; Fiedler & Maschwitz 1989a; Leimar & Axén 1993). This gland is situated dorsally on the seventh abdominal segment and, upon solicitation by the attendant ants, secretes a fluid (Malicky 1970) which the ants readily take up. The nutritive contents of these secretions were only poorly known (Cushman et al. 1994; DeVries 1988; Maschwitz et al. 1975; Nomura et al. 1992; Pierce 1983; Pierce & Nash 1999). Until recently (chapter 4), nectar secretion composition was almost exclusively known from obligately myrmecophilous species, i.e. lycaenid species that are almost always tended and experience a high mortality if deprived from their ants. While benefits and costs for the lycaenid side of the interaction were more often investigated (literature listed in Pierce et. al. 2002, p. 745), the ant side has less often been analysed. The attendant ants are rewarded with nectar secretions (Cushman et al. 1994; Fiedler & Hagemann 1995; Fiedler & Maschwitz 1988; Pierce et al. 1987), but few experimental studies have addressed whether such rewards really transfer into fitness benefits for the ants. Workers of some species do live longer, if access to larval nectar secretions is granted (Cushman et al. 1994; Fiedler & Saam 1995), and enhanced colony growth could be shown in one case, where caterpillars produce copious amounts of nectar (Nash 1989).

Here possible energetic benefits derived from the DNO secretions of facultatively myrmecophilous lycaenid species for tending ants are compared. Facultatively myrmecophilous lycaenid species are in most geographical regions and phylogenetic lineages far more numerous than obligately myrmecophilous species (Fiedler 1991; Fiedler 1997; Pierce et al. 2002), but the possible benefits for ants which tend those species are so far very poorly analysed and documented (Fiedler & Hagemann 1995; Fiedler & Maschwitz 1988; Fiedler & Saam 1995). As currency the energy content of these secretions was chosen. Energy gains of workers translate into energy gains for the whole colony. Possible benefits of mutualisms should ideally be expressed in offspring produced by the partners. Energetic equivalents reported for ant worker production in the literature were thus selected as exemplars to estimate possible benefits.

Nutrient contents of the nectar secretions of the lycaenid species *Polyommatus coridon*, *P. icarus* and *Zizeeria knysna* were used for the calculations. The former two species possess a functional DNO from the third instar onwards, and from then on they can initiate and maintain associations (Malicky 1969). Sometimes *Z. knysna* larvae do have a DNO already in the second instar (Clark & Dickson 1971, own observations), but it is unclear if this gland is already functional at that time. Third instar larvae of *Z. knysna* are definitely capable of nectar delivery.

6.3 Model

A comparative chemical analysis of the dorsal nectar gland secretions of the lycaenid species *Polyommatus coridon*, *P. icarus* and *Zizeeria knysna* provided values of mean sugar and amino acid content. For rearing methods and details of the chemical analysis see chapters 3.4 and 4. For sugars a physiological calorimetric value of 17.6kJ/g was assumed, for amino acids 17.8kJ/g, which accounts for the energy loss due to uric acid excretion (Schmidt-Nielsen 1997). This allowed to calculate the minimum energy content of the nectar secretions (Table 6.1). For *P. coridon* thus an energetic value of 1.01J/µl nectar secretion was obtained, for *P. icarus* 1.33J/µl and for *Z. knysna* 1.17J/µl were calculated.

Table 6.1 Calculated calorimetric value of nectar secretions of three facultatively ant-associated lycaenid species. The number of samples for chemical analysis of sugar and of amino acid content, respectively, in parentheses. ^{1}reared on *H. comosa* ^{2}median

species		mean total sugar content [g/l]	mean total amino acid content [g/l]	total calorimetric value [J] per µl secretion
P. coridon	(23/42)	47.38±19.28^1	9.68±3.39	1.01
P. icarus	(2/2)	74.2^2	1.2^2	1.33
Z. knysna	(8/6)	68.3±22.6	0.3±0.17	1.2

During harvesting of the nectar secretions droplet size was measured, too. Details are given in chapter 3.4. The mean droplet size in the first secretion bout at the initiation of an ant-association was in *P. coridon* 3.7nl (median, quartiles 1.8-6.2nl, n=91 caterpillars, fourth instar caterpillars up to non-feeding, still moving prepupae), in *P. icarus* it was 2.0nl (median, quartiles 0.6-3.6nl, n=13, late fourth instar caterpillars and moving prepupae), in *Z. knysna* 1.4nl (median, quartiles 1.0-2.3, n=72, fourth instar up to moving prepupae).

P. coridon does secrete 6.5-10.5droplets/h as fourth instar caterpillar and 21.5-29.5droplets/h as non-feeding prepupa ((Fiedler & Maschwitz 1989a) and revision in (Fiedler & Hagemann 1995). Third instars are assumed to secrete 25% of the quantity produced by older caterpillars. *P. coridon* (individuals of an artificial induced second generation) needed from hatching until pupation 38.5±1.9d (n=6) under a permanent temperature of 26°C (L:D 16:8) and 43.4±5.9d (n=15) under simulated day-night temperature change (26°C:15°C, L:D 16:8). Thus third and fourth instars were assumed to last two weeks each. This is comparable to the natural situation, where the first third instar caterpillars can be found in the midst of may and the last fourth instar caterpillars at the end of June (Pfeuffer 2000; SBN 1987), own observations), or the slightly shorter time periods for these instars in the closely related *P. bellargus* (Fiedler & Saam 1995). Duration of still-mobile prepupae was assumed as further three days (own observations). With the values for number of droplets secreted per hour as reported in the literature (Fiedler & Hagemann 1995; Fiedler & Maschwitz 1988), the total lifetime number of droplets under a tending regime of 8 hours per day or 24 hours per day respectively, and thus the total lifetime nectar secretion volume could be calculated. Multiplication of the total calorimetric value per µl secretion with these quantities resulted in a total calorimetric value of the lifetime secretion amount of 5.5-8.2J under eight hours tending or 16-24.4J under permanent tending (Table 6.2).

The fourth larval instar of *P. icarus* reared on *Lotus corniculatus* as natural foodplant lasts 5.4 days at 25°C and 16:8 L:D regime (Burghardt & Fiedler 1996a). Under realistic field conditions both the third and fourth instars lasts about one week (SBN 1987), K. Fiedler pers. comm.). Thus fourth and third instars were assumed as seven days, and prepupal duration is about half a day. Third instar caterpillars were assumed to exhibit 25% of the productivity of older caterpillars. Secretion rate per hour was taken from Burghardt & Fiedler (1996a), too. The energetic value of total lifetime secretion quantity was thus 1.9-5.5J under eight hours tending and 4.6-14.2J under continuous tending (Table 6.2).

Duration of the third instar of *Z. knysna* was assumed to be four days (Clark & Dickson 1971, own observations), with a nectar productivity of 25% of older caterpillars. Any possible nectar production in the second instar was ignored for the model here. Fourth instar was assumed to take five days (four days under favourable temperature conditions (own observations, Fiedler & Hagemann (1995), while Clark & Dickson (1971) report six days). Prepupal duration was further half a day. The number of droplets per hour was reported in Fiedler & Hagemann (1995) for different ant tending regimes. Hence, total calorimetric value of lifetime secretion amounts was 0.7-1.1J under eight hours tending, and 1.7-2.8J under permanent tending (Table 6.2).

Table 6.2 Estimated lifetime secretion amounts calculated from secretion rates (droplets/h) and droplet size, and resulting total calorimetric value of these secretions of three facultatively ant-associated lycaenid species a)fourth instar ß)non-feeding prepupae ?)lowest rate with normal survival, caterpillars reared on *S. varia* leaves d)highest rate, reared on *S. varia* flowers e)5 ants tending ?)15 ants tending a)this study b)Fiedler & Maschwitz 1988 c)Burghardt & Fiedler 1996a d)Fiedler & Hagemann 1995

species	mean droplet size [µl]	droplets per hour	estimated lifetime secretion amount [µl] (8h tending)	**total calori-metric value [J]**	estimated lifetime secretion amount [µl] (24h tending)	**total calori-metric value [J]**
P. coridon	$0.0037^{a)}$	$6.5\text{-}10.5^{a)\ b)d)}$	5.4-8.1	**5.45-8.18**	15.8-24.2	**15.96-24.44**
		$21.5\text{-}29.5^{ß)\ b)d)}$				
P. icarus	$0.002^{a)}$	$6.1^{?)}\text{-}21.3^{d)a)\ c)}$	1.4-4.2	**1.92-5.52**	3.4-10.7	**4.58-14.23**
		$36.9^{?)}\text{-}73.1^{d)ß)\ c)}$				
Z. knysna	$0.0014^{a)}$	$5.5^{e)}–9.5^{?)a)\ d)}$	$0.6^{e)}\text{-}0.9^{?)}$	**0.72-1.08**	$1.4^{e)}\text{-}2.3^{?)}$	**1.68-2.76**
		$16.5^{e)}\text{-}25.5^{?)ß)\ d)}$				

In order to assess the energetic value of larval biomass as nutrition for ants, fourth instar caterpillars of *P. coridon* and *Z. knysna* were weighed to the nearest 0.01mg (Sartorius MC 210P). After killing by short time freezing at –20°C the caterpillars were dissected in still frozen condition in small petri dishes. The body parts were separated into a cuticular fraction (cuticle, tarsae, remains of head capsule, transferred to another Petri dish) and a remaining fraction for which a potential usage by predacious ants can be assumed (all soft body parts, hemolymph). Both fractions were dried for three days at 60°C and weighed afterwards. This procedure allowed an estimate of the proportion of the caterpillar's body fresh weight potentially convertible to pure energy by the ants. In *P. coridon* therefore 9.5% (median, quartiles 7.8-10.9% n=4) of a caterpillar's fresh weight comprises digestible matter, for *Z. knysna* the respective fraction is 15.6% (median, quartiles 15.2-17.5% n=10).

As I did not have precise data for the mean fresh weight of early *P. coridon* third instar caterpillars, data from the very closely related *P. bellargus* was used (Saam 1993). Weights of prepupal *P. coridon* and of the various *Z. knysna* instars are based on own data. Lepidopteran caterpillars from several taxa have a calorimetric value of 23.15J per mg body weight (d.w.) (Winter 1972). Combining this figure with the values for the percentage of potentially digestible body parts the calorimetric value of the various caterpillars could be calculated. Early *P. coridon* third instar caterpillars thus have an estimated calorimetric value of 17.59J per individual, while for prepupae the figure is 655.97J. *Z. knysna* early second instar caterpillars yield potentially 0.95J, third instars 3.21J, and prepupae 83.13J per individual (Table 6.3).

Table 6.3 Estimated calorimetric value of different instars of three lycaenid species, *P. coridon* & *P. bellargus* digestible content as 9.5%, *Z. knysna* as 15.6% of fresh weight [a)]still moving, non feeding prepupae [b)]this study [c)]Saam 1993

Species	larval instar	fresh weight [mg]	n	**estimated calorimetric value** [J/individual]	± 1 standard deviation
P. bellargus	young 3rd	4.87±1.22[c]	46	**17.59**	(13.18-21.99)
P. coridon	moving prepupae[a]	181.64±26.25[b]	34	**655.97**	(561.18-750.77)
Z. knysna	young 2nd	0.43±0.21[b]	13	**0.95**	(0.48-1.4)
	young 3rd	1.46±0.57[b]	21	**3.21**	(1.96-4.46)
	moving prepupae[a]	37.8±4.46[b]	13	**83.13**	(73.32-92.94)

Thus energetically a third instar *P. coridon* caterpillar is worth more than the secretions it will deliver to the ants over its whole lifetime, if attended by ants. Only at the upper end of the calculated range of calorimetric values of the lifetime secretion amount, ants derive more energy from harvesting the nectar secretions than from preying on the caterpillar. Prepupae (and fourth instars as well) are energetically far more valuable as prey than the calorimetric value of the secretions they will deliver over the course of their remaining lifespan. In *Z. knysna* only the early second instar caterpillars are energetically worth less than the expected lifetime calorimetric value of the secretions. From early third instar caterpillars onwards more energetic value could be obtained by preying on caterpillars than they will (potentially) ever deliver through nectar secretions. Prepupae contain even markedly more energy in their biomass than the nectar secretions they will deliver in their remaining short lifespan.

6.4 Discussion

These model calculations show clearly that lycaenid caterpillars are energetically worth more than the nectar secretion amount they (will) deliver to the ants. Naturally, models depend fundamentally on the assumptions and values used for calculation. It is therefore important to assess how realistic the model parameters are. The values for droplet delivery per hour might be a slight overestimation, as caterpillars secrete less frequently and in smaller amounts over the course of a long-lasting ant-association (Axén et al. 1996; Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler et al. 1994; Leimar & Axén 1993). The droplet sizes used in the model are the mean over one bout (volume of bout/number of droplets), so these are also slightly overestimated. For the calculations in the models rather short larval durations were selected, to compensate for these possible overestimations. On the other hand the energy content of the nectar secretions themselves might be underestimated. Possibly not all sugars were identified or their content determined quantitatively. For example, erlose amount could not be determined in the HPLC procedure used for most samples (possibly occurring in one *P. icarus* and one *Z. knysna* nectar sample), but showed up in high quantity (32.6g/l, 27%) in an additional sample of *Z. knysna* (semi-synthetic diet under short day conditions, large individuals) analysed on a different HPLC equipment (R. Wiedemann, pers. comm.)). Thus total energy content of nectar might be slightly higher actually, than assumed in my models. Moreover, if proteins or lipids are contained in the nectar secretions, these contribute also to a higher calorimetric value than calculated here. As no precise data is available, any secretions from the pore cupola organs and the other setal structures are not taken into account. These might contribute some small amounts of nutrients and energy to ants (Pierce 1983), however. Estimation of the energetic value of caterpillar biomass includes the gut contents, as removal of these was not feasible. Therefore the digestible fraction of caterpillars might be overestimated. However, ants actually might also use pre-digested plant material. Overall, these over- and under estimations probably level each other out. Thus, for nectar secretions the calorimetric equivalents calculated (Table 6.1) reflect at least the order of magnitude correctly, whereas the estimates of the energetic values of lifetime secretion amount (Table 6.2) have a higher error. For example, nectar secretion rates of lycaenid caterpillars strongly depend on the number and identity of attendant ants, on the stability of the ant association, on the disturbance level of the larvae and other behavioural and ecological settings (Agrawal & Fordyce 2000; Axén 2000; Axén & Pierce 1998; Axén et al. 1996; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Leimar & Axén 1993). Comparison of earlier models (Fiedler & Hagemann 1995; Fiedler & Maschwitz 1988) with the values in this study (Table 6.1 and Table 6.2) shows, that these overestimated the lifetime secretion amounts by a factor of 2-4, and the energetic values, based on the study by Maschwitz et al. (1975), by a factor of 4-28.

The model presented here analyses only the energetic side of the association. Ant workers can therefore maximally harvest an energetic per capita value of nectar secretions of <24.4J from *P. coridon* caterpillars, <14.3J from *P. icarus* and <2.8J from *Z. knysna* caterpillars, respectively. *Lasius alienus*, an ant which tends *P. coridon* and *P. icarus*, produces one new worker ant at a cost of 4.9J. During June (soil temperature 18°C) a nest population of 13000 worker ants required 3260J/d or 0.25J/d per ant (Nielsen 1972; Peakin & Josens 1978). Thus, the nectar secretions of one *P. coridon* caterpillar tended from the third instar onwards, would cover the maintenance of 0.7-3.2 workers on each day throughout the whole tending period or the production of 1.1-5 new ants, depending on the estimate chosen from Table 6.1 and ignoring tending costs (see below). *P. icarus* nectar would allow daily maintenance of 0.5-3.9 workers or the production of 0.4-2.9 new workers, while nectar from *Z. knysna*, if tended throughout, would yield daily maintenance of 0.3-1.1 workers or the production of 0.1-0.5 new workers (Further calculations are given in Table 6.4). These calculated maintenance values for *P. icarus* are in good accordance with survival tests with ants (Fiedler & Saam 1995), where survival of two or less worker ants (*L. niger*, *L. flavus* and *M. rubra*) on *P. icarus* secretions as sole nutrition source was better than survival of five ants (*L. flavus*).

Table 6.4 Comparison of the benefits derived in number of ants maintained daily or number of newly produced ants for two species with the total energetic value of nectar secretions harvested over the whole secretion period of one caterpillar from three facultatively myrmecophilous lycaenid species. Further comparisons in the text. Secretion rate assumed as constant over whole period for sake of calculation simplicity 1: in June (Nielsen 1972; Peakin & Josens 1978) 2: (Brian 1973) (sucrose assumed as 17.6kJ/g: 0.39J/d for worker, 0.70J/d for queens)

	days harvesting possible	over this period continuous daily maintenance of a number of			total energetic value over whole tending period would allow production of a number of
species		*Lasius alienus* (18°C)[1]	*Myrmica rubra* (19°C)[2]		*Lasius alienus*[1]
		Workers	workers[2]	queens[2]	workers
P. coridon	31	0.7-3.2	0.5-2.0	0.3-1.1	1.1-5.0
P. icarus	14.5	0.5-3.9	0.3-2.5	0.2-1.4	0.4-2.9
Z. knysna	9.5	0.3-1.2	0.2-0.7	0.1-0.4	0.1-0.5

Using a general regression between ant size and energy consumption (Nielsen 1986), nectar secretions of one *P. coridon* caterpillar would be adequate to sustain a 1mg ant worker 6.3-28.3days (at 0.036J/h at 25°C), and *P. icarus* nectar secretions would suffice for 2.2-16.5days, *Z. knysna* nectar secretions for 20h-3.2days. Although the comparison of several energetic studies of ants has to be considered with caution (Nielsen 1986), and the physiological condition of workers depends also on the state of the colony (Peakin & Josens 1978), and references therein), these calculations show that secretions of a *P. coridon* caterpillar may contribute considerably to the nutritional uptake and production of ants, similarly, but to a smaller degree, also *P. icarus*. The contribution of *Z. knysna* is minute, but this species is normally tended by much smaller ant species such as *Tapinoma* and *Pheidole* species.

Of course, nectar secretions do not just provide energy like carbohydrates. Especially in *P. coridon* a rich mixture of amino acids is delivered (chapter 4). These might be physiologically more valuable than reflected just by their energetic equivalent. Nevertheless, if the ants would kill a caterpillar and feed its body which is rich in amino acids and fat to the brood their profit would be higher.

For a comprehensive cost-benefit analysis, the costs of tending are also to be taken into account. These are composed of the time and energy costs of locating a caterpillar for the first time, the actual travel costs from and back to the nest, and finally the costs of time and energy spent tending. There are surprisingly few studies which cover the ants' costs of the association with lycaenid caterpillars. *Iridomyrmex anceps* ants foraging on and tending the obligately myrmecophilous *Jalmenus evagoras* caterpillars for eight minutes at a distance of less than one meter from the nest at a temperature of 28°C are estimated to pay a cost of about 10mJ per whole trip (Nash 1989). This is small if compared to the energetic benefit harvested from *J. evagoras* secretions, but the purely energetic benefit/cost ratio of other sources, e.g. aphid honeydew is better (Degen et al. 1986).

Energetic costs of locomotion can be expressed as the gross cost of transport (COT), netto cost of transport (NCOT) or minimum cost of transport (MCOT) (Lighton et al. 1993). Only the last measure is independent of running speed and temperature, and allows therefore comparison between different groups of animals (Lighton & Feener 1989). The most ecological relevant measure would be COT. However, running speeds are species specific and temperature dependent, furthermore they decrease if more obstacles are encountered and with higher loads (Bartholomew et al. 1988; Fewell et al. 1996; Weier & Feener 1995). To calculate, which running distance and how many foraging trips would be possible with the nectar energy harvested from one caterpillar, MCOT values from different ant species were selected. MCOT was chosen for comparability and as load costs can be neglected (mean amount per caterpillar <1µl/d), although this slightly underestimates the actual costs (real trip cost: 5.3J, MCOT*running distance=4.1J in (Fewell et al. 1996)). As foraging distance two meters (linear distance without obstacles) was assumed, a typical foraging range for the European *Myrmica rubra* (Als et al. 2002) or *Lasius niger* (Savolainen & Vepsäläinen 1988). However, no locomotion cost determinations exist for typical attendant species of European Lycaenidae, therefore one large nectar harvester species with internal load carriage, one giant nectar harvester species with external load carriage and one large seed harvester species with highly efficient external load carriage were chosen (Table 6.5). The comparison shows that considerable distances can be run with the energy harvested from secretions of facultatively lycaenid caterpillars. Furthermore, smaller ants gain a greater benefit than larger ants with higher locomotion costs.

Table 6.5 Comparison of maximum travel distance and maximum number of foraging trips (2m single distance) possible for different ant species with the harvest of the daily mean nectar energy from one caterpillar of three different lycaenid species. 1: MCOT 158.2J*kg^{-1}*m^{-1} , 6.04mg, (Duncan & Lighton 1994). 2: MCOT 212.9J*kg^{-1}*m^{-1} , 155.1mg, (Fewell et al. 1996). 3: MCOT 158.0J*kg^{-1}*m^{-1}, 12.96mg, (Lighton et al. 1993)

ant species	type		*P. coridon*	*P. icarus*	*Z. knysna*
		mean energy harvest [J*d^{-1}]	0.2-0.8	0.1-1.0	0.1-0.3
Myrmecocystus mendax[1]	honeypot ant, internal nectar carriage	maximum distance [m]	188-827	136-1026	84-303
		number of trips	47-207	34-257	21-76
Paraponera clavata[2]	giant tropical ant, external nectar carriage	maximum distance [m]	5-24	4-30	2-9
		number of trips	1-6	1-7	0.6-2
Pogonomyrmex rugosus[3]	seed harvester efficient external carriage	maximum distance [m]	88-386	63-479	39-142
		number of trips	22-96	16-120	10-35

The calculations are necessarily coarse estimates, as caterpillars here are assumed to secrete equal amounts over the whole time, but prepupae have been shown to produce more secretions (Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995). The calculations were determined with one ant individual attending one caterpillar, but *P. coridon* often aggregates (Fiedler & Maschwitz 1989a, own observations), and all three lycaenid species normally are attended by several ant individuals simultaneously. At least in the laboratory situation a high number of ants are recruited to single and groups of *P. coridon* larvae over the duration of one hour (Fiedler & Maschwitz 1989a). Thus the harvest from the caterpillars would be divided upon more ants. The actual daily turnover rate of ant individuals at the caterpillars in the natural situation is unknown, therefore the number of movements back and forth from the nest exhibited by the ants are also unknown. The actual costs of movement might be higher due to obstacles, and especially in the confined space of the ants' nest (Vogt & Appel 2000).

Seed harvester ants are relatively effective load carriers (Fewell 1988; Lighton & Duncan 2002; Nielsen & Baroni-Urbani 1990; Weier & Feener 1995), therefore these ants are limited in time, while energetic costs of movement are relatively small, especially compared to the large energetic content of seeds. Nectarivorous species however, are less efficient load carriers, regardless of external or internal carriage (Duncan & Lighton 1994), therefore in these species energetic costs of movement play an important role, especially in the larger species (Fewell et al. 1996). For the typical attendant ant species of the lycaenids considered, no data are available, but surely energy and time spent on the movement and the tending must be taken into account. Especially owing to the lower secretion rates per unit time and caterpillar than in obligate myrmecophilous lycaenids, individual attending times of ants are most likely longer at facultatively myrmecophilous species. A further cost is the mortality risk of foraging ants, e.g. it was estimated as 6% for *Myrmica rubra* (Brian 1973). This risk is communicated along foragers in several ant species ((Nonacs & Dill 1991), and references therein) and patches are chosen accordingly. Therefore it may have little effect or is balanced against the benefit of the particular patch (Nonacs & Dill 1991), on the other hand, if ants are lost on the way or during tending, these would have to be subtracted from the gain in number of ants which can be produced with the energy gain from lycaenid secretions (Table 6.4).

In the models presented here, benefits accruing to ants were calculated for the "average" individuals of the three lycaenid species. To really assess and ultimately understand the ecology and evolution of these interactions the possible conditional outcomes have to be considered (Bronstein 1994). These depend here in particular on the density of caterpillars which are available and the size of the ant colony which attempts to harvest nutrients from these. The costs for tending ants may not be high, if these come from a colony with enough workers, so that some can be "spent" on rather unproductive tasks. In contrast small nests might not be able to afford the absence of workers. On the other hand the net reward is greater for smaller ant colonies and ant species which have lower upkeep costs. Minute ants, like *Plagiolepis* sp. can cover the requirements of the colony (<250 workers) with only harvesting *P. coridon* caterpillars (Fiedler & Maschwitz 1988).

The disparity between the energetic value of the nectar secretions possibly harvested in the future and the worth of the fourth instar caterpillar biomass is marked. This ultimately lends to the question, why do the ants not kill the caterpillar? Or at least do so in the prepupal phase, when the caterpillar has reached the maximum weight and therefore energy content and will spend yet greater droplets, but only for a short remaining time period? One possible mechanistic explanation is the unusual body form combined with an exceptionally thick cuticle, which makes it almost impossible for *Lasius niger* and smaller sized ants to kill *P. coridon* or *P. icarus* larvae (Malicky 1969; Malicky 1970). *Myrmica rubra* and *Lasius flavus* however, are sometimes able to kill *Z. knysna* ("accidents" during own observations) and predation of ants on lycaenid caterpillars has also been observed occasionally in many other species combinations (Fiedler et al. 1996; Malicky 1969). One further explanation are the semiochemicals probably derived from the pore cupolas and other setal structures on the caterpillar integument (Henning 1983; Malicky 1969). Also the eversible tentacle organs, which many myrmecophilous lycaenid caterpillars possess and evert during associations, serve to bind the ants (Axén et al. 1996; DeVries 1988; Fiedler & Maschwitz 1987). All these structures appease ants so that these view the caterpillar no longer as potential prey. Typically ants which tend caterpillars are a subset of those species that regularly collect liquid carbohydrate food like extrafloral nectar or Homopteran honeydew (DeVries 1991b; Eastwood & Fraser 1999; Fiedler 1991; Fiedler 2001). Specialized predators (with the possible exception of *Myrmecia* (Eastwood & Fraser 1999)) never exhibit tending behaviour, and also other ants with specialist feeding habits (seed harvester, fungus growers, inquiline social parasites) do not or very rarely engage in trophobiotic behaviours (Malicky 1969). Specialized secretion foragers among the Formicidae might be naturally less likely to exhibit predatory tendencies. The ants might furthermore not be able to escape a possible chemical fooling (Pierce 1987), although a specialized chemical manipulation is unlikely to occur in facultatively myrmecophilous species (see below).

The ants may not be maximizing foraging benefits, they might "just" take whatever is available or monopolize it due to competition with other ant colonies (Baroni-Urbani & Nielsen 1990), including nectar secretions from facultatively myrmecophilous lycaenid caterpillars, even if the benefits are rather small. Furthermore for the defence of resources often more ants are recruited, than would be optimal energetically (Dreisig 1988). Lycaenid caterpillars occurring in groups are known to save secretions if more ants are tending, because the amount of secretion spent per individual caterpillar decreases (Axén & Pierce 1998; Leimar & Axén 1993), as also the per capita visitation of the caterpillars decreases (Axén & Pierce 1998). Most facultatively myrmecophilous species do live solitary and might, as *P. icarus*, exhibit cannibalistic tendencies, whereas more strongly associated species like *P. coridon* often occur in aggregations (Fiedler & Maschwitz 1989a, own observations, chapter 2, Plate 1, E). Obligately myrmecophilous species often occur in highly clumped aggregations (Fiedler & Maschwitz 1989b; Pierce & Elgar 1985; Pierce et al. 1987; Seufert & Fiedler 1996). Thus aggregating might serve primarily as a strategy to enhance the profitability of the secretions for the ants, which was shown here to be not that high, and likewise reduce the per capita investment of the individual caterpillar. For European lycaenids a significant correlation between larval aggregation and myrmecophily could not be shown, but this might be due to a lack of sufficient number of highly myrmecophilous and aggregative species.

However, as the per capita secretion amount per ant worker is lower when tending an aggregation (Weith 1998), the ants do not endlessly increase the number of ants tending a trophobiotic aggregation (Breton & Addicott 1992; Fiedler & Maschwitz 1989a; Weith 1998), suggesting they enact some form of cost-control in these interactions. Nevertheless, even a single lycaenid caterpillar, especially if held in own earth pavilions (Fiedler & Maschwitz 1988; Pfeuffer 2000; Pierce 1983, own observations), might be a reliable and predictable source for the future (Pierce 1987), usable even, if for example foraging for prey outside is difficult in adverse weather conditions such as heavy rain. Furthermore, as tending can increase lycaenid population size (Pierce & Easteal 1986), ants as long-living superorganisms would then have a predictable food source in several years.

Obligately myrmecophilous lycaenid species are over-proportionally tended by the most dominant species within the nectar foragers (Eastwood & Fraser 1999). Shifts from subordinate to more dominant species can be observed, if a trophobiont increases its quality (Peterson 1993). In this regard, it is interesting that both *P. coridon* and *P. icarus* are frequently visited by *L. niger* and the closely related species *L. alienus*, *L. paralienus* and *L. platythorax* and by various species of the genus *Formica*. In xerothermic limestone slopes these ants usually dominate the ant community in Central Europe (compare (Andersen 1997; Mabelis 1984; Seifert 1996)). Thus the caterpillars of facultatively myrmecophilous species in Europe are highly attractive to ants. Almost all older *P. coridon* larvae in nature are tended by ants, and also *P. icarus* immatures are regularly found in company with ants (Ebert & Rennwald 1991; SBN 1987).

In conclusion, facultatively myrmecophilous lycaenid species exhibit not outright “fraud”, but nevertheless lend ants to engage in marginally beneficial activities. From the perspective of the lycaenid butterfly larva the very low lifetime investment into nectar secretions emphasizes that ant associations bear low costs, at least in facultatively myrmecophilous lycaenids. This also might explain the frequent occurrence of facultative lycaenid-ant interactions both taxonomically and geographically. The costs to the ants, on the other hand, are most likely small, as non-parasitic ant-associations are ancient (DeVries & Poinar 1997). In contrast specialized lycaenids, whose larvae act as parasites of ants are regarded as young clades in evolutionary time (Fiedler 1998) and possibly even evolutionary dead ends (Pierce 2001). One would also expect strong selection against these facultative associations if the costs for the ants were too high, unless the lycaenid caterpillars would have managed to break the ants’ communication code at a rather important and vulnerable point, like the imitation of brood pheromones. However, among the Lycaenidae brood odour mimicry is only known from a few parasitic and nest-inquiline species (Akino et al. 1999; Elmes et al. 2002; Henning 1983; Schlick-Steiner et al. 2004; Schönrogge et al. 2004). Such a chemical mimicry is not even to be expected in facultatively myrmecophilous lycaenid species, whose ant visitors are recruited from different ant genera and subfamilies.

6.5 References

Ackery, P. R., de Jong, R. & Vane-Wright, R. I. 1999: The butterflies: Hedyloidea, Hesperioidea and Papilionoidea. In: Lepidoptera, Moths and Butterflies, Vol. 1 (Kristensen, N. P. ed). W deGruyter, Berlin, New York. pp. 263-300.

Addicott, J. F. 1986: Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. Oecologia **70**, 486-494.

Agrawal, A. A. & Fordyce, J. A. 2000: Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B **267**, 1857-1861.

Akino, T., Knapp, J. J., Thomas, J. A. & Elmes, G. W. 1999: Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. Proc R Soc Lond B **266**, 1419-1426.

Als, T. D., Nash, D. R. & Boomsma, J. J. 2002: Geographical variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. Ecol Entomol **27**, 403-414.

Andersen, A. N. 1997: Functional groups and patterns of organization in North American ant communities: a comparison with Australia. J Biogeogr **24**, 433-460.

Axelrod, R. & Hamilton, W. D. 1981: The evolution of cooperation. Science **211**, 1390-1396.

Axén, A. H. 2000: Variation in behavior of lycaenid larvae when attended by different ant species. Evol Ecol **14**, 611-625.

Axén, A. H. & Pierce, N. E. 1998: Aggregation as a cost-reducing strategy for lycaenid larvae. Behav Ecol **9**, 109-115.

Axén, H. A., Leimar, O. & Hoffman, V. 1996: Signalling in a mutualistic interaction. Anim Behav **52**, 321-333.

Baroni-Urbani, C. & Nielsen, M. G. 1990: Energetics and foraging behaviour of the European seed harvesting ant *Messor capitatus* II. Do ants optimize their harvesting? Physiol Entomol **15**, 449-461.

Bartholomew, G. A., Lighton, J. R. B. & Feener, D. H. j. 1988: Energetics of trail running, load carriage, and emigration in the column-raiding army ant *Eciton hamatum*. Physiol Zool **61**, 57-68.

Bendor, J. & Swistak, P. 1995: Types of evolutionary stability and the problem of cooperation. Proc Natl Acad Sci USA **92**, 3596-3600.

Boucher, D. H. 1982: The ecology of mutualism. Ann Rev Ecol Syst **13**, 315-347.

-. 1985: The idea of mutualism, past and future. In: The Biology of Mutualism - Ecology and Evolution (Boucher, D. H. ed). Crown Helm, London. pp. 1-28.

Boyd, R. & Lorberbaum, J. P. 1987: No pure strategy is evolutionary stable in the repeated Prisoner's Dilemma game. Nature **327**, 58-59.

Brembs, B. 1996: Chaos, cheating and cooperation: potential solutions to the prisoner's dilemma. Oikos **76**, 14-24.

Breton, L. M. & Addicott, J. F. 1992: Density-dependent mutualism in an aphid-ant interaction. Ecology **73**, 2175-2180.

Brian, M. V. 1973: Feeding and growth in the ant *Myrmica*. J Anim Ecol **42**, 37-53.

Bronstein, J. L. 1994: Conditional outcomes in mutualistic interactions. Trends Ecol Evol **9**, 214-217.

-. 2001: The exploitation of mutualism. Ecol Lett **4**, 277-287.

Bull, J. J. & Rice, W. R. 1991: Distinguishing mechanisms for the evolution of co-operation. J theor Biol **149**, 63-74.

Burghardt, F. & Fiedler, K. 1996a: The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). Ecol Entomol **21**, 1-8.

-. 1996b: Myrmecophilous behaviours in caterpillars of the butterfly, *Polyommatus icarus* (Rottemburg, 1775): temporal patterns and age dependency (Lepidoptera: Lycaenidae). Nota lepid **19**, 212-229.

Clark, G. C. & Dickson, C. G. C. 1971: Life histories of South African lycaenid butterflies. Purnell, Cape Town.

Cushman, J. H., Rashbrook, V. K. & Beattie, A. J. 1994: Assessing benefits to both participants in a lycaenid-ant association. Ecology **75**, 1031-1041.

Degen, A. A., Gersani, M., Avivi, Y. & Weisbrot, N. 1986: Honeydew intake of the weaver ant *Polyrhachis simplex* (Hymenoptera: Formicidae) attending the aphid *Chaitophorous populialbae* (Homoptera: Aphididae). Insectes Sociaux **33**, 211-215.

DeVries, P. J. 1988: The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants. Zool J Linnean Soc **94**, 379-393.

-. 1991a: Evolutionary and ecological patterns in myrmecophilous ridodinid butterflies. In: Ant plant interactions (Huxley, C. R. & Cutler, D. F. eds). Oxford University Press, Oxford. pp. 143-171.

-. 1991b: Mutualism between *Thisbe irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. Biol J Linnean Soc **43**, 179-195.

DeVries, P. J. & Poinar, G. O. 1997: Ancient butterfly ant symbiosis: direct evidence from Dominican amber. Proc R Soc Lond B **264**, 1137-1140.

Doebeli, M. & Knowlton, N. 1998: The evolution of interspecific mutualism. Proc Natl Acad Sci USA **95**, 8676-8680.

Dreisig, H. 1988: Foraging rate of ants collecting honeydew or extrafloral nectar, and some possible constraints. Ecol Entomol **13**, 143-154.

Dugatkin, L. A. 1997: Cooperation among animals. Oxford University Press, New York.

Duncan, F. D. & Lighton, J. R. B. 1994: The burden within: the energy cost of load carriage in the honeypot ant, *Myrmecocystus*. Physiol Zool **67**, 190-203.

Eastwood, R. & Fraser, A. M. 1999: Associations between lycaenid butterflies and ants in Australia. Aust Ecol **24**, 503-537.

Ebert, G. & Rennwald, E. 1991: Die Schmetterlinge Baden-Württembergs. Eugen Ulmer Verlag, Stuttgart.

Elmes, G. W., Akino, T., Thomas, J. A., Clarke, R. T. & Knapp, J. J. 2002: Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. Oecologia **130**, 525-535.

Fewell, J. H. 1988: Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. Behav Ecol Sociobiol **22**, 401-408.

Fewell, J. H., Harrison, J. F., Lighton, J. R. B. & Breed, M. D. 1996: Foraging energetics of the ant, *Paraponera clavata*. Oecologia **105**, 419-427.

Fiedler, K. 1991: Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionidae). Bonner Zoologische Monographien **31**.

-. 1997: Geographical patterns in life-history trait of Lycaenidae butterflies - ecological and evolutionary implications. Zoology **100**, 336-347.

-. 1998: Lycaenid-ant interactions of the *Maculinea* type: tracing their historical roots in a comparative framework. J Insect Conservation **2**, 3-14.

-. 2001: Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. Divers Distrib **7**, 45-60.

Fiedler, K. & Hagemann, D. [1995]: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K., Hölldobler, B. & Seufert, P. 1996: Butterflies and ants: the communicative domain. Experientia **52**, 14-24.

Fiedler, K. & Hummel, V. 1995: Myrmecophily in the brown argus butterfly, *Aricia agestis* (Lepidoptera: Lycaenidae): Effects of larval age, ant number and persistence of contact with ants. Zoology **99**, 128-137.

Fiedler, K. & Maschwitz, U. 1987: Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) III. New aspects of the function of the retractile tentacular organs of lycaenid larvae. Zool Beitr N F **31**, 409-416.

-. 1988: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) II. Lycaenid larvae as trophobiotic partners of ants - an quantitative approach. Oecologia **75**, 204-206.

-. 1989a: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. Ethology **80**, 71-80.

-. 1989b: The symbiosis between the weaver ant, *Oecophylla smaragdina* and *Anthene emolus*, an obligate myrmecophilous butterfly. J Nat Hist **23**, 833-846.

Fiedler, K. & Saam, C. 1995: Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study. Oecologia **104**, 316-322.

Fiedler, K., Schurian, K. G. & Hahn, M. 1994: The life history and myrmecophily of *Polyommatus candalus* (Herrich-Schäffer) from Turkey (lep., Lycaenidae). Linn Belg **14**, 315-332.

Hacker, S. D. & Gaines, S. D. 1997: Some implications of direct positive interactions for community species diversity. Ecology **78**, 1990-2003.

Henning, S. F. 1983: Chemical communication between lycaenid larvae (Lepidoptera: Lycaenide) and ants (Hymenoptera: Formicidae). J ent Soc sth Afr **46**, 341-366.

Herre, E. A., Knowlton, N., Mueller, U. G. & Rehner, S. A. 1999: The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol Evol **14**, 49-53.

Hoeksema, J. D. & Bruna, E. M. 2000: Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. Oecologia **125**, 321-330.

Leimar, O. & Axén, A. H. 1993: Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav **46**, 1177-1182.

Lighton, J. R. B. & Duncan, F. D. 2002: Energy cost of locomotion: validation of laboratory data by in-situ respirometry. Ecology **83**, 3517-3522.

Lighton, J. R. B. & Feener, D. H. j. 1989: A comparison of energetics and ventilation of desert ants during voluntary and forced locomotion. Nature **342**, 174-175.

Lighton, J. R. B., Weier, J. A. & Feener, D. H. j. 1993: The energetics of locomotion and load carriage in the desert harvester ant *Pogonomyrmex rugosus*. J exp Biol **181**, 49-61.

Mabelis, A. A. 1984: Interference between Wood Ants and Other Ant Species (Hymenoptera Formicidae). Neth J Zool **34**, 1-20.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

-. 1970: New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymneoptera). J Lep Soc **24**, 190-202.

Maschwitz, U., Wüst, M. & Schurian, K. 1975: Bläulingsraupen als Zuckerlieferanten. Oecologia **18**, 17-21.

Nash, D. R. 1989: Cost-benefit analysis of a mutualism between lycaenid butterflies and ants. Ph.D. thesis, University of Oxford, Oxford.

Nielsen, M. G. 1972: An attempt to estimate energy flow through a population of workers of *Lasius alienus* (Först) (Hymenoptera: Formicidae). Natura Jutlandica **16**, 97-107.

-. 1986: Respiratory rates of ants from different climatic habitats. J Insect Physiol **32**.

Nielsen, M. G. & Baroni-Urbani, C. 1990: Energetics and foraging behaviour of the European seed harvesting ant *Messor capitatus* I. Respiratory metabolism and energy consumption of unloaded and loaded workers druing locomotion. Physiol Entomol **15**, 441-448.

Noe, R. & Hammerstein, P. 1994: Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behav Ecol Sociobiol **35**, 1-11.

-. 1995: Biological markets. Trends Ecol Evol **10**, 336-339.

Nomura, K., Hirukawa, N., Yamaoka, R. & Imafuku, M. 1992: Problems on the symbiosis between the lycaenid butterfly larva, *Niphanda fusca shijimia* and the ant *Camponotus* (*Camponotus*) *japonicus*. Tyo to Ga **43**, 138-143.

Nonacs, P. & Dill, L. M. 1991: Mortality risk versus food quality trade-offs in ants: patch use over time. Ecol Entomol **16**, 73-80.

Peakin, G. J. & Josens, G. 1978: Respiration and energy flow. In: Production ecology of ants and termites (Brian, M. V. ed). Cambridge University Press, Cambridge. pp. 111-164.

Peterson, M. A. 1993: The nature of ant attendance and the survival of larval *Icaricia acmon* (Lycaenidae). J Lepid Soc **47**, 8-16.

Pfeuffer, E. 2000: Zur Ökologie der Präimaginalstadien des Himmelblauen Bläulings (*Lysandra bellargus* ROTTEMBURG 1775) und des Silbergrünen Bläulings (*Lysandra coridon* PODA 1761), unter besonderer Berücksichtigung der Myrmekophilie. Ber Naturwiss Ver Schwaben **104**, 72-98.

Pierce, N. E. 1983: The ecology and evolution of the symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University.

-. 1987: The evolution and biogeography of associations between lycaenid butterflies and ants. Oxford Surveys in Evolutionary Biology **4**, 89-116.

-. 2001: Peeling the onion: Symbioses between ants and blue butterflies. In: Model Systems in Behavioral Ecology (Dugatkin, L. A. ed). Princeton University Press, Princeton. pp. 41-56.

Pierce, N. E., Braby, M. F., Huxley, C. R., Lohman, D. J., Mathew, J., Rand, D. B. & Travassos, M. A. 2002: The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol **47**, 733-771.

Pierce, N. E. & Easteal, S. 1986: The selective advantage of attendant ants for the larvae of a lycaenid butterfly, *Glaucopsyche lygdamus*. J Anim Ecol **55**, 451-462.

Pierce, N. E. & Elgar, M. A. 1985: The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. Behav Ecol Sociobiol **16**, 209-222.

Pierce, N. E., Kitching, R. L., Buckley, R., Taylor, M. F. J. & Benbow, K. F. 1987: The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. Behav Ecol Sociobiol **21**, 237-248.

Pierce, N. E. & Nash, D. R. 1999: The Imperial Blue, *Jalmenus evagoras* (Lycaenide). In: Biology of Australian Butterflies, Vol. 6 (Kitching, R. L., Scheermeyer, E., Jones, R. E. & Pierce, N. E. eds). CSIRO Publishing, Melbourne. pp. 279-317.

Saam, C. 1993: Untersuchungen zum Einfluß der Ameisenassoziationen auf die Individualentwicklung einiger europäischer Bläulingsarten. Diploma thesis, Bayer. Julius-Maximilians-Universität, Würzburg.

Savolainen, R. & Vepsäläinen, K. 1988: A competition hierarchy among boreal ants: impact on resource partitioning and community structure. Oikos **51**, 135-155.

SBN. 1987: Tagfalter und ihre Lebensräume. Schweizerischer Bund für Naturschutz. Fotorotar AG Egg/ZH, Basel.

Schlick-Steiner, B. C., Steiner, F. M., Höttinger, H., Nikiforov, A., Mistrik, R., Schafellner, C., Baier, P. & Christian, E. 2004: A butterfly's chemical key to various ant forts: intersection-odour or aggregate-odour multi-host mimicry? Naturwissenschaften, **91**, 209-214.

Schmidt-Nielsen, A. 1997: Animal physiology. Cambridge University Press, Cambridge.

Schönrogge, K., Wardlaw, J. C., Peters, A. J., Everett, S., Thomas, J. A. & Elmes, G. W. 2004: Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. J Chem Ecol **30**, 91-107.

Schwartz, M. W. & Hoeksema, J. D. 1998: Specialization and resource trade: biological markets as a model of mutualisms. Ecology **79**, 1029-1038.

Seifert, B. 1996: Ameisen: beobachten, bestimmen. Naturbuch Verlag, Augsburg.

Seufert, P. & Fiedler, K. 1996: The influence of ants on the patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a south-east Asian tropical rain forest. Oecologia **106**, 127-136.

Stachowicz, J. J. 2001: Mutualism, facilitation and the structure of ecological communities. Bioscience **51**, 235-246.

Trivers, R. L. 1971: The evolution of reciprocal altruism. Q Rev Biol **46**, 35-57.

van Beneden, P. J. 1875: Les Commensaux et les parasites. Biblio Sci Int, Paris.

Vogt, J. T. & Appel, A. G. 2000: Metabolic cost of spontaneous movement in the red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology **35**, 89-98.

Weier, J. A. & Feener, D. H. j. 1995: Foraging in the seed harvester ant genus *Pogonomyrmex*: are energy costs important? Behav Ecol Sociobiol **36**, 291-300.

Weith, W. 1998: Mutualismen von Bläulingen (Lepidoptera: Lycaenidae) und Ameisen (Hymenoptera: Formicidae). Diploma thesis, Universität Bayreuth, Bayreuth.

Williams, G. C. 1966: Adaptation and natural selection. University Press, Princeton.

Winter, K. 1972: Zum Energieumsatz phytophager Insekten im Buchenwald. Untersuchungen an Lepidopterenpopulationen. Ph.D. thesis, Georg August Universität, Göttingen.

7 Synopsis

H. Daniels

Department of Animal Ecology I, University of Bayreuth, D-95440 Bayreuth, Germany

The significance of mutualism for the structuring of ecological communities (e.g. Hacker & Gaines 1997; Stachowicz 2001) has been largely ignored for a long time, as opposed to antagonistic interactions such as competition and predation. However, mutualisms are ubiquitous both in evolutionary and geographical terms (Boucher 1982; Boucher 1985; Bronstein 1998; Herre et al. 1999, and references therein). Ants as an important selective force in terrestrial ecosystems interact in a mutualistic manner with a wide array of organisms, like plants, fungi and trophobiotic insects (Hölldobler & Wilson 1990). Trophobionts generally comprise a vast number of honeydew-producing homopteran species, but also the nectar-secreting caterpillars of many lycaenid and riodinid butterfly species. Caterpillar-ant associations might be myrmecoxenous, i.e. the caterpillars are largely ignored and not preyed upon by the ants. Myrmecoxenous interactions are not mutualistic and clearly show asymmetries with regard to the cost-benefit balance, since only the butterfly larvae gain advantages through fraud and manipulation. Truly myrmecophilous associations may be facultative or obligate, depending on the butterfly species. Obligately myrmecophilous species among lycaenids essentially require specific ants for their survival, be it as protectors against enemies or as a food source. These associations are also highly asymmetric, as the ants do not depend on the caterpillars. Obligate butterfly-ant associations are characterised by a high degree of specificity and intimacy, accompanied by numerous behavioural specialisations of the caterpillars and frequently also the pupae and adult butterflies. Facultatively myrmecophilous lycaenid species do not depend on their association with ants. Thus such relationships are less asymmetric and less specific (Fiedler 2001). Nevertheless, with regard to the intensity of associations a broad variability can be observed in this class of interactions. The mechanisms underlying this variation had been unclear at the onset of this thesis work. Central for (potentially) mutualistic caterpillar-ant interactions is the dorsal nectar organ of the butterfly larvae (Fiedler & Maschwitz 1989a). One might therefore expect that nectar composition would contribute to explaining differences in the intimacy and specificity of facultative caterpillar-ant interactions, where the unpredictability of partner combinations selects against the evolution of species-specific mimics of chemical signals (in contrast to parasitic lycaenid-ant associations (Akino et al. 1999; Henning 1983)).

The first major obstacle to surmount was the collection of sufficiently large nectar samples for precise chemical analysis. As caterpillars have strong control over their secretion behaviour various attempts to artificially stimulate nectar secretion failed (**chapter 3.4)**. Although caterpillars belonging to facultatively myrmecophilous species cannot predict which ant species out of a large set of potential visitors might eventually interact with them, they nevertheless do employ some kind of ‘ant’ template. Lycaenid larvae are very rarely fooled by non-ant arthropods (Downey 1965; Gilbert 1976; Schurian et al. 1993), and in most cases they obviously recognize that the stimulation is not caused by an ant. One attribute lacking in the experiments to stimulate nectar delivery might have been the vibration caused by ant movements in the ground or the air. I repeatedly observed *Polyommatus coridon* larvae to evert their tentacle organs at the moment when ants were placed several centimetres (>6cm) behind them. Immatures of some lycaenid species are known to communicate with ants by means of substrate borne vibrations, although the mechanisms behind and the significance of this phenomenon are still largely unclear. Pupae and caterpillars of myrmecophilous species can enhance ant tending by sound production (DeVries 1990; DeVries 1991; Downey 1966; Pierce et al. 2002), and references therein). Possibly call production might be universal in the Lycaenidae, since the phenomenon also occurs among pupae and larvae of myrmecoxenous lycaenid species (Pierce et al. 2002), however only infrequently in myrmecoxenous riodinid larvae (DeVries 1991). Vibratory capacities might originally have evolved as defence and only subsequently became integrated into communicative function for ant association (Fiedler 1992). The functions are still inconclusive (Travassos & Pierce 2000), and the vibratory capacity might serve more for enhancing and maintaining an ant association than initiating it (Fiedler et al. 1996). Although the acoustic signals of ants, which serve a variety of functions (Baroni-Urbani et al. 1988; Hölldobler & Wilson 1990; Roces et al. 1993), and lycaenids are similar, they lack fine level similarity even in highly specialized parasitic lycaenid species (DeVries et al. 1993). This issue was not further analysed in this thesis.

In an attempt to avoid the dependence on tending-motivation by the ants in the laboratory assays, and to further characterise the antennal drumming behaviours of ants tending lycaenid caterpillars, an “artificial ant” (**chapter 3.5**) was developed. An ‘astable multivibrator’ consisting of one timer element and an array of resistors, generated defined switching patterns in a common magnetic relay. A silicone tube with a humane hair glued to the end was fixed at the relay and thus exhibited a regular swinging movement. This termed out to the best artificial effector at the caterpillars’ integument so far. The “artificial ant” was sometimes sufficient to elicit secretion behaviour, and this approach might be worth testing with obligately myrmecophilous lycaenid species to obtain more information regarding the releasers of secretion behaviour.

In order to rear large numbers of caterpillars of the strongly myrmecophilous lycaenid *P. coridon*, which as univoltine species obligately undergoes egg diapause throughout the winter and therefore was available as caterpillar for a short time-span only, a method to break the diapause was established (**chapter 3.3**). The success rate of 60-70% larvae which hatch subitaneously at a moderate work effort demonstrated that the normally strictly univoltine *P. coridon* has the potential to develop without diapause.

For the purpose of the present study the only successful way of obtaining sufficient nectar samples was to milk caterpillars with a microcapillary while being attended by *Lasius flavus* ants. Despite many methodological improvements detailed in **chapter 3.4** this method remained extremely time-consuming. For comparative nectar analyses one strongly myrmecophilous species was used, the European *P. coridon*, and two moderately myrmecophilous species, the Palaearctic *P. icarus* and the (sub-)tropical *Z. knysna*. Both *Polyommatus* species are rather closely related (Wiemers 2003), whereas *Z. knysna* is a far more distant relative (Eliot 1973; Scott & Wright 1990). All three species secreted only minute droplets form their dorsal nectar glands. The mean droplet size was 3.7nl in *P. coridon*, 2nl in *P. icarus* and only 1.4nl in *Z. knysna*. Due to the low secretion rates collating a single sample thus required up to eight weeks in the less strongly myrmecophilous species.

Comparative chemical analyses of the nectar secretions of the three facultatively myrmecophilous lycaenid species by means of HPLC (**chapter 4**) showed that the secretions contain substantial amounts of nutrients like sugars and amino acids. The food sources available to the caterpillars had an impact on the number of amino acids in *S. varia* reared *P. coridon* caterpillars, and on the homogeneity of the amino acid pattern and the sucrose content in *P. coridon*. Rearing the caterpillars on a semi-synthetic diet with defined homogenous composition resulted in nectar secretions of homogenous composition. As it was also most rich in nutrients and water, the semi-synthetic diet ‘allowed’ for a higher sucrose content in the nectar. But overall no ‘dramatic’ influence of larval food on nectar quality could be observed. Thus the secretion composition seems to be relatively independent of the larval food source, as long as no qualitatively very poorly or very richly food is presented (Burghardt & Fiedler 1996a; Fiedler 1990). In the obligately myrmecophilous lycaenid *Jalmenus evagoras* higher larval attendance was shown on fertilised plants compared to caterpillars reared on unfertilised plants (Baylis & Pierce 1991). Extending from the results of the chemical analyses presented here, it would be interesting to test if the higher sugar concentration in nectar secretions of *P. coridon* caterpillars fed semi-synthetic diet would translate into a preference by attendant ants compared to *P. coridon* larvae reared on *S. varia*.

The effects of larval food on lycaenid nectar were thus not very pronounced. This is in contrast with the Homoptera, where effects of different host plants or artificial diets on honeydew composition have been demonstrated (concentration of sugars (Byrne et al. 2003; Febvay et al. 1999; Fischer & Shingleton 2001; Hendrix et al. 1992) and amino acids (Febvay et al. 1999)).

It was proposed that within the Lycaenidae myrmecophilous species would preferentially feed on protein rich plants, particularly on nitrogen-fixing plant species (Pierce 1985; Pierce & Elgar 1985). In particular it has been argued that more strongly ant-associated species secrete rich amino acid mixtures (Pierce 1983), richness in three species later shown by (Cushman et al. 1994; DeVries 1988; Nomura et al. 1992). Thus the need as to meet their own developmental requirements and to simultaneously synthesize high quality nectar should then select for utilization of host plants of the highest possible quality (Baylis & Pierce 1993), (except the species in the small group of entomophagous species, review in Cottrell (1984)). However, the widespread affiliation of many lycaenid species with legumes might also reflect an ancestral host plant use (Fiedler 1991). Furthermore, the connection between attractiveness of myrmecophilous species and legume feeding is not as straightforward as assumed (Fiedler 1995; Fiedler 1996; Fiedler & Saam 1994). The experiments presented in this thesis revealed only subtle nectar variation in *P. coridon* mediated by larval food. This suggests that, at least in facultatively myrmecophilous lycaenids, food quality may not be of major importance in maintaining the ant mutualism, unless host plants of extremely low quality are offered (e.g. (Burghardt & Fiedler 1996a; Fiedler 1990). Moreover, host plant quality might affect the quantity of nectar secretions more strongly than nectar quality.

Of the three examined species *Polyommatus coridon* is most strongly associated with ants and these larvae secrete, as expected, a mixture of amino acids (**chapter 4**). However, the total amino acid content in *P. coridon* nectar was the highest thus far recorded in any lycaenid species, even higher than in obligately myrmecophilous species. This surprisingly rich mixture was an unexpected result. The two obligate ant mutualists *Paralucia aurifera* and

Jalmenus evagoras secrete more sugar than *P. coridon* in their nectar (Cushman et al. 1994; Pierce 1983; Pierce & Nash 1999), and therefore offer energetically more attractive rewards. The ant-parasitic *Niphanda fusca* was comparable to *P. coridon* in sugar and inferior in amino acid content of larval nectar (at least concerning the report by Nomura et al. 1992). In this case however, manipulative signals from the caterpillar can be assumed to exist, thus releasing it from the obligation to present an extremely appealing nectar secretion in order to ensure high attention by its host-ant (*Camponotus japonicus*). The other two species examined here, *P. icarus* and *Z. knysna*, spend considerably less amino acids, both in quantity and diversity. The amino acid pattern is relatively similar in the two closely related *Polyommatus* species. However, this species similarity is not mirrored in the sugar pattern, where the two less strongly ant-associated, but more distantly related, species *P. icarus* and *Zizeeria knysna* are more similar to each other. Even though data on lycaenid nectar composition remain sparse, it appears that these secretions reflect both, phylogenetic relatedness (similarities in amino acid patterns within *Polyommatus*) and ecological context (host plant effects, sugar composition in less strongly ant-associated species).

Overall nectar of myrmecophilous lycaenid species (**chapter 4**, **Table 4.3**) contains about 5-10% sugar. A possible exception is the Australian obligate ant-mutualist *Paralucia aurifera* (Cushman et al. 1994), but the unusually high sugar content reported in that study might also hint to a lack of control with regard to evaporation. No details are given as to how the nectar of *P. aurifera* had been harvested. The amino acid content of nectar seems to be determined by the intimacy of associations with attendant ants, more strongly myrmecophilous species providing a richer mixture. An extreme example of high amino acid content is provided by the riodinid *Thisbe irenea* (DeVries 1988). Examination of additional facultatively ant-associated species and further (re-)examinations of the secretions of obligately ant-associated species (including ant-parasites like *Maculinea* or *Lepidochrysops*) with modern HPLC technique might be rewarding, to corroborate or reject this pattern. Nectar of the strong, but facultative myrmecophilous *P. coridon* is not qualitatively inferior to nectar secretions of obligate and specific myrmecophiles in the Lycaenidae. Thus the main difference between strong facultative and obligate myrmecophiles might rest only on the secretion rates of the particular species. Obligately myrmecophilous species exhibit a much higher secretion rate (Fiedler & Maschwitz 1989b; Pierce 1983; Pierce & Nash 1999; Seufert & Fiedler 1996a; Seufert & Fiedler 1996b) compared to facultatively myrmecophilous species (Fiedler & Hagemann 1995; Fiedler & Maschwitz 1988; Fiedler & Saam 1994). Also in aphids the honeydew excretion rate was more important than a higher nutrient content (Völkl et al. 1999). Different ant species can elicit variable secretion rates in lycaenids, and secretion rates are also highly plastic within species according to larval age, host plant quality and density of ant mutualists available (Agrawal & Fordyce 2000; Axén 2000; Axén & Pierce 1998; Axén et al. 1996; Burghardt & Fiedler 1996b; Fiedler & Hagemann 1995; Fiedler & Hummel 1995; Fiedler et al. 1994; Leimar & Axén 1993). As a result, lycaenid larvae might regulate the size of their ant guard depending on the ecological and behavioural context (discussion in Axén (2000).

Tests of the nectar components with ant species from three different subfamilies showed (**chapter 5**) that rich mixtures of amino acids are more attractive than pure sucrose solution, especially if the contents are balanced. Due to the tremendous species-specific differences in gustatory preferences among the Formicidae (Knight & Rust 1991), no *universal* amino acid key compound seems to exits. For certain species such ‘key compounds’ apparently exist, e.g. leucine for *Lasius niger* (this study) and *Solenopsis* (Ricks & Vinson 1970), phenylalanine for *Myrmica rubra* (this study) and *Oecophylla smaragdina* (Blüthgen & Fiedler 2004), or glycine for *Camponotus japonicus* (Wada et al. 2001). Amino acids often loose their attractiveness if their concentration exceeds a certain level. Furthermore preferences of ants are also influenced by the actual requirements of a colony, and preferences for particular amino acids can decrease if they are offered in high concentrations over several days (Blüthgen & Fiedler 2004). Thus the lack of a universal key amino acid compound and a universally preferred concentration is a further incentive for lycaenid larvae to keep the amino acid mixture in their nectar balanced. The alternative way to increase interests by ants is to offer sugars, particularly sucrose. Increasing the sugar concentration is clearly a way of enhancing attractiveness to ants. This is not valid for amino acids, because at least some individual amino acids presented in higher amounts tend to decrease preferences. Sugar concentration in nectar is limited by two factors. First the caterpillars need to allocate carbohydrates between their own metabolic demands and requirements for their ant-association. Second, higher sugar concentrations increase the viscosity of the nectar, which prolongs feeding time at high concentrations (Hainsworth et al. 1991; Josens et al. 1998). The strongly ant-associated species, *P. coridon*, exhibits the first alternative of presenting a rich mixture of amino acid with a lower concentration of sugars, whereas the two less strongly ant-associated species follow the second option with high sugar concentrations and limited investment in nectar amino acids.

In a new model calculation, the energetic benefits derived from harvesting nectar secretions were compared to the possible energetic gain from preying upon the caterpillar (**chapter 6**). The calculation demonstrated that the latter behavioural alternative would pay more. The energetic benefits of secretions of facultatively myrmecophilous species calculated in earlier models were shown to be overestimations and had to be corrected by one order of magnitude. Nevertheless ants derive some benefits which translate into worker maintenance or new worker production. Workers of some ant species do live longer, if access to larval nectar secretions is granted (Cushman et al. 1994; Fiedler & Saam 1995), The results of the latter experimental study match to the predictions from the models presented here. Enhanced ant colony growth was shown in the case of *Jalmenus evagoras*, whose caterpillars produce copious amounts of nectar (Nash 1989). Nevertheless the energetic benefit/cost ratio of other sources, e.g. aphid honeydew, is very probably much better (Degen et al. 1986). In general, the degree to which a preference is exhibited for lycaenid secretions is dictated by the nutritional state of the colony and the access to alternative sources as homopterans or extrafloral nectar (Axén et al. 1996; Pierce & Nash 1999). Under heavy competition (as is usual in natural ant communities (Andersen 1997; Savolainen & Vepsäläinen 1988)) even the utilization of marginal food resources will pay in the long run. For example, even extrafloral nectaries of plants with low productivity are usually visited by ants in rich communities with strong asymmetric competition (Blüthgen et al. 2004). More specialized lycaenid species secrete a very rich amino acid mixture (*P. coridon*, this study, *Thisbe irenea,* (DeVries 1988), and therefore offer resources which are very probably worth even more than just their energetic benefit. A competitive effect between extrafloral nectaries (EFN) and homopteran aggregations and thus a higher attractiveness of the EFN was proposed as a defence measure of plants (Becerra & Venable 1989; Becerra & Venable 1991). Contrary to the latter two studies, however, nectar from EFN is not highly predictable in time and space, and the ideas of Becerra and Venable were rejected by empirical studies (Del-Claro & Oliveira 1993; Fiala 1990). However, foraging strategies of different ant species might also be influenced by their anatomical crop structure, and thus the ant species differ in their benefit to EFN bearing plants, the topic might be further complicated (Davidson et al. 2004). Obligate myrmecophilous lycaenid species (Fiedler 1992) and riodinid species (DeVries 1988; DeVries & Baker 1989) are able to subtract ants from EFN, demonstrating their superiority in attracting ants by means of nectar secretion in concert with chemical communication.

The purely energetic gain might be higher in smaller ant species and depends also on the size of the ant colony and the costs of tending (viz. travel time, number of workers tending and thus unavailable compared to total number of foragers, actual demand of colony, tending time). Foraging costs for ants have relatively seldom been analysed (Fewell et al. 1996), and the few studies were mainly considered with large sized seed harvesters, leaf-cutter, army or honeypot ants. It is still unclear, if ants do maximise energy gain (energy (benefits – costs) / foraging time) or energy efficiency (energy (benefits-cost) / foraging energy costs). The topic is further complicated by the recruitment behaviour of ants, as both these aspects of foraging efficiency are simultaneously maximized (Baroni-Urbani & Nielsen 1990) when nestmates are recruited to rewarding resource spots (Beckers et al. 1993; Bonser et al. 1998; Mailleux et al. 2000; Roces & Hölldobler 1993; Sumpter & Beekman 2003). Seed harvesters are surprisingly efficient load carriers (Fewell 1988; Fewell et al. 1996; Lighton et al. 1993; Nielsen & Baroni-Urbani 1990; Weier & Feener 1995), so that these species are mainly time limited (Fewell 1988; Weier & Feener 1995) while energetic costs are unlikely to play a major role. Nectar has a lower energy density than seeds, thus nectarivorous species, especially large ones, are probably more sensitive to costs. Therefore these ant species should minimize their costs and maximize efficiency rather than gain (Fewell et al. 1996). Until studies for typical formicine species of small size are available, the exact costs of tending lycaenid caterpillars for European ants cannot be estimated exactly, however.

The keyword “conditionality” enters once again the stage, as the outcomes of a mutualism, especially if the interaction is facultative for both sides, depends on the actual conditions, viz. the identity of the interacting species, their density (and resulting aggregation size, if applicable), the density of enemies, competition among the ants, and possibly competition between nectar producers (Bronstein 1994; Del-Claro & Oliveira 2000). Many studies, concerning especially homopterans, have shown conditionality (Addicott 1978; Addicott 1979; Addicott 1985; Addicott 1986; Breton & Addicott 1992; Cushman & Addicott 1989; Del-Claro & Oliveira 2000; Itioka & Inoue 1996).

Facultative lycaenid-ant interactions can thus not generally be considered as mutualisms. Moreover this depends on the actual conditions. Lycaenid species, which exhibit a high secretion rate and possibly occur aggregated, are more rewarding than single feeding caterpillars which secrete only a few droplets per hour. Small ant species are more likely to benefit than large ants due to the higher net reward, as upkeep costs are lower for small ant species and colonies. Indeed, minute ants, like *Plagiolepis* sp., have been shown to cover the requirements of the colony (<250 workers) with harvests of *P. coridon* caterpillars as only food source (Fiedler & Maschwitz 1988). Most of the facultatively myrmecophilous association of ants with lycaenids are therefore only marginally beneficial for the attendant ants.

If the costs of tending exceed the food rewards the ants should decide to prey on the caterpillars (Pierce 1987). But ant species from the genera *Lasius* or *Myrmica* normally cannot penetrate the exceptionally thick cuticle of lycaenid caterpillars (Malicky 1969), and this applies to many more of the (usually small-sized) ants that attend lycaenid larvae (Fiedler 2001). Furthermore, several lines of evidence suggest that chemical manipulation plays a role also in facultative myrmecophiles. Lycaenid caterpillars parasitised by braconid wasps remain attractive for some days even after emergence of the parasitoid adults from the caterpillars carcass (Baumgarten & Fiedler 1998; Fiedler et al. 1995). Pupae which do not longer possess a functional dorsal nectar organ are attractive to ants (Malicky 1969) and likewise *P. coridon* caterpillars with experimentally capped dorsal nectar organ are still attractive (Fiedler & Maschwitz 1989a), although in the latter case recruitment of ants towards these caterpillars stops. Thus 'defection' by the caterpillar does not immediately lead to abandoning by the ants. In contrast, if a parasite disrupts larval behaviour considerably, as in the case of the tachinid *Aplomya* flies (Diptera), the ants will desert (Baumgarten & Fiedler 1998; Fiedler et al. 1995). Thus the chemical fooling works only to a certain degree in ensuring attendance, but still the caterpillars are not attacked by the ants. Likewise this does not happen, if occasionally individual caterpillar do not secrete at all (own observations, Burghardt & Fiedler 1996a) these are only abandoned, whereas ants sometimes prey on unproductive aphids (Sakata 1995).

Likely candidates for the source of chemical fooling in the Lycaenidae are the pore cupola organs (Kitching & Luke 1985; Malicky 1969; Malicky 1970), whose secretions (Pierce 1983) still await a thorough chemical investigation. In addition the eversible tentacle organs (Kitching 1983; Kitching & Luke 1985), have an exciting and binding effect upon ants (Axén et al. 1996; DeVries 1988; Fiedler & Maschwitz 1987). No successful attempt to identify the presumable volatiles from the tentacle organs of lycaenids and riodinids have thus far been published, although ant alarm pheromone components have been suspected as the effective substances in the tentacle organs (Henning 1983). Alarm pheromones are the least specific of all ant pheromone classes (Vander Meer & Alonso 1998), thus synthesis and release of these substances might be a possible option for lycaenids. As alarm pheromones typically have a concentration dependent gradation of aggressiveness released in the specific ants (Hölldobler & Wilson 1990; Vander Meer & Alonso 1998; Wilson 1958), the difficulty remaining might then be the requirement to meet the appropriate concentration. Possibly the concentration might be minute and build up through repeated eversions, until ant attention is high enough, which the caterpillars may perceive by the running speed of the ants on their integument and through the associated substrate vibrations (see above). Ants (*Lasius niger*, *L. flavus*) habituate to tentacle organ eversions (Axén et al. 1996), and facultatively myrmecophilous lycaenid larvae may regulate the number of attendant ants with these organs (Axén et al. 1996; Fiedler & Maschwitz 1987; Leimar & Axén 1993). Thus the tentacle organs appear to serve for a subtle manipulation.

The existence of true brood pheromones in ants is discussed controversely (Morel & Vander Meer 1988; Vander Meer & Alonso 1998) and their definitive existence has still to be proven in experiments with appropriate controls for confounding food or colony odour responses. Recently, however, n-alkanes have been proposed as possible retrieval signal for ant foragers (Viana et al. 2001), but the signals are presumably coupled with special behavioural cues (Morel & Vander Meer 1988; Vander Meer & Alonso 1998). Malicky (1969) was the first to discuss the role of ant brood odours in the lycaenid-ant interactions. A possible ant brood pheromone was first found in extracts of the integument of an obligate ant-parasitic nest inquiline among the Lycaenidae (Henning 1983). This type of chemical mimicry is not to be expected in the facultatively myrmecophilous species examined here, as such facultatively associated lycaenid caterpillars cannot produce a pheromone which would match all possible ant visitors. More recent studies show that ant-parasitic *Maculinea* species (and other ant-parasites (Elgar & Allan 2004) and social-parasitic ants (D'Ettore et al. 2002)) often exhibit cuticular hydrocarbon patterns similar to those of their host ants (Akino et al. 1999; Elmes et al. 2002; Schlick-Steiner et al. 2004), although fully successful adoption requires further cues (Akino et al. 1999; Elmes et al. 2004; Schönrogge et al. 2004). The cuticular hydrocarbon pattern of facultatively myrmecophilous caterpillars cannot be matched to all visitors, especially as these are recruited from different ant genera and subfamilies. Indeed, hexane whole body extracts and solid phase extractions of cuticular hydrocarbons of mature *P. coridon* and *Z. knysna* larvae seem not to fit to the cuticular hydrocarbon pattern of *L. flavus* ant workers and a transfer of hydrocarbons from *Lasius flavus* workers to *P. coridon* or *Z. knysna* caterpillars after continuous tending for ten days seems not to happen (own unpublished data). These preliminary data support the view that chemical mimicry (or camouflage) by cuticular hydrocarbons is not a crucial element in the communication between facultative ant-associated lycaenid larvae and ants (Fiedler et al. 1996), while more and more evidence is accumulating that such sophisticated ways of manipulation are essential in obligate and host-specific ant-associations.

The evolution of mutualism and the stability of such interactions in evolutionary times can be described in different theoretical approaches. One of these is the game theory, with the famous prisoners' dilemma transferred to biology in its iterated version (IPD) (Axelrod & Hamilton 1981; Trivers 1971, reviewed e.g. Brembs 1996; Dugatkin 1997). These models consider mainly the question: when does it pay to defect? Furthermore, they are limited to competition between individuals of the own class, all payments are fixed (Doebeli & Knowlton 1998), and at least originally active partner choice was not included (Bull & Rice 1991). Thus these models do not match exactly the situation observed in ant-lycaenid interactions. A further class are the models concerned with indirect reciprocity in the interactions. These require individual recognition of the partners or at least of the 'standing' of the partner met (for critical discussion see e.g. Leimar & Hammerstein (2001)). However, individual recognition in lycaenid-ant interactions is extremely unlikely (Pierce 1987). *A*nts have to been observed to show some fidelity to individual *Glaucopsyche lygdamus* caterpillars in tending (Pierce 1989). Although memory effects of the workers might be involved, the marking of profitable mutualists is equally possible (Glinwood et al. 2003; Sakata 1994) or workers may just lay individual pheromone trails (Hanley & Lovett 1999). Thus, the relevance of these models for lycaenid-ant interactions is questionable. Doebeli and Knowlton (1998) constructed the first PD model explicitly for mutualisms. According to their model, if competition occurs within different trader classes, mutualism evolves instead of disruption of associations. The introduction of the effects of space creates some analogy to another class of models, the biological market models (an early precursor is Janzen (1985)); explicitly formulated (Noe & Hammerstein 1994; Noe & Hammerstein 1995)). Here mutualisms are viewed as interactions between different classes of traders which offer each other commodities. These commodities are difficult or impossible to obtain by the other respective class. Specialization of both classes on their own easily obtained commodity pays (Schwartz & Hoeksema 1998), in time establishing a market following the rules of supply and demand. In fact, lycaenid-ant interactions are more complex than IPDs (Brembs 1996), thus market models seem to be superior for explaining lycaenid-ant interactions. They suffer, however, insofar as defection is not included.

The association between lycaenids and ants shares components of both model classes, (potential) protection is exchanged for food (DeVries 1988; Maschwitz et al. 1975; Nomura et al. 1992; Pierce 1983; Pierce & Nash 1999; Wagner 1994; this study), although the benefits differs according to the lycaenid species (Pierce et al. 2002, and references therein). Conditionality of mutualism shares traits with a supply and demand situation. Obligately myrmecophilous species bear some costs of the association, whereas in facultatives costs are minor (Pierce et al. 2002; Stadler et al. 2001 and references therein). The costs for the ants are not clear, but lycaenids would not pay time costs until ants desert them, whereas the ants have time costs tending less rewarding caterpillars. The benefit for the ants harvesting secretions from facultatively myrmecophilous species is possibly lower than the benefit from preying on the caterpillars would be (see above), but the extent of behavioural and chemical manipulation involved is unclear (see above). Furthermore, if melezitose might act as secondarily evolved advertisement substance for few ant species (see discussion chapter 5), indicating profitable carbohydrate sources, then *P. icarus* and especially *Z. knysna* would falsely announce a good quality source. Both species secrete only small quantities of nectar (*P. icarus* profitable individuals ca. <0.03-0.09µl/h, *Z. knysna* profitable individuals ca. <0.01-0.03µl/h) with low sugar content compared to highly myrmecophilous aphids (0.11-0.77µl/h, Woodring et al. in press) (chapter 4). The ants typically tending the three lycaenid species examined here are more scavengers than actively prey-killing predators (Fiedler 2001). Furthermore they might monopolize also less rewarding resources when competing with other ants. Thus both sides might try to maximize their benefits, displaying a mutualism, which is a reciprocal exploitation with net benefits on either side, but susceptible to changes in surrounding conditions (Bronstein 1994; Herre et al. 1999). To judge the extent of exploitation, and factors constraining exploitation, requires exact identification of the costs and benefits of the respective interaction (Herre et al. 1999). The present study contributed, especially with the comparative chemical analysis of the nectar secretions, a further important step in this direction.

Increased pressure by predators and parasitoids might select towards more nutritious rewards in facultative myrmecophilous species. But at the same time there are considerable costs which select against this trend. A specialised mutualism with ants can even enhance parasitism risk, as attendant ants act as cues for the parasite, simplifying its search for the butterfly host species (Nash 1989; Pierce 1987; Seufert & Fiedler 1996b; Seufert & Fiedler 1999). Since obligately myrmecophilous species often occur in highly clumped aggregations (Pierce & Elgar 1985; Pierce et al. 1987; Seufert & Fiedler 1996a), they are a rich resource for a parasitoid, once found (Seufert & Fiedler 1999). A further cost of specialisation on certain ant species simultaneously decreases the possible distribution range, as the butterflies need not only to search for their host plants, but additionally for the matching ant species (Pierce 1987). Such ant-dependent oviposition has been shown mainly in obligately myrmecophilous species (Fiedler 1991; Fiedler & Maschwitz 1989b; Pierce & Elgar 1985; Seufert & Fiedler 1996a; Seufert & Fiedler 1996b; Smiley et al. 1988), even up to a discrimination of different populations of the respective host ant (Fraser et al. 2002). One facultatively myrmecophilous species has been shown to be unusually specialized to a specific subset of *Lasius* ant species and occurrence of this species (*Plebejus argus*) is closely correlated to patches with its host ants (Jordano et al. 1992; Jordano & Thomas

1992; Seymour et al. 2003), although this is highly exceptional for facultative myrmecophiles. In *obligate* myrmecophilous species a more pronounced preference for the appropriate host ant than for the appropriate host plant can even widen the host plant range (Bell 1915-20) in (Fiedler 1994; Pierce 1984; Pierce & Elgar 1985). Obligate myrmecophiles might further diversify, if after shifts to novel ant clades they subsequently radiate, the ants acting therefore as template against which the *obligate* lycaenids diversify (Pierce et al. 2002, but see Als et al. 2002; Costa et al. 1996). The radiation would be mediated by higher divergence between populations, enforced by small population sizes and fragmented distributions, which are both linked to the specific requirements concerning both ants and host plants in obligate myrmecophilous species (Atsatt 1981; Costa et al. 1996; Jordano & Thomas 1992; Smiley et al. 1988). Parasitic myrmecophily has also been proposed to contribute to cryptic speciation, as there is strong selection to specialise on local hosts without appreciable morphological divergence (Schönrogge et al. 2002). Yet, the conditions of small population sizes and fragmented distributions pose simultaneously great costs, as such highly patchily distributed species are especially susceptible to local extinction (Costa et al. 1996; Thomas & Elmes 1998). Host ant specialization among myrmecophilous insects is thus to be expected mostly with dominant, long lived and widespread ant species (Eastwood & Fraser 1999; Fiedler 1991; Fiedler 2001; Stadler et al. 2001). Furthermore ant species differ in protection ability for their throphobionts (Addicott 1979; Buckley 1987; Buckley & Gullan 1991; Gibernau & Dejean 2001; Itioka & Inoue 1996; Katayama & Suzuki 2003; Messina 1981), a necessary, but not sufficient condition for specialisation on ant species (Fraser et al. 2001). Such specialization in interactions on partners that are particularly efficient may pay temporarily, but spatial and temporal variation in the potential partner species will usually select against the evolution of very specific interactions (Hoeksema & Bruna 2000). Similar considerations seem to be valid for many other potentially mutualistic systems, too, e.g. ants and homopterans (Bristow 1991; Stadler et al. 2001; Stadler et al. 2003) or plant-pollinator systems (Schemske & Horvitz 1984; Waser et al. 1996). In most of these systems temporal and special variation decrease the potential for specialization. Well known exceptions are only seen in highly co-evolved plant-pollinator systems such as yucca moths (Pellmyr & Krenn 2002), or fig wasps (Cook & Rasplus 2003).

From the perspective of the lycaenid caterpillar the investment into nectar secretions clearly pays. In facultative myrmecophiles in particular, this investment bears low costs ((Fiedler & Saam 1994), this study). Storing secretions in the dorsal gland to offer them to ants, if the caterpillar happens to be visited by ants serves at least as protection against ant harassment. This strategy permits the caterpillar not only to access to an ‘enemy free space’ (Atsatt 1981; Lenz 1917), which also myrmecoxenous caterpillar can achieve (Fiedler 1995). In addition, the capacity to produce nectar further opens up the potential of being actively tended and therefore to be protected against natural enemies. Thus, even at relatively low secretion rates and if associations with ants may be unstable or unpredictable, it usually pays to keep the dorsal nectar organ as a cost effective life insurance, which is more reliable than the possession of just pore cupola organs. In fact, systematic comparisons suggest that complete losses of the dorsal nectar organ are not that common among the Lycaenidae and are often associated with life in peculiar habitats (Fiedler 1991). Thus being a low cost strategy ((Burghardt & Fiedler 1996a; Wagner 1993), this study) combined with high flexibility and opportunism (see also (Schmidt & Rice 2002) well explains the enormous taxonomic and also geographical distribution of facultative myrmecophily in the species-rich butterfly family Lycaenidae.

References

Addicott, J. F. 1978: Competition for mutualists: aphids and ants. Can J Zool **56**, 2093-2096.

-. 1979: A multispecies aphid-ant association: density dependence and species specific effects. Can J Zool **57**, 558-569.

-. 1985: Competition in mutualistic systems. In: The Biology of Mutualism - Ecology and Evolution (Boucher, D. H. ed). Crown Helm, London. pp. 217-247.

-. 1986: Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. Oecologia **70**, 486-494.

Agrawal, A. A. & Fordyce, J. A. 2000: Induced indirect defence in a lycaenid-ant association: the regulation of a resource in a mutualism. Proc R Soc Lond B **267**, 1857-1861.

Akino, T., Knapp, J. J., Thomas, J. A. & Elmes, G. W. 1999: Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. Proc R Soc Lond B **266**, 1419-1426.

Als, T. D., Nash, D. R. & Boomsma, J. J. 2002: Geographical variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. Ecol Entomol **27**, 403-414.

Andersen, A. N. 1997: Functional groups and patterns of organization in North American ant communities: a comparison with Australia. J Biogeogr **24**, 433-460.

Atsatt, P. R. 1981: Lycaenid butterflies and ants: selection for enemy-free space. Am Nat **118**, 638-654.

Axelrod, R. & Hamilton, W. D. 1981: The evolution of cooperation. Science **211**, 1390-1396.

Axén, A. H. 2000: Variation in behavior of lycaenid larvae when attended by different ant species. Evol Ecol **14**, 611-625.

Axén, A. H. & Pierce, N. E. 1998: Aggregation as a cost-reducing strategy for lycaenid larvae. Behav Ecol **9**, 109-115.

Axén, H. A., Leimar, O. & Hoffman, V. 1996: Signalling in a mutualistic interaction. Anim Behav **52**, 321-333.

Baroni-Urbani, C., Buser, M. W. & Schilliger, E. 1988: Substrate vibration during recruitment in ant social organization. Insectes Sociaux **35**, 241-250.

Baroni-Urbani, C. & Nielsen, M. G. 1990: Energetics and foraging behaviour of the European seed harvesting ant *Messor capitatus* II. Do ants optimize their harvesting? Physiol Entomol **15**, 449-461.

Baumgarten, H. T. & Fiedler, K. 1998: Parasitoids of lycaenid butterfly caterpillars: Different patterns in resources use and their impact on the Hosts' symbiosis with ants. Zool Anz **236**, 167-180.

Baylis, M. & Pierce, N. E. 1991: The effect of host-plant quality on the survival of larvae and oviposition by adults of an ant-tended lycaenid butterfly, *Jalmenus evagoras*. Ecol Entomol **16**, 1-9.

-. 1993: The effects of ant mutualism on the foraging and diet of lycaenid caterpillars. In: Caterpillars (Stamp, N. E. & Casey, T. M. eds). Chapman & Hall, New York. pp. 404-421.

Becerra, J. X. & Venable, D. L. 1989: Extrafloral nectaries: a defense against ant-Homoptera mutualisms. Oikos **55**, 276-280.

-. 1991: The role of ant-Homoptera mutualisms in the evolution of extrafloral nectaries. Oikos **60**, 105-106.

Beckers, R., Deneubourg, J.-L. & Goss, S. 1993: Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. J Insect Behav **6**, 751-759.

Bell, T. R. 1915-20: The common butterflies of the plains of India. J Bombay Nat Hist Soc **1915-1920**.

Blüthgen, N. & Fiedler, K. 2004: Preferences for sugars and amino acids in a diverse nectar-feeding ant community. J Anim Ecol **73**, 155-166.

Blüthgen, N., Stork, N. E. & Fiedler, K. 2004: Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. Oikos **106(2)**, 344-358.

Bonser, R., Wright, P. J., Bament, S. & Chukwu, U. O. 1998: Optimal patch use by foraging workers of *Lasius fuliginosus*, *L. niger* and *Myrmica ruginodis*. Ecol Entomol **23**, 15-21.

Boucher, D. H. 1982: The ecology of mutualism. Ann Rev Ecol Syst **13**, 315-347.

-. 1985: The idea of mutualism, past and future. In: The Biology of Mutualism - Ecology and Evolution (Boucher, D. H. ed). Crown Helm, London. pp.1-28.

Brembs, B. 1996: Chaos, cheating and cooperation: potential solutions to the prisoner's dilemma. Oikos **76**, 14-24.

Breton, L. M. & Addicott, J. F. 1992: Density-dependent mutualism in an aphid-ant interaction. Ecology **73**, 2175-2180.

Bristow, C. M. 1991: Why are so few aphids ant-tended. In: Ant-plant interactions (Huxley, C. R. & Cutler, D. F. eds). Oxford University Press, Oxford. pp. 104-119.

Bronstein, J. L. 1994: Conditional outcomes in mutualistic interactions. Trends Ecol Evol **9**, 214-217.

-. 1998: The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica **30**, 150-161.

Buckley, R. 1987: Interactions involving plants, Homoptera, and ants. Ann Rev Ecol Syst **18**, 111-135.

Buckley, R. & Gullan, P. 1991: More aggressive ant species(Hymenoptera: Formicidae) provide better protection for soft scales and mealybugs (Homoptera: Coccidae, Pseudococcidae). Biotropica **23**, 282-286.

Bull, J. J. & Rice, W. R. 1991: Distinguishing mechanisms for the evolution of co-operation. J theor Biol **149**, 63-74.

Burghardt, F. & Fiedler, K. 1996a: The influence of diet on growth and secretion behaviour of myrmecophilous *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenidae). Ecol Entomol **21**, 1-8.

-. 1996b: Myrmecophilous behaviours in caterpillars of the butterfly, *Polyommatus icarus* (Rottemburg, 1775): temporal patterns and age dependency (Lepidoptera: Lycaenidae). Nota lepid **19**, 212-229.

Byrne, D. N., Hendrix, D. L. & Livy, H. W. I. 2003: Presence of trehalulose and other oligosaccharids in Hemiptera honeydew, particularly Aleyrodidae. Physiol Entomol **28**, 144-149.

Cook, J. M. & Rasplus, J.-Y. 2003: Mutualists with attitude: coevolving fig wasps and figs. Trends Ecol Evol **18**, 241-248.

Costa, J. T., McDonald, J. H. & Pierce, N. E. 1996: The effect of ant association on the population genetics of the Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae). Biol J Linnean Soc **58**, 287-306.

Cottrell, G. B. 1984: Aphytophagy in butterflies: its relationship to myrmecophily. Zool J Linnean Soc **79**, 1-57.

Cushman, J. H. & Addicott, J. F. 1989: Intra- and interspecific competition for mutualists: ants as limited and limiting resource for aphids. Oecologia **79**, 315-321.

Cushman, J. H., Rashbrook, V. K. & Beattie, A. J. 1994: Assessing benefits to both participants in a lycaenid-ant association. Ecology **75**, 1031-1041.

Davidson, D. W, Cook, S. C. & Snelling, R. R. 2004: Liquid-feeding performances of ants (Formicidae): ecological and evolutionary implications. Oecologia **139**, 255-266.

Degen, A. A., Gersani, M., Avivi, Y. & Weisbrot, N. 1986: Honeydew intake of the weaver ant *Polyrhachis simplex* (Hymenoptera: Formicidae) attending the aphid *Chaitophorous populialbae* (Homoptera: Aphididae). Insectes Sociaux **33**, 211-215.

Del-Claro, K. & Oliveira, P. S. 1993: Ant-Homoptera interaction: do alternative sugar sources distract ants? Oikos **68**, 202-206.

-. 2000: Conditional outcome in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. Oecologia **124**, 156-165.

D'Ettore, P., Mondy, N., Lenoir, A. & Errard, C. 2002: Blending in with the crowd: social parasites integrate into their host colonies using a flexible chemical signature. Proc R Soc Lond B **269**, 1911-1918.

DeVries, P. J. 1988: The larval ant-organs of *Thisbe irenea* (Lepidoptera: Riodinidae) and their effects upon attending ants. Zool J Linnean Soc **94**, 379-393.

-. 1990: Enhancement of symbioses between butterfly caterpillars and ants by vibrational communication. Science **248**, 1104-1106.

-. 1991: Call production by myrmecophilous riodinid and lycaenid butterfly caterpillars (Lepidoptera): morphological, acoustical and evolutionary patterns. Am Mus Nov **3025**, 1-23.

DeVries, P. J. & Baker, I. 1989: Butterfly exploitation of an ant-plant mutualism: adding insult to herbivory. J New York Entomol Soc **97**, 332-340.

DeVries, P. J., Cocroft, R. B. & Thomas, J. A. 1993: Comparison of acoustical signals in *Maculinea* butterfly caterpillars and their obligate host *Myrmica* ants. Biol J Linnean Soc **49**, 229-238.

Doebeli, M. & Knowlton, N. 1998: The evolution of interspecific mutualism. Proc Natl Acad Sci USA **95**, 8676-8680.

Downey, J. C. 1965: Thrips utilize Exudations of Lycaenidae. Entomol News **76**, 25-27.

-. 1966: Sound production in pupae of Lycaenidae. J Lepid Soc **20**, 129-155.

Dugatkin, L. A. 1997: Cooperation among animals. Oxford University Press, New York.

Eastwood, R. & Fraser, A. M. 1999: Associations between lycaenid butterflies and ants in Australia. Aust Ecol **24**, 503-537.

Elgar, M. A. & Allan, R. A. 2004: Predatory spider mimics acquire colony-specific cuticular hydrocarbons from their ant model prey. Naturwissenschaften **91**, 143-147.

Eliot, J. N. 1973: The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. Bull Brit Mus Nat Hist (Entomol) **28**, 371-505.

Elmes, G. W., Akino, T., Thomas, J. A., Clarke, R. T. & Knapp, J. J. 2002: Interspecific differences in cuticular hydrocarbon profiles of *Myrmica* ants are sufficiently consistent to explain host specificity by *Maculinea* (large blue) butterflies. Oecologia **130**, 525-535.

Elmes, G. W., Wardlaw, J. C., Schönrogge, K., Thomas, J. A. & Clarke, R. T. 2004: Food stress causes differential survival of socially parasitic caterpillars of *Maculinea rebeli* integrated in colonies of host and non-host *Myrmica* ant nests. Ent exp & appl **110**, 53-63.

Febvay, G., Rahbe, Y., Rynkiewicz, M., Guillaud, J. & Bonnot, G. 1999: Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, *Acyrtosiphon pisum*, reared on different diets. J exp Biol **202**, 2639-2652.

Fewell, J. H. 1988: Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. Behav Ecol Sociobiol **22**, 401-408.

Fewell, J. H., Harrison, J. F., Lighton, J. R. B. & Breed, M. D. 1996: Foraging energetics of the ant, *Paraponera clavata*. Oecologia **105**, 419-427.

Fiala, B. 1990: Extrafloral nectaries vs ant-Homoptera mutualisms: a comment on Becerra and Venable. Oikos **59**, 281-282.

Fiedler, K. 1990: Effects of larval diet on myrmecophilous qualities of *Polyommatus icarus* caterpillars (Lepidoptera: Lycaenide). Oecologia **83**, 284-287.

-. 1991: Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionidae). Bonner Zoologische Monographien **31**.

-. 1992: The life history of *Surendra florimel* Doherty 1889 (Lepidoptera: Lycaenidae) in West Malaysia. Nachr entomol Ver Apollo Frankfurt N F **13**, 65-92.

-. 1994: Lycaenid butterflies and plants: is myrmecophily associated with amplified host diversity. Ecol Entomol **19**, 79-82.

-. 1995: Lycaenid butterflies and plants: Is myrmecophily associated with particular hostplant preferences? Ethol Ecol Evol **7**, 107-132.

-. 1996: Host-plant relationships of lycaenid butterflies: large scale patterns, interactions with plant chemistry, and mutualism with ants. Ent Exp appl **80**, 259-267.

-. 2001: Ants that associate with Lycaeninae butterfly larvae: diversity, ecology and biogeography. Divers Distrib **7**, 45-60.

Fiedler, K. & Hagemann, D. [1995]: The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue Butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae). J Res Lepid **31 (3-4)**, 213-232.

Fiedler, K., Hölldobler, B. & Seufert, P. 1996: Butterflies and ants: the communicative domain. Experientia **52**, 14-24.

Fiedler, K. & Hummel, V. 1995: Myrmecophily in the brown argus butterfly, *Aricia agestis* (Lepidoptera: Lycaenidae): Effects of larval age, ant number and persistence of contact with ants. Zoology **99**, 128-137.

Fiedler, K. & Maschwitz, U. 1987: Functional analysis of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) III. New aspects of the function of the retractile tentacular organs of lycaenid larvae. Zool Beitr N F **31**, 409-416.

-. 1988: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) II. Lycaenid larvae as trophobiotic partners of ants - an quantitative approach. Oecologia **75**, 204-206.

-. 1989a: Functional analyses of the myrmecophilous relationships between ants (Hymenoptera: Formicidae) and Lycaenids (Lepidoptera: Lycaenidae) I. Release of food recruitment in ants by lycaenid larvae and pupae. Ethology **80**, 71-80.

-. 1989b: The symbiosis between the weaver ant, *Oecophylla smaragdina* and *Anthene emolus*, an obligate myrmecophilous butterfly. J Nat Hist **23**, 833-846.

Fiedler, K. & Saam, C. 1994: Does ant-attendance influence development in 5 European Lycaenide butterfly species ? (Lepidoptera). Nota lepid **17**, 5-24.

-. 1995: Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study. Oecologia **104**, 316-322.

Fiedler, K., Schurian, K. G. & Hahn, M. 1994: The life history and myrmecophily of *Polyommatus candalus* (Herrich-Schäffer) from Turkey (lep., Lycaenidae). Linn Belg **14**, 315-332.

Fiedler, K, Seufert, P., Pierce, N. E., Pearson, J. G. & Baumgarten, H.-T. [1995]: Exploitation of lycaenid-ant mutualisms by braconid parasitoids. J Res Lepid **31**, 153-168.

Fischer, M. K. & Shingleton, A. W. 2001: Host plants and ants influence the honeydew sugar composition of aphids. Funct Ecol **15**, 544-550.

Fraser, A. M., Axén, A. H. & Pierce, N. E. 2001: Assessing the quality of different ant species as partners of a myrmecophilous butterfly. Oecologia **129**, 452-460.

Fraser, A. M., Tregenza, T., Wedell, N., Elgar, M. A. & Pierce, N. E. 2002: Oviposition tests of ant preference in a myrmecophilous butterfly. J Evol Biol **15**, 861-870.

Gibernau, M. & Dejean, A. 2001: Ant protection of a Heteropteran trophobiont against a parasitoid wasp. Oecologia **126**, 53-57.

Gilbert, L. E. 1976: Adult resources in butterflies: African lycaenid *Megalopalpus* feeds on larval nectary. Biotropica **8**, 282-283.

Glinwood, R., Willekens, J. & Pettersson, J. 2003: Discrimination of aphid mutualists by an ant based on chemical cues. Acta Agr Scand BS P **53**, 177-182.

Hacker, S. D. & Gaines, S. D. 1997: Some implications of direct positive interactions for community species diversity. Ecology **78**, 1990-2003.

Hainsworth, F. R., Precup, E. & Hamill, T. 1991: Feeding, energy processing rates and egg production in painted lady butterflies. J exp Biol **156**, 249-265.

Hanley, R. S. & Lovett, J. P. 1999: Behavior and subcaste specialization among workers of the giant tropical ant *Paraponera clavata* (Hymenoptera: Formicidae: Ponerinae). In: Entomological Contributions in Memory of Byron A. Alexander, Vol. 24 (Byers, G. W., Hagen, R. H. & Brooks, R. W. eds). University of Kansas Natural History Museum Special Publications, Kansas. pp. 45-50.

Hendrix, D. L., Wei, Y.-A. & Leggett, J. E. 1992: Homopteran honeydew sugar composition is determined by both the insect and plant species. Comp Biochem Physiol B **101**, 1/2.

Henning, S. F. 1983: Chemical communication between lycaenid larvae (Lepidoptera: Lycaenide) and ants (Hymenoptera: Formicidae). J ent Soc sth Afr **46**, 341-366.

Herre, E. A., Knowlton, N., Mueller, U. G. & Rehner, S. A. 1999: The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol Evol **14**, 49-53.

Hoeksema, J. D. & Bruna, E. M. 2000: Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. Oecologia **125**, 321-330.

Hölldobler, B. & Wilson, E. O. 1990: The ants. Harvard University Press / Springer, Heidelberg.

Itioka, T. & Inoue, T. 1996: Density-dependent ant attendance and its effects on the parasitism of a honeydew-producing scale insect, *Ceroplastes rubens*. Oecologia **106**, 448-454.

Janzen, D. H. 1985: The natural history of mutualisms. In: The Biology of Mutualism - Ecology and Evolution (Boucher, D. H. ed). Crown Helm, London. pp. 40-99.

Jordano, D., Rodríguez, J., Thomas, C. D. & Haeger, J. F. 1992: The distribution and density of a lycaenid butterfly in relation to *Lasius* ants. Oecologia **91**, 439-446.

Jordano, D. & Thomas, C. D. 1992: Specificity of an ant-lycaenid interaction. Oecologia **91**, 431-438.

Josens, R. B., Farina, W. M. & Roces, F. 1998: Nectar feeding by the ant *Camponotus mus*: intake rate and crop filling as a function of sucrose concentration. J Insect Physiol **44**, 579-585.

Katayama, N. & Suzuki, N. 2003: Bodyguard effects for aphids of *Aphis craccivora* Koch (Homoptera: Aphididae) as related to the activity of two ant species, *Tetramorium caespitum* Linnaeus (Hymenoptera: Formicidae) and *Lasius niger* L. (Hymenoptera: Formicidae). Appl Ent Zool **38**, 427-433.

Kitching, R. L. 1983: Myrmecophilous organs of the larvae and pupae of the lycaenid butterfly *Jalmenus evagoras*. J Nat Hist **17**, 471-481.

Kitching, R. L. & Luke, B. 1985: The myrmecophilous organs of the larvae of some British Lycaenidae (Lepidoptera): a comparative study. J Nat Hist **19**, 259-276.

Knight, R. L. & Rust, M. K. 1991: Efficacy of formulated baits for control of the Argentine Ant (Hymenoptera: Formicidae). J Econ Entomol **84**, 510-514.

Leimar, O. & Axén, A. H. 1993: Strategic behaviour in an interspecific mutualism: interactions between lycaenid larvae and ants. Anim Behav **46**, 1177-1182.

Leimar, O. & Hammerstein, P. 2001: Evolution of cooperation through indirect reciprocity. Proc R Soc Lond B **268**, 745-753.

Lenz, F. 1917: Der Erhaltungsgrad der Myrmekophilie. Z indukt Abstammungs Vererbungsl **19**, 44-46.

Lighton, J. R. B., Weier, J. A. & Feener, D. H. j. 1993: The energetics of locomotion and load carriage in the desert harvester ant *Pogonomyrmex rugosus*. J exp Biol **181**, 49-61.

Mailleux, A. C., Deneubourg, J.-L. & Detrain, C. 2000: How do ants assess food volume? Anim Behav **59**, 1061-1069.

Malicky, H. 1969: Versuch einer Analyse der ökologischen Beziehungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr Ent **112**, 213-298.

-. 1970: New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymneoptera). J Lep Soc **24**, 190-202.

Maschwitz, U., Wüst, M. & Schurian, K. 1975: Bläulingsraupen als Zuckerlieferanten. Oecologia **18**, 17-21.

Messina, F. J. 1981: Plant protection as a consequence of an ant-membracid mutualism: interactions on goldenrod (*Solidago* sp.). Ecology **62**, 1433-1440.

Morel, L. & Vander Meer, R. K. 1988: Do ant brood pheromones exist? Ann Entomol Soc Am **81**, 707-709.

Nash, D. R. 1989: Cost-benefit analysis of a mutualism between lycaenid butterflies and ants. Ph.D. thesis, University of Oxford, Oxford.

Nielsen, M. G. & Baroni-Urbani, C. 1990: Energetics and foraging behaviour of the European seed harvesting ant *Messor capitatus* I. Respiratory metabolism and energy consumption of unloaded and loaded workers druing locomotion. Physiol Entomol **15**, 441-448.

Noe, R. & Hammerstein, P. 1994: Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behav Ecol Sociobiol **35**, 1-11.

-. 1995: Biological markets. Trends Ecol Evol **10**, 336-339.

Nomura, K., Hirukawa, N., Yamaoka, R. & Imafuku, M. 1992: Problems on the symbiosis between the lycaenid butterfly larva, *Niphanda fusca shijimia* and the ant *Camponotus* (*Camponotus*) *japonicus*. Tyo to Ga **43**, 138-143.

Pellmyr, O. & Krenn, H. W. 2002: Origin of a complex key innovation in an obligate insect-plant mutualism. Proc Natl Acad Sci USA **99**, 5498-5502.

Pierce, N. E. 1983: The ecology and evolution of the symbioses between lycaenid butterflies and ants. Ph.D. thesis, Harvard University.

-. 1984: Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants. Symp R Entomol Soc London **11**, 197-200.

-. 1985: Lycaenid butterflies and ants: selection for nitrogen-fixing and other protein-rich food plants. Am Nat **125**, 888-895.

-. 1987: The evolution and biogeography of associations between lycaenid butterflies and ants. Oxford Surveys in Evolutionary Biology **4**, 89-116.

-. 1989: Butterfly-ant mutualisms. In: Towards a more exact ecology (Grubb, P. J. & Whittaker, J. eds). Blackwell Scientific, Oxford. pp. 299-324.

Pierce, N. E., Braby, M. F., Huxley, C. R., Lohman, D. J., Mathew, J., Rand, D. B. & Travassos, M. A. 2002: The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu Rev Entomol **47**, 733-771.

Pierce, N. E. & Elgar, M. A. 1985: The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. Behav Ecol Sociobiol **16**, 209-222.

Pierce, N. E, Kitching, R. L., Buckley, R., Taylor, M. F. J. & Benbow, K. F. 1987: The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. Behav Ecol Sociobiol **21**, 237-248.

Pierce, N. E. & Nash, D. R. 1999: The Imperial Blue, *Jalmenus evagoras* (Lycaenide). In: Biology of Australian Butterflies, Vol. 6 (Kitching, R. L., Scheermeyer, E., Jones, R. E. & Pierce, N. E. eds). CSIRO Publishing, Melbourne. pp. 279-317.

Ricks, B. L. & Vinson, S. B. 1970: Feeding acceptability of certain insects and various water-soluble compounds to two varieties of the imported Fire ant. J Econ Entomol **63**, 145-148.

Roces, F. & Hölldobler, B. 1993: Leaf density and a trade-off between load-size selection and recruitment behaviorin the ant *Atta cephalotes*. Oecologia **97**, 1-8.

Roces, F., Tautz, J. & Hölldobler, B. 1993: Stridulation in leaf-cutting ants. Naturwissenschaften **80**, 521-524.

Sakata, H. 1994: How an ant decides to prey on or attend aphids. Res Popul Ecol **36**, 45-541.

-. 1995: Density-dependent predation of the ant *Lasius niger* (Hymenoptera: Formicidae) on two attended aphids *Lachnus tropicalis* and *Myzocalis kuricola* (Homoptera: Aphididae). Res Popul Ecol **37**, 159-164.

Savolainen, R. & Vepsäläinen, K. 1988: A competition hierarchy among boreal ants: impact on resource partitioning and community structure. Oikos **51**, 135-155.

Schemske, D. W. & Horvitz, C. C. 1984: Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science **225**, 519-521.

Schlick-Steiner, B. C., Steiner, F. M., Höttinger, H., Nikiforov, A., Mistrik, R., Schafellner, C., Baier, P. & Christian, E. 2004: A butterfly's chemical key to various ant forts: intersection-odour or aggregate-odour multi-host mimicry? Naturwissenschaften, **91**, 209-214.

Schmidt, D. J. & Rice, S. J. 2002: Association of ants with juvenile *Ogyris amaryllis amaryllis* (Hewitson) (Lepidoptera: Lycaenidae) in south-eastern Queensland. Aust J Entomol **41**, 164-169.

Schönrogge, K., Barr, B., Wardlaw, J. C., Napper, E., Gardener, M. G., Breen, J., Elmes, G. W. & Thomas, J. A. 2002: When rare species become endangered: cryptic speciation in myrmecophilous hoverflies. Biol J Linnean Soc **75**, 291-300.

Schönrogge, K., Wardlaw, J. C., Peters, A. J., Everett, S., Thomas, J. A. & Elmes, G. W. 2004: Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. J Chem Ecol **30**, 91-107.

Schurian, K. G., Fiedler, K. & Maschwitz, U. 1993: Parasitoids exploit secretions of myrmecophilous lycaenid butterfly caterpillars (Lycaenidae). J Lepid Soc **47**, 150-154.

Schwartz, M. W. & Hoeksema, J. D. 1998: Specialization and resource trade: biological markets as a model of mutualisms. Ecology **79**, 1029-1038.

Scott, J. A. & Wright, D. M. 1990: Butterfly phylogeny and fossils. In: Butterflies of Europe Vol 2: Introduction to lepidopterology (Kudrna, O. ed). Aula Verlag, Wiesbaden.

Seufert, P. & Fiedler, K. 1996a: The influence of ants on the patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a south-east Asian tropical rain forest. Oecologia **106**, 127-136.

-. 1996b: Life history diversity and local coexistence of three closely related lycaenid butterflies (Lepidoptera: Lycaenidae) in Malaysian rainforests. Zool Anz **234**, 229-239.

-. 1999: Myrmecophily and parasitoid infestation of south-east asian lycaenid butterfly larvae. Ecotropica **5**, 56-94.

Seymour, A. S., Gutiérrez, D. & Jordano, D. 2003: Dispersal of the lycaenid *Plebejus argus* in response to patches of its mutualist ant *Lasius niger*. Oikos **103**, 162-174.

Smiley, J. T., Atsatt, P. R. & Pierce, N. E. 1988: Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. Oecologia **76**, 416-422.

Stachowicz, J. J. 2001: Mutualism, facilitation and the structure of ecological communities. Bioscience **51**, 235-246.

Stadler, B., Fiedler, K., Kawecki, T. J. & Weisser, W. W. 2001: Costs and benefits for phytophagous myrmecophiles: when ants are not always available. Oikos **92**, 467-478.

Stadler, B., Kindlmann, P., Smilauer, P. & Fiedler, K. 2003: A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance. Oecologia **135**, 422-430.

Sumpter, D. J. T. & Beekman, M. 2003: From nonlinearity to optimality: pheromone trail foraging by ants. Anim Behav **66**, 273-280.

Thomas, J. A. & Elmes, G. W. 1998: Higher productivity at the cost of increased host specificity when *Maculinea* butterfly larvae exploit ant colonies through trophallaxis rather than by predation. Ecol Entomol **23**, 457-464.

Travassos, M. A. & Pierce, N. E. 2000: Acoustics, context and function of vibrational signalling in a lycaenid butterfly-ant mutualism. Anim Behav **60**, 13-26.

Trivers, R. L. 1971: The evolution of reciprocal altruism. Q Rev Biol **46**, 35-57.

Vander Meer, R. K. & Alonso, L. E. 1998: Pheromone directed behavior in ants. In: Pheromone communication in social insects (Vander Meer, R. K., Breed, M. D., Espelie, K. E. & Winston, M. L. eds). Westview Press, Boulder. pp. 159-192.

Viana, A. M. M., Frézard, A., Malosse, C., Della Lucia, T. M. C., Errard, C. & Lenoir, A. 2001: Colonial recognition of fungus in the fungus-growing ant *Acromyrmex subterraneus subterraneus* (Hymenoptera: Formicidae). Chemoecology **11**, 29-36.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M. W. & Hoffmann, K. H. 1999: Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia **118**, 483-491.

Wada, A., Isobe, Y., Yamaguchi, S., Yamaoka, R. & Ozaki, M. 2001: Taste-enhancing effects of glycine on the sweetness of glucose: a gustatory aspect of symbiosis between the ant, *Camponotus japonicus*, and the larvae of the lycaenid butterfly, *Niphanda fusca*. Chem Senses **26**, 983-992.

Wagner, D. 1993: Species-specific effects of tending ants on the development of lycaenid butterfly larvae. Oecologia **96**, 276-281.

-. 1994: Species-specific effects of tending ants on the life history of *Hemiargus isola*, a North American lycaenid butterfly. Ph.D. thesis, Princeton University, Princeton.

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. & Ollerton, J. 1996: Generalization in pollination systems and why it matters. Ecology **77**, 1043-1059.

Weier, J. A. & Feener, D. H. j. 1995: Foraging in the seed harvester ant genus *Pogonomyrmex*: are energy costs important? Behav Ecol Sociobiol **36**, 291-300.

Wiemers, M. 2003: Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: *Polyommatus*) - a molecular phylogenetic approach. Ph.D. thesis, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn.

Wilson, E. O. 1958: A chemical releaser of alarm and digging behaviour in the ant *Pogonomyrmex badius*. Psyche **65**, 41-51.

8 Summary

The present thesis investigates the significance of variation in the nectar secretions of facultatively ant-associated (myrmecophilous) lycaenid butterfly larvae. Three species were selected, the strongly myrmecophilous European *Polyommatus coridon*, and two moderately myrmecophilous species, the Palaearctic *P. icarus* and the (sub-)tropical *Zizeeria knysna*. Both *Polyommatus* species are rather closely related to each other, whereas *Z. knysna* is a far more distant relative.

? For the rearing of a sufficiently high number of caterpillars a new method for breaking the egg diapause of the univoltine *P. coridon* was established (**chapter 3.3**). This resulted in 60-70% subitaneously hatching larvae under moderate work effort.

? Various attempts to induce nectar secretion by artificial stimulation failed. Based on observations of ant behaviour at the caterpillars a new "artificial ant" was assembled, which in a few cases even elicited secretion behaviour. An improved version may be used to further study the stimulatory antennation patterns of ants in interactions with lycaenid caterpillars (**chapters 3.4**, **3.5**).

? Nectar was harvested from caterpillars being attended by *Lasius flavus* ants with a microcapillary. This method was optimised in terms of timing the encounters with ants number of attendant ants and larval age. This approach allowed determination of the size of individual secretion droplets size based on large samples. Mean droplet size was 3.7nl in *P. coridon*, 2nl in *P. icarus* and 1.4nl in *Z. knysna*, which in the latter two species is 65-79% smaller than previously reported (**chapter 3.4**).

? Comparative chemical analyses (**chapter 4**) with HPLC technique revealed sucrose as main sugar component in nectar of all three species. In *P. coridon* sucrose was accompanied by glucose and rarely by further sugars in minor quantities, but never by melezitose. In *P. icarus* and *Z. knysna* melezitose was the second most-important component, followed by fructose and glucose. Total sugar contents were 43.6±14.8g/l for *P. coridon*, 74.2g/l for *P. icarus* and 68.3±22.6g/l for *Z. knysna*.

P. coridon nectar contained up to 14 amino acids. The major component was always leucine (50% of total), further important amino acids were tyrosine, proline, arginine, and phenylalanine. *P. icarus* nectar comprised up to six amino acids, dominated by tyrosine and phenylalanine. *Z. knysna* nectar contained only alanine and proline. Total sugar contents were 9.7±3.4g/l for *P. coridon*, 1.2g/l for *P. icarus* and 0.3±0.17g/l for *Z. knysna*.

Nectar composition was considerably different from hemolymph, which in all three species contained trehalose, accompanied by a smaller amounts of sucrose. Up to 16 different amino acids occurred relatively regularly, dominated by histidine, serine and proline.

Larval food had minor influence on *P. coridon* nectar composition. Caterpillars fed with semi-synthetic diet secreted more sucrose, with a trend towards higher total sugar content. Furthermore these caterpillars produced nectar with a more homogeneous amino acid pattern than larvae reared on natural host plants (*Hippocrepis comosa*, *Securigera varia*).

? Bioassays with ants from three different subfamilies (*Lasius niger* (Formicinae), *Myrmica rubra* (Myrmicinae), *Tapinoma melanocephalum* (Dolichoderinae)) (**chapter** 5) demonstrated a preference for the disaccharide sucrose (standard concentration 0.1mol/l, as in *P. coridon* nectar) over monosaccharides. Melezitose in nectar concentration (*P. icarus*) was not preferred to sucrose. Some single amino acids in sucrose solutions were preferred over pure sucrose, e.g. leucine by *L. niger*, or phenylalanine and tyrosine by *M. rubra*. In general, raising the amino acid concentration did not enhance preferences and even reduced them in some cases. Mixtures of four or eleven amino acids mixed in sucrose and complete nectar analoga were preferred to sucrose. *L niger* preferred a balanced mixture over an energetically similar, but less balanced mixture.

Due to the tremendous variability in gustatory preferences exhibited by the broad range of largely unpredictable ant visitors, lycaenid caterpillars should either decide on a balanced mixture, containing possible amino acid 'key compounds' in moderate concentrations, or if this investment does not pay, should secrete sugar-rich nectars.

? Based on the HPLC analyses the energetic value of the secretion was calculated for *Polyommatus coridon* as 1.0J/µl, for *P. icarus* secretions as 1.3J/µl, and for *Z. knysna* secretions as 1.2J/µl (**chapter 6**). Using the new droplet size measurements combined with published studies on secretion rates allowed estimations of the lifetime energetic value of secretions of individual caterpillars. One *P. coridon* caterpillar would deliver 5.45-24.44J, one *P. icarus* caterpillar 1.92-14.23J and one *Z. knysna* caterpillar 0.72-2.76J. Dissection and gravimetric analysis of the larvae allowed estimation of the caloric equivalent of their biomass. One early *P. coridon* third instar (i.e. at the onset of nectar secretion) would yield 17.6J, and moving prepupae 656J. *Z. knysna* second instars would yield 0.95J, third instars 3.2J, and moving prepupae 83.1J. Thus preying on the caterpillars rather than harvesting their secretions would be of greater energetic benefit. Comparisons of these new model with further reports show the rather low benefit accruing to the ants from tending facultatively myrmecophilous lycaenids for the ants. These considerations also underpin that manipulation of ants (probably by means of still unknown chemicals) must also be involved in the putatively mutualistic interactions between ants and facultatively myrmecophilous lycaenid caterpillars. The model data also suggest that the mutualistic nature of a facultative caterpillar-ant association will not always be granted, and will strongly depend on the density of caterpillars, alternative nectar sources, behavioural context and physiological status of the caterpillars and ants.

Data on nectar composition support the view that in myrmecophilous lycaenid species secretions rich in amino acids are related to intimate and often obligate ant-associations, whereas facultative and unspecific myrmecophiles rely more on carbohydrates. The degree of myrmecophily seems to be a better predictor of secretion content than taxonomic relatedness. The low investment costs of the caterpillars into nectar secretions well explain the enormous taxonomic and geographical distribution of facultative myrmecophily.

9 Zusammenfassung (German Summary)

Mutualismen zwischen Angehörigen unterschiedlicher Arten sind ubiquitär und haben einen fundamentalen Einfluß auf allen Ebenen der biologischen Organisation. Trotz ihrer Bedeutung fristeten interspezifische Mutualismen den größten Teil des 20. Jahrhunderts in der Evolutionsökologie ein Schattendasein neben antagonistischen Interaktionen wie Prädation und Konkurrenz. Erst in den letzten drei Jahrzehnten rückten sie wieder in den Blickpunkt.

Ameisen haben einen bedeutenden selektiven Einfluß in terrestrischen Ökosystemen und interagieren in interspezifischen Mutualismen mit einer Vielzahl von Arten, darunter Pflanzen, Pilze und trophobiotischen Insekten. Neben vielen Arten der Homoptera finden sich unter den Trophobionten auch Schmetterlingslarven der Familien Lycaenidae und Riodinidae. Dies mag überraschen, denn Schmetterlingsraupen gelten als ein Prototyp von Ameisenbeute. Die Larven der Lycaenidae besitzen jedoch Anpassungen in Morphologie und Verhalten, die eine friedfertige Interaktion ermöglichen. Wirkungsvolle chemische Signale werden durch mehrere Organe vermittelt. Das wichtigste Organ für die Interaktionen echter Myrmekophiler ist das dorsale Nektarorgan, mediodorsal auf dem siebten Segment gelegen. Diese Drüse besitzt ein eigenes Reservoir. Nach intensivem Betrillern durch Ameisen mittels ihrer Antennen werden winzige Tropfen eines Sekrets abgegeben, welche die Ameisen bereitwillig aufnehmen. Die Sekretabgabe steht dabei unter der Kontrolle durch die Raupe.

Die Interaktionen mit Ameisen kann myrmekoxen sein, d.h. die Raupen werden ignoriert und nicht angegriffen. Diese Interaktionen sind nicht mutualistisch, da lediglich die Raupen den Vorteil des Nichtangriffs genießen, während die Ameisen manipuliert werden. Echte myrmekophile Interaktionen dagegen können obligat oder fakultativ sein. Obligate Myrmekophile bedürfen ihrer Ameisen essentiell, sei es als Futterquelle oder zum Schutz gegenüber Feinden der Raupen. Diese Interaktionen sind hochgradig asymmetrisch, da die Ameisen nicht der Lycaeniden bedürfen. Sie sind in der Regel spezifisch, Vergesellschaftungen werden nur mit einer kleinen Anzahl Ameisenarten eingegangen und sind gekennzeichnet durch eine hohe Intimität und Stabilität. Fakultativ myrmekophile Interaktionen hingegen sind nicht durch eine hohe Spezifität hinsichtlich der beteiligten Ameisen gekennzeichnet, obwohl manche Ameisenarten häufiger an solchen Raupen gefunden werden als andere. Fakultativ myrmekophile Interaktionen, die beim Großteil aller myrmekophilen Lycaenidae-Arten (ca. 80%) zu beobachten sind, variieren in ihrer Stärke und Stabilität in weiten Grenzen. Diese Unterschiede könnten im Nährstoffgehalt der Sekrete (Zucker und freie Aminosäuren) der dorsalen Nektardrüse begründet sein. Die Sekrete wurden bisher nur sehr lückenhaft unter Angabe weniger Fakten und fast ausschließlich an obligat myrmekophilen Lycaeniden analysiert. Die Bedeutung der qualitativen und quantitativen Variabilität der Nektarsekrete für die Interaktionen fakultativ myrmekophiler Lycaenidae mit Ameisen wurde in der vorliegenden Arbeit zum ersten Mal systematisch und vergleichend untersucht.

Hierzu wurde ein stark myrmekophiler Bläuling, der europäische *Polyommatus coridon*, und zwei schwächer myrmekophile Arten, der paläarktische *P. icarus* und der (sub-)tropische *Zizeeria knysna*, ausgewählt. Die beiden *Polyommatus* Arten sind nah verwandt, während *Z. knysna* lediglich ein entfernter Verwandter innerhalb der Polyommatini ist.

? Um eine ausreichende Anzahl von Raupen zur Verfügung zu haben, wurde eine neue Methode etabliert, welche die Ei-Diapause des normalerweise univoltinen *P. coridon* mit einer Erfolgsquote von 60-70% Subitanentwicklern bei moderatem Arbeitsaufwand ermöglicht (**Kapitel 3.3**).

? Die Hauptschwierigkeit bestand im Ernten ausreichender Sekretmengen für eine chemische Analyse. Verschiedene Versuche zur künstlichen Stimulationen der Lycaenidenraupen scheiterten (**Kapitel 3.4**). Ausgehend von Verhaltensbeobachtungen an den Ameisen, speziell der Antennenbewegungen auf dem Raupenintegument, wurde eine „künstliche Ameise“ entwickelt (**Kapitel 3.5**). Diese bestand aus einer ‚astabilen Multivibrator’-Schaltung, welche die präzise Ansteuerung eines gewöhnlichen Magnetrelais ermöglichte. Dadurch wurde ein Silikonschlauch mit einem menschlichen Haar an der Spitze in regelmäßige Schwingungen versetzt, das Bewegungen auf der Raupenoberfläche ausübte. Die „künstliche Ameise“ löste (selten) Sekretabgaben aus und mit einer verbesserten Version könnte das Auslöseverhalten speziell an obligat myrmekophilen Lycaeniden-Raupen studiert werden.

? Letztlich war das Abernten nur mit Mikrokapillaren in Vergesellschaftung einzelner Lycaenidenraupen mit Ameisen der Art *Lasius flavus* erfolgreich. Trotz Verbesserung des zeitlichen Abnahmemusters mußten die Proben vor allem für die schwach myrmekophilen Arten gepoolt werden. Das Zusammentragen einer einzelnen Probe benötigte bis zu zwei Monate.

Es gelang erstmals eine präzise Größenbestimmung der Nektarsekrettropfen anhand hoher Stichproben. Die Tropfengröße beträgt 3.7nl bei *P. coridon*, 2nl bei *P. icarus* und 1.4nl bei *Z. knysna*. Für die letzteren beiden Arten ist sie damit deutlich niedriger (65-79%) als bisher in der Literatur angegebene Schätzungen (**Kapitel 3.4**).

? Die vergleichende chemische Analyse (**Kapitel 4**) mittels HPLC zeigte, daß Saccharose Zuckerhauptkomponente der Nektare aller drei Arten ist. *P. coridon*-Nektar enthielt noch Glucose und selten weitere Zucker in kleinen Mengen, jedoch keine Melezitose. Bei *P. icarus* und *Z. knysna* war Melezitose die zweitwichtigste Komponente, gefolgt von Fructose und Glucose. Die Gesamtzuckergehalte betrugen 43.6±14.8g/l bei *P. coridon*, 74.2g/l bei *P. icarus* und 68.3±22.6g/l bei *Z. knysna*.

P. coridon-Nektar enthielt bis zu 14 verschiedene Aminosäuren. Hauptkomponente ist in allen Fällen Leucin (50% des Gesamtgehalts), weitere wichtige Aminosäuren waren Tyrosin, Proline, Phenylalanin und Arginin. *P. icarus*-Nektar enthält bis zu sechs Aminosäuren, dominiert von Tyrosin und Phenylalanin. *Z. knysna*-Nektar enthielt Alanin und Prolin. Der Gesamtgehalt freier Aminosäuren betrug 9.7±3.4g/l bei *P. coridon*, 1.2g/l bei *P. icarus* und 0.3±0.17g/l bei *Z. knysna*.

Die Zusammensetzung der Hämolymphe war bei allen drei Arten sehr ähnlich. Hauptzucker war Trehalose, begleitet von Saccharose. 16 Aminosäuren traten sehr regelmäßig auf in einem Gesamtgehalt von 10.81±7.92g/l, dominiert von Histidin, Serin und Prolin. Die Aminosäurezusammensetzung unterschied sich signifikant von jener der Nektare.

Aufzucht auf verschiedenen Futtermedien hatte nur einen leichten Einfluß auf die Nektarzusammensetzung bei *P. coridon*. Fütterung mit semi-synthetischer Diät führte zu signifikant erhöhter Saccharosemenge im Nektar, mit einem Trend zu höherem Gesamtzuckergehalt. Die definierte homogene Zusammensetzung des semi-synthetischen

Futters erzeugte eine homogenere Aminosäurezusammensetzung des Nektars als bei Aufzucht mit natürlichen Wirtspflanzen (*Hippocrepis comosa*, *Securigera varia*), die unter Freilandbedingungen gezogen wurden.

Hinsichtlich der Aminosäurezusammensetzung wurde Ähnlichkeit zwischen den *Polyommatus* Arten festgestellt, während die Zuckerzusammensetzung der nicht nahverwandten schwach myrmekophilen Arten ähnlich ist. Der Grad der Ameisenassoziation bestimmt damit zumindest die Zuckerzusammensetzung des Nektars in größerem Maße als phylogenetische Verwandtschaft.

? Bioassays mit Ameisenarten (**Kapitel 5**) aus drei unterschiedlichen Unterfamilien (*Lasius niger* (Formicinae), *Myrmica rubra* (Myrmicinae), *Tapinoma melanocephalum* (Dolichoderinae)) zeigten, daß das Disaccharid Saccharose (Standard Konzentration 0.1mol/l, ähnlich zu *P. coridon*-Nektar) gegenüber Monosacchariden bevorzugt wird. Melezitose in Nektarkonzentration (*P. icarus*) wurde nicht gegenüber Saccharose bevorzugt. Bestimmte Aminosäuren in Saccharoselösungen wurden gegenüber reiner Saccharose bevorzugt, beispielweise Leucin (in Nektarkonzentration von *P. coridon*) von *L. niger* und Tyrosin oder Phenylalanin von *M. rubra*. Im Allgemeinen führte das Erhöhen der Aminosäurekonzentration nicht zwangsläufig zu höherer Präferenz, Leucin wurde z.B. in hoher Konzentration von *L. niger* abgelehnt. Mixturen von vier bis elf Aminosäuren in Saccharose und komplette Analoga der Nektare der drei untersuchten Lycaeniden wurden gegenüber reiner Saccharose bevorzugt. Beimischung von Melezitose erhöhte die Attraktivität einer Mischung von vier Aminosäuren nicht. *L. niger* bevorzugte ein ausgewogenes Gemisch gegenüber einem energetisch gleichwertigem, aber unausgewogenerem Gemisch.

Die extrem hohe Variabilität gustatorischer Präferenzen verschiedener Ameisenarten (sogar innerhalb gleicher Gattungen und auch verschiedener Kolonien) zusammen mit der Unvorhersagbarkeit der Ameisenbesucher führt dazu, daß fakultativ myrmekophile Lycaenidenarten keine optimale Nektarzusammensetzung produzieren können, die für alle Ameisenbesucher gleichermaßen attraktiv wäre. Sie sollten entweder ein ausgewogenes Gemisch, eventuell mit Schlüsselaminosäuren in moderaten Mengen, oder – falls diese Investition sich nicht lohnt – einen zuckerreichen Nektar anbieten.

? Aus den Ergebnissen der chemischen Analyse kann der energetische Wert der Sekrete berechnet werden (**Kapitel 6**). Er beträgt bei *P. coridon* 1.0J/µl, bei *P. icarus* 1.3J/µl und bei *Z. knysna* 1.2J/µl. Die Ergebnisse der Tropfengrößenbestimmung zusammen mit Sekretabgaberaten aus Literaturangaben erlauben eine Abschätzung der Energiemenge, die ein einzelnes Raupenindividuum im Laufe seiner gesamten Lebenszeit abgeben kann. Diese beträgt bei *P. coridon* 5.45-24.44J, bei *P. icarus* 1.92-14.23J und bei *Z. knysna* 0.72-2.76J.

Mittels Präparation und gravimetrischer Analyse wurde das kalorische Äquivalent der Biomasse von Lycaenidenraupen bestimmt. Eine *P. coridon*-Raupe im dritten Larvalstadium, dem frühstmöglichen Zeitpunkt der Sekretabgabe, liefert 17.6J, eine noch sekretierende *P. coridon*-Präpuppe 656J, während das dritte Larvalstadium von *Z. knysna* 3.2J und eine noch sekretierende Präpuppe 83.1J Energiegehalt aufweist. Es wäre also deutlich lohnender, die Raupe selbst als Beute zu verwerten, anstatt die Sekrete zu melken. Vergleichende Studien zu Metabolismusraten und Fouragierkosten bei Ameisen zeigen den relativ geringen Nutzen, den Ameisen aus der Interaktion mit fakultativ myrmekophilen Lycaenidae ziehen. Auch beim Zustandekommen fakultativer Bläulings-Ameisen-Interaktionen müssen deshalb manipulierende Signale beteiligt sein. Dies stellt die allgemein akzeptierte Vorstellung in Frage, daß solche Interaktionen generell mutualistischen Charakter haben. Ob es sich im Einzelfall wirklich um einen Mutualismus handelt, hängt sehr stark ab von der Dichte der Raupen, dem Vorhandensein alternativer Nektarquellen, dem Verhaltenskontext und dem physiologischen Status der Raupe und der Ameisen.

Insgesamt zeigt die vorliegende Arbeit, daß bei myrmekophilen Lycaeniden aminosäurereiche Nektarsekrete Kennzeichen enger und oftmals obligater Vergesellschaftungen mit Ameisen sind, während fakultative und unspezifische Myrmekophile zuckerreiche Nektare anbieten. Die Nektarzusammensetzung ist auch durch phylogenetische, jedoch stärker durch eine ökologische Komponente charakterisiert.

Die geringen Kosten, die den Raupen aus der Investition in die Sekrete erwachsen, erklären die weite taxonomische und geographische Verbreitung fakultativer Myrmekophilie als evolutionär stabile Life-history-Strategie bei Bläulingen.

10 Darstellung des Eigenanteils

Note for international readers: Please ignore this chapter, as it is a necessary technicality concerning the procedure of attaining the Ph.D. degree at the University of Bayreuth.

Diese Doktorarbeit folgt dem allgemein gebräuchlichen internationalen Standard einer Ph.D.-Thesis. Sie ist in separate Kapitel gegliedert, die auch gelesen werden können, ohne vorhergehende Kapitel studieren zu müssen, und die dennoch eine geschlossene Einheit bilden (kumulative Form).

Sämtliche Auswertungen in dieser Arbeit und auch die Literaturrecherche wurden von mir durchgeführt.

In Kapitel 1 führe ich allgemein in das Thema Mutualismus und den Spezialfall der Interaktionen zwischen Bläulingen und Ameisen ein, während ich in Kapitel 2 in Kürze die Biologie der untersuchten Arten erläutere. Beide Kapitel sind ausschließlich von mir geschrieben.

Die Kapitel 3-6 enthalten sämtlich Originaldaten und sind in ihrem Erscheinungsbild an Manuskripte für wissenschaftliche Veröffentlichungen angelehnt. Gemäß den Anweisungen der Promotionskommission enthält jedes Kapitel seine eigenen Literaturquellenangaben, auch wenn dies die Redundanz erhöht. Kapitel 3.1-3.4 enthalten meine Originaldaten und –ideen und wurden vollständig von mir verfaßt. Zu Kapitel 3.5 (Eigenanteil ~85%) trug T. Geister intellektuell bei (~15%). Sämtliche Daten in Kapitel 4 (Eigenanteil ~70%) wurden von mir erhoben, G. Gottsberger stellte die HPLC zur Verfügung (~15%), und K. Fiedler hatte ergänzende Vorschläge für wenig bekannte, jedoch zur Ergebnisfindung beitragende statistische Verfahren (~15%). Kapitel 5 (Eigenanteil ~85%) präsentiert meine Originaldaten, die zum Teil von einer Praktikantin und einem technischen Angestellten unter meiner Anleitung miterhoben wurden, K. Fiedler trug intellektuell zur Diskussion bei (~15%). Kapitel 6 (Eigenanteil ~95%) präsentiert meine Daten und Berechnungen, zu denen K. Fiedler noch Diskussion beitrug (5%).

In Kapitel 7 als Synopsis fasse ich die Verbindungen der Teilaspekte zusammen und erläutere deren Bedeutung. Dieses Kapitel wurde allein von mir geschrieben.

11 Acknowledgements

Foremost I have to express my sincerest thanks to Prof. Dr. Konrad Fiedler, who not only shared his enormous knowledge and provided interesting details on lycaenids from all over the world, but was always ready to enter into fruitful discussion or to debate the fine details of statistics.

I am grateful to Prof. Dr. Gerhard Gottberger (University of Ulm) for cooperation with the HPLC analyses and Hans Malchus for technical assistance (chapter **4**).

The district government of Oberfranken kindly issued permits to collect individuals in the field for starting of laboratory populations. Dr. Klaus G. Schurian kindly provided *Zizeeria knysna* larvae and pupae from the Canary Islands (chapter 3, 4).

Dr. Peter Hartmann helped acquiring the *Myrmica rubra* nests in his own garden. The Umweltbundesamt (Berlin), namely Gabriele Schrader, kindly provided the *Tapinoma melanocephalum* nests (chapter **5**).

The function generator used in the artificial stimulation experiments was kindly supplied by the Department of Experimental Physics V, University Bayreuth, Prof. Dr. Georg Eska (chapter **3.4**). The meteorological station in the Botanical Garden of the University of Bayreuth, namely Dr. Johannes Lüers, provided climate data for the period during which the bio-assays with *L. niger* were conducted (chapter **5**).

Annick Servant helped for some periods with the rearing of *Polyommatus icarus* and *Zizeeria knysna*, especially during the summer season, when the work effort was highest.

Bernd Kornmaier, Dagmar Isheim and Thorin Geister contributed valuable discussion to several drafts (based on different construction principles) of the “artificial ant“. Thorin Geister built the final version of the “artificial ant” and performed most of the experiments with it (chapter **3.5**). Under guidance Franziska Kuhlmann performed the first half of the bioassays with *L. niger* and Jörg Hager helped executing some of the last bioassays with *M. rubra* and *T. melanocephalum* (chapter **5**).

Prof. Dr. Gerhard Rambold, PD Dr. Manfred Kaib and Dr. Nico Blüthgen deserve thanks for valuable discussion of some aspects of this thesis.

I thank my colleagues in the Graduiertenkolleg, especially Stefan Dötterl, Florian Lengyel and Robert Wiedemann for their readiness to cooperate and exchange ideas. My colleagues in and outside the department, notably Claudia Ruf, Dirk Süßenbach, Heike Ackermann, Nadine Hilt and Xaver Schratt deserve thanks for several favours that made life a whole lot easier. Further indirect help and interest was abundantly supplied by people too numerous to mention.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft and was conducted in the Graduiertenkolleg 678 (“Ecological Significance of Natural Compounds and other Signals in Insects – from Structure to Function”) under co-supervision by Prof. Dr. Klaus H. Hoffmann, PD Dr. Wolfgang Völkl and Prof. Dr. Carlo Unverzagt.

My warmest thanks go to my beloved wife Wiltrud Daniels for her never ending support, discussion, encouragement, tons of editing and patience regarding the lack of weekends and the chaos in our home over a period of three years.

12 Appendix

12.1 “Artificial ant” (chapter 3.5)

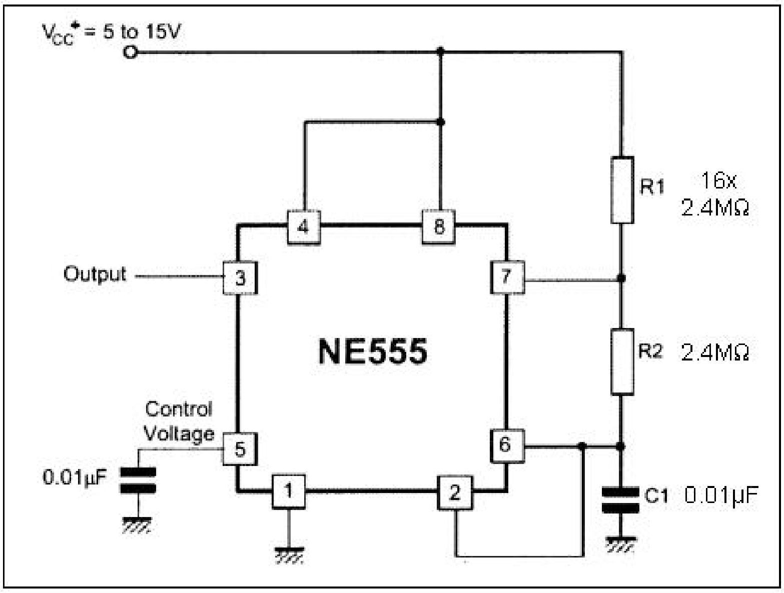


Fig. 12.1 Diagram of the circuit used for the astable multivibrator of the artificial ant. Values used for resistors and the capacitor denoted to the right.

The frequency f results out the relationship between the resistors and the capacitor, as follows:

$$f = \frac{1}{(R_1 + 2R_2)C_1}$$

thus generating frequencies from 3-20Hz (Table 12.1).

Table 12.1 Switch position and resulting frequencies in the astable multivibrator.

switch position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
resistance sum [MO]	2.4	4.8	7.2	9.6	12.0	14.4	16.8	19.2	21.6	24.0	26.4	28.8	31.2	33.6	36.0	38.4	40.8	43.2
resulting frequency [Hz]	20.0	15.0	12.0	10.0	8.57	7.5	6.66	6.0	5.45	5.0	4.61	4.28	4.0	3.75	3.52	3.33	3.15	3.0

12.2 Semi-synthetic diet formulation (“artificial food”) (chapter 4)

This semi-synthetic is based on a diet formulation after Burghardt and Fiedler 1996a, further optimised and modified for the requirements of three different species.

The basic substances are to be thoroughly ground, and finely sieved. All other substances from Table 12.2 are added and the resulting base mixture is thoroughly blended.

Plants are dried at 50°C for three days, the leaves are stripped and ground until consistency of fine powder is achieved (*Hippocrepis comosa* or *Securigera varia* for *Polyommatus coridon*, *Medicago sativa* or other Fabaceae for *P. icarus* and *Zizeeria knysna*). The plant material is to be blended with the basic mixture. One half of the water quantity (Table 12.3) and the wheat germ oil is added, the remaining water and agar are used to solidify the final diet mixture. *P. coridon* can be fed a diet of more solid consistency, as it feeds naturally on the relatively tough *H. comosa* leaves, while *P. icarus* and *Z. knysna* thrive better on a diet with soft consistency.

The final diet mixture can be stored in the refrigerator for up to two weeks. In the freezer storage for several months is possible, but after defrosting the diet looses consistency.

Table 12.2 Basic mixture for the semi-synthetic diet "artificial food". 1: optionally [ICN]: ICN Biomedicals Inc. Ohio, USA [Roth]: Karlsruhe, Germany [Fluka]: Sigma-Aldrich Chemie, Steinheim, Germany

	substance	producer	amount [g]
base substances	peas, finely ground	-	75
	wheat germs, finely ground	-	15
	"*Torula* yeast" (*Candida utilis*)	[ICN]	15
additives	Wesson salt mixture	[ICN]	5
	ascorbic acid	[Roth]	2.5
	Vanderzandt vitamin mixture for insects	[ICN]	5
	cholesterol	[Fluka]	0.5
	quercetine[1]	[Roth]	0.7
preserving agents	methyl-4-hydro-benzoate	[Fluka]	0.5
	sorbic acid	[Fluka]	0.5
antibiotic	tetracycline	[Fluka]	0.25

Table 12.3 Plant matter, oil and water quantities for the semi-synthetic diet, dependent on butterfly species

species	*Polyommatus coridon*	*P. icarus, Zizeeria knysna*
water	425ml	425ml
wheat germ oil	2.5ml	2.5ml
dried, pulverized plant matter	10g *Hippocrepis comosa* or *Securigera varia*	10g *Medicago sativa* or other Fabaceae
agar	3.75-up to 7.5g	3.75g

12.3 Erklärung

Hiermit erkläre ich, daß ich die vorliegende Arbeit selbständig verfaßt und dabei keine anderen als die angegebenen Hilfsmittel verwendet habe.

Ferner erkläre ich, daß ich diese Arbeit weder einer anderen Prüfungsbehörde vorgelegt noch anderweitig mit oder ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

Bayreuth, den

Holger Daniels