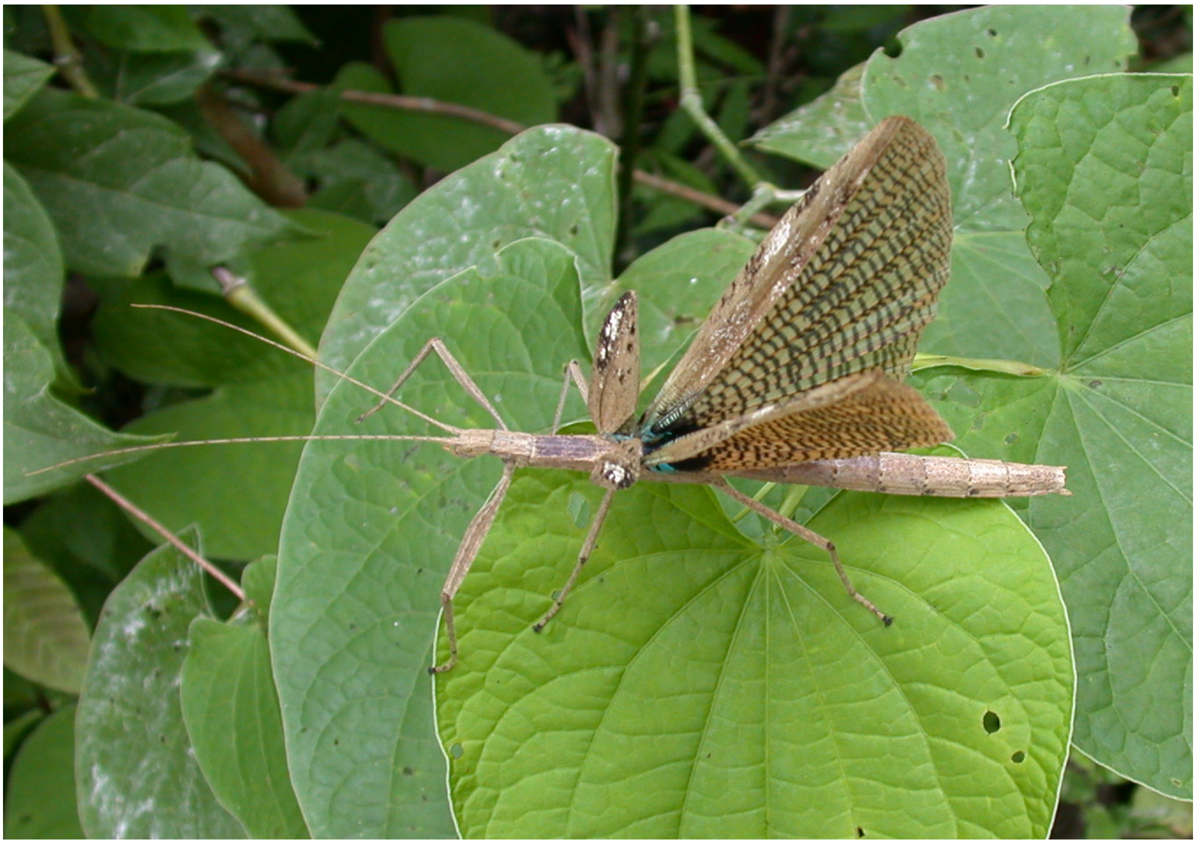
ECOLOGY OF PHASMIDS (PHASMATODEA) IN A MOIST NEOTROPICAL FOREST:

A STUDY ON LIFE HISTORY, HOST-RANGE AND BOTTOM-UP *VERSUS* TOP-DOWN REGULATION



Dissertation zur Erlangung des
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vorgelegt von

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Cover: A female of *Metriophasma diocles* displays all its beauty (on *Piper marginatum*; © by the Author).

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Phasmids, the most peculiar beasts,
'*between the Devil and the Deep Blue Sea*'
(Lawton & McNeill 1979)

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List of Abbreviations

%	percent
ANOVA	Analysis of Variance
BCI	Barro Colorado Island
BCNM	Barro Colorado Nature Monument
cf.	confer
cm	centimeter
CTFS	Center for Tropical Forest Science
d	day
df	degrees of freedom
e.g.	*"exempli gratia"* [Lat.]; ‘example given’ or ‘for example’
g	gram
GOF	Goodness of Fit
ha	hectare
i.e.	*"id est"* [Lat.]; ‘that is’
ind.	individual
m	meter
mg	milligram
mm	millimeter
N	Nitrogen
N	sample size
ºC	degree Celsius
P	probability
pers. obs.	Personal observation
SE	Standard-Error
SLW	specific leaf weight
StDev	Standard-Deviation
STRI	Smithsonian Tropical Research Institute
TAE	Tannic Acid Equivalent

Abbreviations used in particular statistical or analytical methods are explained in the text.

1 Introduction

1.1 General aspects

Tropical rainforests are renowned for their vast biodiversity and high productivity. For understanding of the factors that structure these communities and maintain ecosystem stability, the relationships among trophic levels are of particular interest. Increasing attention has been paid to plant-animal interactions, and herbivory is discussed as a key agent in maintaining forest dynamics and ecosystem stability (Lowman 1984; Brokaw 1985; Schowalter *et al.* 1985; Brown *et al.* 1987). In tropical forests, herbivores consume up to 11 percent of annual leaf production (Coley & Barone 1996). Such strong pressure is thought to select for the evolution of plant defenses and high plant biodiversity (Ehrlich & Raven 1964; Janzen 1970; Connell 1971; Rhoades & Cates 1976; Coley *et al.* 1985). In return, high variety and high regimes of plant defenses are thought to favor high diversity of herbivores in the tropics (Janzen 1973; Coley & Aide 1991). Alternatively, natural enemies may account for selection towards narrow niches of their prey and thereby promote high diversity of tropical faunas (Pianka 1966; Price *et al.* 1980; Bernays & Graham 1988). Whether populations at different trophic levels are limited by resources (bottom-up, *sensu* Murdoch 1966; White 1978; McNeill & Southwood 1978) or due to consumption by higher trophic levels (top-down, *sensu* Hairston *et al.* 1960) is subject to ongoing debate.

Given the high biodiversity and productivity of tropical rainforests, it is most likely that processes that structure tropical communities may be fundamentally different than processes in temperate ecosystems. As a consequence, ecological paradigms developed in temperate systems may not be useful for understanding tropical systems (Dyer & Coley 2001). In fact, relationships among trophic levels seem to be converse depending on latitude. Tropical plants compared to temperate species seem to be richer in plant defenses both in variability and amounts (*e.g.*, Coley & Kursar 1996). But the impact of plant defenses appears to be greater in temperate regions (Coley & Barone 1996). The opposite pattern emerged for predators. There is evidence that the impact of predators on herbivores is stronger in the tropics than in the temperate zone (reviewed in Dyer & Coley 2001). However, there is consensus that tropical herbivores are kept at bay by both bottom-up and top-down regulation mechanisms (Pace *et al.* 1999; Persson 1999; Polis 1999). In other words: herbivores are between the devil (natural enemies) and the deep blue sea (poor food) (Lawton & McNeill 1979). In the debate about the relative roles of resource limitation and predators in controlling tropical herbivore populations, evidence suggests that natural enemies may be the major control factor (Dyer & Coley 2001).

Regardless whether strong impact from higher or lower trophic levels limits herbivore populations, current theories assume that this selection pressure has led to narrow niches (Pianka 1966; MacArthur & Wilson 1967). Since food is one of the most important dimensions of the niche (Krebs 1989), niche breadth is generally expressed in terms of feeding specialization of herbivores (Bernays & Chapman 1994). This debate is of broad interest because estimates of global biodiversity are largely based on the degree of specialization of tropical insects. Just recently global biodiversity estimates were corrected from formerly stated 31 million (Erwin 1982) to about 4 to 10 million species (Ødegard 2000; Novotny *et al.* 2002a) because increasing research effort indicates that specialization is less pronounced than formerly supposed (Basset 1996, 1999; Basset *et al.* 1992, 1996; Barone 1998; Novotny *et al.* 2002a).
Generally, these studies state that most tropical insect herbivores are rare (Basset 1996, 1999; Basset *et al.* 1992, 1996; Barone 1998; Novotny *et al.* 2002b), but they represent the plants view of herbivore specialization, *i.e.* the herbivore communities of one or several plant species are sampled, and thus may cover only a fraction of density and distribution patterns of insect herbivores.
There is an enormous lack of research which addresses the densities of insect communities, let alone the densities and distribution of single insect herbivore species in tropical forests. The scarce information on this topic derives from studies using trap techniques (Wolda 1978, 1980, 1983, 1992; Smythe 1982), or describing the overall arthropod community (Janzen & Schoener 1968; Elton 1973). Beyond doubt, these studies contributed important baseline data for the development of general ideas, but they either incorporate species particularly prone to a certain trapping device, or all insect feeding guilds. Surprisingly few studies have reported the most basic demographic parameters for tropical insect herbivores, distribution and population density (Smiley 1978; Willig *et al.* 1986, 1993; Willig & Camilo 1991).

1.2 Current knowledge about tropical phasmid ecology

Although phasmids are common herbivores in many tropical ecosystems, little is known about the biology of these hemimetabolous insects (Bedford 1978; Van den Bussche *et al.* 1989; Willig *et al.* 1986, 1993). The order Phasmatodea (walkingsticks and leaf insects) is one out of nine orders of phytophagous insects and contains approximately 3000 described species that occur worldwide with a concentration in tropical regions (Whiting *et al.* 2003). Generally, phasmids are considered as herbivore generalists although empirical studies on diet breadth of natural populations are missing (Bedford 1978; Willig *et al.* 1986, 1993; Sandlin & Willig 1993).

The significance of phasmids as herbivores has attracted some interest as they can reach plague numbers and may cause severe damage to forests (temperate forests: Graham 1937; Australian Eucalypt forests: Campbell 1960, 1961, 1974; Campbell & Hadlington 1967) or to agricultural plantations (Pacific coconut plantations: Paine 1968; Swaine 1969). Such herbivores exerting strong pressure may be key agents in maintaining forest dynamics and ecosystem stability (Lowman 1984; Brokaw 1985; Schowalter *et al.* 1985; Brown *et al.* 1987). Their relevance as herbivores in tropical forests was confirmed in studies on the abundant endemic Puerto Rican species *Lamponius portoricensis* REHN. Population density of this polyphagous species seems to temporally vary to an enormous extent (Willig *et al.* 1986, 1993; Willig & Camilo 1991). Yet, besides drastic reductions in population densities following natural disturbance of high intensity (Willig & Camilo 1991) the causal factors of these fluctuations largely remain unknown. Host plant densities partly explained distribution patterns and patch densities of *L. portoricensis* (Willig *et al.* 1993), and individuals displayed differential preference among hosts depending on age, sex and prior experience (Sandlin & Willig 1993). This preference may be the result of specific nutritional constraints but could also reflect selection by predation pressure: Sandoval (1994) showed that color morphs of an endemic Californian species suffered differential predation on varying host plants thereby propagating specialization and speciation.

A variety of behavioral and morphological adaptations underline the importance of predation-related selection on phasmid evolution. Most phasmids species exhibit a repertory of predation-avoidance behaviors (Robinson 1968a, 1968b, 1969; Bedford 1978). They live cryptic, resembling sticks and leaves, and the majority of species is active at nights (Bedford 1978). Most phasmid species dispose of glands (Bedford 1978) whose secretions were shown to deter predators (Eisner 1965; Eisner *et al.* 1997). Many phasmid eggs attract ants with a protein rich cap (Capitulum) and may thereby be protected from parasitoid wasps (Compton & Ware 1991; Hughes & Westoby 1992; Windsor *et al.* 1996).

Information on phasmid species of Panamá is restricted to the early taxonomic work of Hebard (1923, 1929, 1933) and the studies about the defensive behavior of several phasmids by Robinson (1968a, 1968b, 1969). Panamanian phasmids have been observed feeding on plant species of the family Araceae (Robinson 1969). And phasmids are reported to feed on *Piper* spp. (Piperaceae) in Costa Rica (Marquis 1991) and Puerto Rico (Willig *et al.* 1986, 1993; Sandlin & Willig 1993).

1.2.1 Design and aims of the study

In this dissertation, I take an approach to contribute to the understanding of the ecological processes affecting distribution and density of tropical insect herbivores. Starting from descriptive information on the phasmid community on Barro Colorado Island (BCI) (Chapter 2), the study then focuses on population parameters of the phasmid *Metriophasma diocles* WESTWOOD (Chapter 2&3) and on experiments on potential control factors related to the bottom-up (Chapter 4) and top-down (Chapter 5) views of population regulation (Figure 1-1).

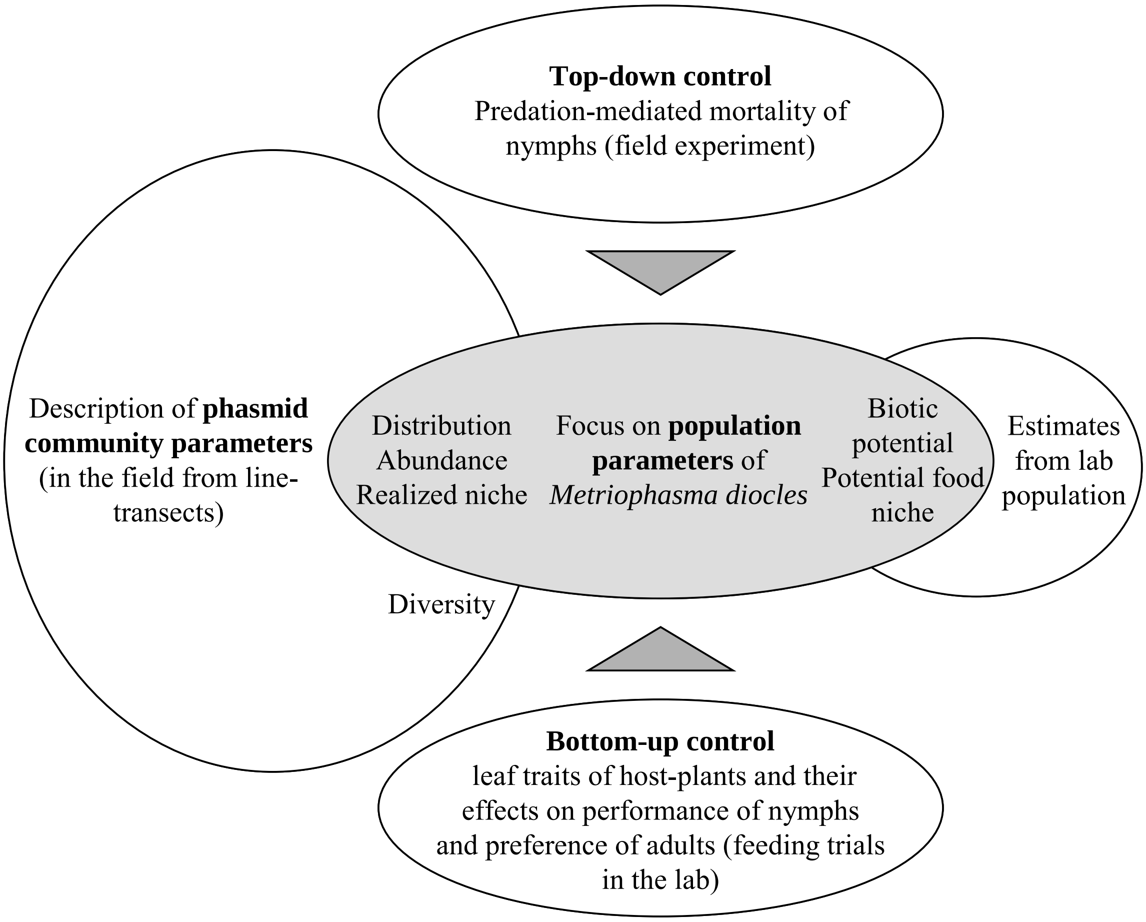

Figure 1-1: Study design: The outer circles represent the main focuses of Chapters 2 to 5.

As a first step, Chapter 2 addresses the structural aspects of the phasmid community on BCI. Distribution and abundance of herbivores may be modulated by resource limitation (*e.g.*, Joern & Gaines 1990), and knowledge about the food niche of herbivores is crucial for an evaluation of resource restrictions. If phasmids behave as specialists, their habitat should generally reflect the habitat of their host plants. In contrast, generalists should be less dependent on host plant distribution. Thus uncovering the diet breadth of herbivores directly relates to understanding habitat choice. In combining information on (1) plant species phasmids were observed on along line-transects with (2) feeding-trials in the lab the realized food niche can be separated from other niche dimensions that are typically incorporated in field studies. The difference between realized and absolute food niche then allows to conclude on other impact factors like climate or natural enemies. The estimation of phasmid densities provides a basis for the following chapters.

The realized population densities reflect an organism in the boundaries of its environment (Begon *et al.* 1996). In Chapter 3, I meet the question what is the inherent potential of the phasmid species *Metriophasma diocles* to reproduce free from environmental limitations. I modeled the potential population growth of *M. diocles* on the basis of life cycle parameters like sex ratio, female fecundity and generation time assessed from a laboratory population. The difference between potential and realized population densities then reflects the impact of control. Population control factors can act pre- or post-natal. Here, I present a first estimate of egg mortality and failed hatching and show the effect of pre-natal mortality on potential population increase of *M. diocles*.

Dietary constraints of *M. diocles* are the subject of Chapter 4, addressing the bottom-up view of herbivore regulation. Nutritious requirements of phasmids may be age or sex-specific (*e.g.*, Cassidy 1978; Sandlin & Willig 1993), and early life stages of herbivorous insects may suffer high mortality depending on the food source and its species-specific physical and chemical leaf characteristics (Joern & Gaines 1990; Belovsky & Slade 1995). In feeding-trials, I observed performance (*sensu* growth and survival) of first instar nymphs and preference of adult females on different host plants reflecting *M. diocles* host range. Performance of nymphs and preference of adults were analyzed with respect to particular defensive leaf traits that potentially reduce digestibility of the foliage or are toxic to the organism.

In Chapter 5 I am concerned with the top-down view of *M. diocles* population regulation. In a predation-exclusion field experiment I assessed the impact of predators on first instar nymphs of *M. diocles*. A general pattern in insects is high mortality of early life stages with high impact of predation, particularly by parasitoids (Cornell & Hawkins 1995; Cornell *et al.* 1998). While these patterns arose from studies on holometabolous insects, knowledge on hemimetabolous herbivores mainly originates from studies on temperate grasshoppers (*e.g.*, Joern & Gaines 1990; Belovsky & Slade 1995; Oedekoven & Joern 1998).

1.3 Site characterization and general methods

1.3.1 Study site, vegetation and climate

The study took place on Barro Colorado Island (BCI; 9°09’N, 79°51’W), a field site of the Smithsonian Tropical Research Institute (STRI) in Panamá (Figure 1-2). The island of 1567 ha is located in the lake Gatun. This freshwater lake was dammed up between 1911 and 1914 during construction of the Panama Canal. The island is the centerpiece of the 5600 ha Barro Colorado Nature Monument (BCNM) that was established in 1978 and includes the adjacent mainland peninsulas. In 1923, BCI was declared as biological reserve and in 1946 it became a unit of the Smithsonian Institution. Since then, the island has become one of the most intensively studied areas in the tropics.

BCI is completely covered with semi-deciduous tropical moist forest of several successional stages (Foster & Brokaw 1982). The northeastern part of the island consists of 100 to 200 year old secondary forest whereas old forest of 200 to 400 years covers most of the southeast of BCI. A small strip of old forest remained south of the laboratory (surrounding Lutz Creek). The area around the laboratory is cleared and the vegetation is dominated by pioneer plant species such as *Cecropia*, *Ochroma*, *Trema*, *Solanum* and *Piper* (Thies 1998). The flora of BCI comprises more than 1200 plant species and is described in detail by Croat (1978).

Annual rainfall on BCI averages 2634 mm (Paton 2002). There is a distinctive seasonality in rainfall with a wet season from May to mid-December and a pronounced dry season approximately from mid-December to the end of April (Figure 1-3). On average, only 293 mm of rain falls during the dry season. The annual average temperature on BCI is 27 ºC, with a diurnal variation of 7-8 ºC. A detailed description on vegetation and climatic conditions of BCI is given in Leigh *et al.* (1990).

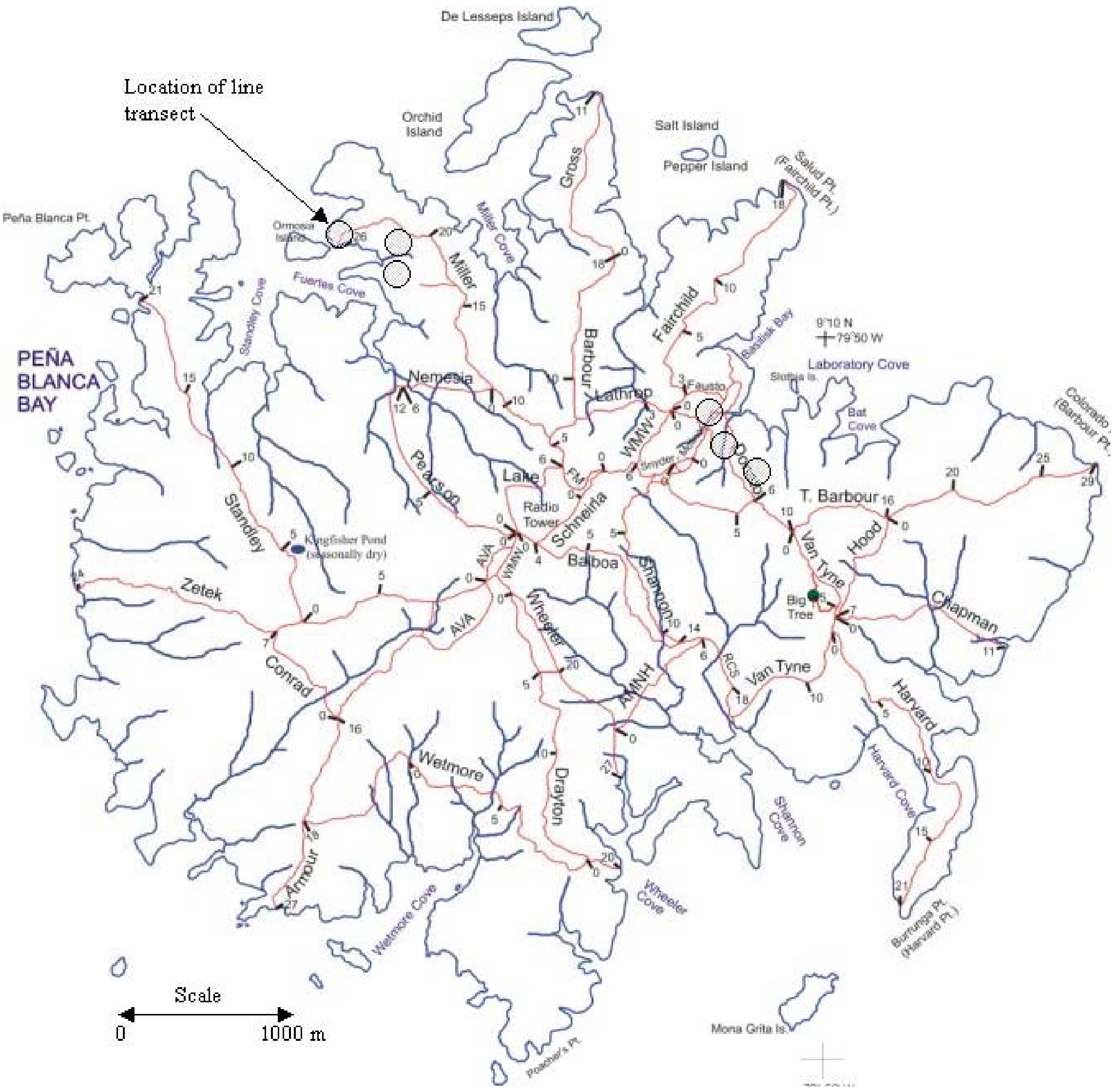

Figure 1-2: Map of Barro Colorado Island (BCI), Panama (source STRI; modified). Lines with names represent trails, numbers assign distances from the trail origin (x100 m). Bold lines illustrate streams. Circles mark locations of line-transects (see Chapter 2).

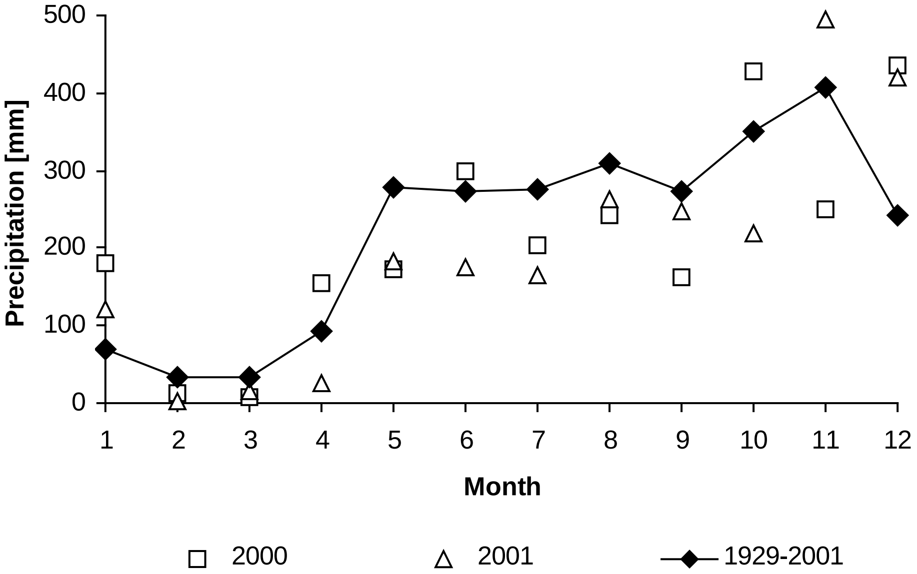

Figure 1-3: Precipitation on Barro Colorado Island in the years 2000 and 2001 compared to the long-term average (1929 to 2001; source Paton 2002).

1.3.2 Maintenance of phasmid species in the laboratory

The phasmids used for setting up lab colonies were collected by hand in a haphazard way during specific night walks in forest parts and clearings other than that used for line-transects. Field collections were made during a cumulative period from July 1999 to December 2001. Lab populations of *Metriophasma diocles* WESTWOOD, *Bacteria ploiaria* (WESTWOOD) and *Otocrania* sp. could be established permanently, while nymphs of *Oncotophasma martini* (GRIFFINI) could be reared from eggs but no females grew to maturity. Consequently the population collapsed in April 2000 and was given up, as it could not be provided with sufficient animals from field collections. Phasmid species were maintained in cages (1 m height, 0.5 m depth, 0.5 m width, covered with mosquito net) in screened and shaded rooms. Cages were sprayed with fresh water daily and provided with edible plants periodically. Eggs were collected weekly when cages were cleaned.

1.3.3 Identification of phasmid and plant species

Phasmid species were identified in cooperation with the taxonomist Sven Bradler at University of Göttingen, Germany. Vouchers were deposited at the STRI insect collection in Panama City, Republic of Panamá. Plant species were identified with the help of Croat’s Flora of BCI (Croat 1978) and verified by Rolando Pérez, Andrés Hernández and Salomón Aguilar who work as field botanists for the Center for Tropical Forest Science (CTFS). Plant Vouchers were deposited at the herbarium of BCI, Republic of Panamá.

1.3.4 Data analysis

For statistical analysis I used the computer-based programs Statistica (StatSoft Inc. 2001) and JMP (SAS Institute Inc. 2001). Data were processed in Excel (Microsoft Corp. 2000). Figures were created in SigmaPlot (SPSS Inc. 1999), Excel and Statistica.

2 Community structure and host range of phasmids on BCI

2.1 Introduction

Biodiversity of tropical forests is markedly higher than in their temperate counterparts (*e.g.*, Mac Arthur 1972; Erwin 1982) and herbivorous insects constitute a major fraction of species (Erwin 1982; Stork 1993; Ødegard 2000; Novotny *et al.* 2002a). The origin of tropical species diversity is partly hypothesized to be based on the arms raise between plants and their consumers. Their impact on leaves is thought to represent an important selective force for the evolution of plant defenses (Ehrlich & Raven 1964; Rhoades & Cates 1976; Coley *et al.* 1985). Also, herbivory on seeds and seedlings has been attributed to the high diversity of tropical plant communities (Janzen 1970; Connell 1971). In return the greater variety and higher regime of plant defenses in tropical forests are discussed to favor narrower diets of tropical insect herbivores thereby leading to a higher arthropod diversity than in temperate forests (Janzen 1973; Coley & Aide 1991).

Aside from plant defenses, natural enemies can select for narrower diets since they have different abilities to locate a herbivore on variable plant species (Price *et al.* 1980, Bernays & Graham 1988).

In contrast, the scarcity of most plant species in tropical forests (Hubbell & Foster 1986) may favor the generalist habit if locating them is difficult and costly for a specialist herbivore, both in terms of time and exposure to predators (Jaenike 1990; Basset 1992; Coley & Barone 1996).

Indeed most tropical insect herbivores seem to be relatively specific. Even though the debate on the degree of host specificity remains controversial (*e.g.* Erwin & Scott 1980; Erwin 1982; Basset 1999; Basset *et al.* 1992, 1996; Barone 1998; Ødegard 2000; Novotny *et al.* 2002a, 2002b) there is evidence that 90 percent of all phytophagous insects feed on plants in less than three different plant families (Bernays & Graham 1988; Marquis & Braker 1994; Barone 1998).

The majority of insect herbivore species in tropical forests is rare (Basset 1996, 1999; Basset *et al.* 1992, 1996; Barone 1998; Novotny *et al.* 2002b) and abundances vary temporally and spatially. In seasonal tropical forests, abundances of many insect species are positively correlated to rainfall (Wolda 1978, 1979, 1982, 1992) and leaf production (Murali & Sukumar 1993). Concerning the maintenance of low overall population densities, the relative roles of lower or higher trophic levels are subject to ongoing debate but evidence suggests that top-down effects are more important (Coley & Barone 1996; Pace *et al.* 1999; Persson 1999; Polis 1999; Dyer & Coley 2001). On a spatial scale insect herbivore densities are generally assumed to be higher in light gaps than in the forest understory, but this has mainly been inferred from leaf damage (*e.g.*, Coley, 1980, 1982, 1983; Coley & Barone 1996 and references therein). Empirical studies on spatial variation in tropical insect herbivore abundances are scarce (*e.g.*, Elton

1973; Janzen & Schoener 1968; Smiley 1978; Willig *et al.* 1986, 1993; Willig & Camilo 1991; Braker 1991).

Studies assessing diversity and host specificity of herbivores are often descriptive and frequently based on collection records (*e.g.* Wood & Olmstead 1984; Janzen 1988; Hodkinson & Casson 1991). Conclusions from such approaches on specialization of herbivores are problematic because they generally are based on the counts of occurrence of insect herbivores on different plant species. Therefore, they depend on the extent of records (Barone 1998). With increasing sample size, there is an inherent increase in plant species observed for a particular herbivore species. But more importantly, collection records do not allow to differentiate if a plant species really is part of an insects diet or not (Basset 1997; Novotny & Basset 2000). Therefore estimates of resource use from field observations represent the *multidimensional realized niche* of herbivores, and plant records may diverge into dimensions like food, mating, oviposition, or just transience (Begon *et al.* 1996).

One possibility to separate a herbivore's food niche from other niche dimensions, and thereby giving a more accurate estimate of specialization levels, is combining field records with feeding trials (*e.g.*, Basset 1996, 1999; Basset *et al.* 1992, 1996; Barone 1998; Novotny *et al.* 2002a, 2002b). Feeding trials result in an estimate of the *absolute food niche* of a herbivore (Krebs 1989). This is the potential host range of a herbivore when released from biotic or abiotic factors which limit the use of palatable plants under natural conditions. Information about the *absolute food niche* together with information of field collection records (representing the multidimensional *realized niche*) allows approaching the *realized food niche*. Differences between absolute and realized food niche then give a first insight into the significance of resources, competition and predation in shaping plant-herbivore systems.

When discussing the factors that may have led to the observed restriction in using food resources, evolutionary and ecological processes have to be considered. A first insight into host range evolution can be gained by applying conventional definitions of host plant range on the results of feeding trials (representing the absolute food niche). According to Bernays & Chapman (1994) the categories of host range usually are recognized as: (1) monophagous, *i.e.* feeding on plants within a single genus; (2) oligophagous, *i.e.* feeding on plants within a single family; and (3) polyphagous, *i.e.* feeding on plants from different families. This concept refers to taxonomic relationships of host-plants and is based on the classical theory that the capability of insects to handle allelochemicals of other plant taxa declines with their increasing phylogenetic distance to the original host plant taxa (Ehrlich & Raven 1964).

On the other hand, restrictions in insect herbivore host range may be associated with chemical plant properties that are not exclusively governed by phylogeny. For example, according to current plant defense theories, mature leaves of shade-tolerant, slow growing plant species (persistent) are better defended than mature leaves of shade-intolerant, fast growing plant species of gaps and forest edges (pioneers) (Coley 1983; *Coley et al.* 1985) and hence are less digestible. Consequently, a generalist herbivore should preferentially feed on leaves of pioneer plant species (Berenbaum *et al.* 1984).

Although phasmids are common herbivores in many tropical systems, little is known of their biology (Bedford, 1978; van den Bussche *et al.*, 1989; Willig *et al.*, 1986, 1993). Generally phasmids are considered as herbivore generalists but specialization ranges from strict monophagy to wide polyphagy (Bedford 1978). With about 3000 species worldwide (Whiting *et al.* 2003) phasmids are comparably poor in diversity and many species are known to occur in low abundances in humid tropical forests (Novotny & Basset 2000; but see Willig *et al.* 1986). Accordingly, studies to date indicate that the Panamanian phasmid community is poor in species (Hebard, 1923, 1929, 1933; Robinson, 1968a, 1968b, 1969) while there is no information available on density, distribution or specialization patterns.

Ecological information for neotropical phasmids is entirely restricted to the studies on the abundant endemic Puerto Rican species *Lamponius portoricensis* (Willig *et al.* 1986, 1993; Willig & Camilo 1991, Sandlin & Willig 1993). Distribution patterns and patch densities of this polyphagous species could partially be explained by host plant availability (Willig *et al.* 1993) and drastic reductions in population densities followed natural disturbance of high intensity (Hurricane Hugo; Willig & Camilo 1991). However, the causal factors of temporal fluctuations in *L. portoricensis* densities largely remain unknown.

Here, I assessed diversity, distribution, and density of the phasmid community on BCI by monitoring line-transects in the forest understory and along forest edges, and I determined the absolute food niche of four phasmid species by conducting feeding-trials.

The possible range of host plants of phasmid species was determined by presenting them with four groups of plants. (1) I tested phylogenetic restrictions in host range by offering plant species out of the same genus, the same family and other families than the original host-plant belonged to (*i.e.* where they were observed on). To test for constraints in habitat selection connected to food digestibility and availability, I presented phasmids (2) with persistent and pioneer plant species representing the forest understory and forest edges or gaps, and (3) with a selection of 10 of the most abundant tree species on BCI (>1000 ind.*50 ha^{-1}; Condit *et al.* 1998). (4) The validity of the assessed host range was tested by offering a random selection of plant species.

2.2 Materials and methods

For details on study site and the maintenance of lab populations please refer to Chapter 1.

2.2.1 Line-transects

To describe diversity, distribution and population densities of phasmid species in forest understory versus forest edge habitats, I collected data along line-transects from May 2000 to July 2001. I established 3 line-transects of 80 m length each in the forest understory and at forest edges (for locations see Figure 1-2). Transects were monitored at night every two weeks. The vegetation was scanned with headlamps up to 2 m heights and in a one-sided width of about 2 m from the line center (to both sides in the understory and to one side at the forest edge). Phasmids were classified to morphotypes, and released again after body length was measured.

2.2.2 No-choice feeding trials

To estimate breadth and overlap of the realized niches of phasmid species I recorded all plants on which phasmids were found along line-transects. As observations of feeding in the field were rare, all plant records were treated as potential host plants. These plant species were then tested in subsequent no-choice feeding trials.

To determine diet breadth (*sensu* absolute food niche) phasmid adults and nymphs were presented with leaves from alternative plant species in a no-choice design. Adult leaves were harvested in the forest, placed in a sealed plastic zip log bag and brought back to the laboratory. Within 2 hours of being collected leaves or leaf discs were presented to phasmids. Feeding trials were conducted in a screened room subject to ambient conditions. According to the size of the animals, plastic containers of different sizes were used as trial arenas. The lid of such arenas was screened to allow for ambient conditions. Each plant species was tested with a minimum of three *M. diocles* individuals. Adult phasmids and large nymphs (instars five and six) were presented with whole leaves. Leaves were offered with water supply by sticking the petiole in a water-filled vial and fastening it with Cotton. Small nymphs (instars one to four) were offered leaf discs (punch of 13 mm diameter) that were stuck into a piece of cardboard to assure free access for the nymph. A piece of humid paper towel helped minimizing desiccation. Feeding trials began in the late afternoon and lasted 24 hours. In cases where the leaf received only a few bites or was not eaten at all, it was considered inedible (see for comparison Barone 1998). As soon as one phasmid individual obviously had fed on a leaf, the according plant species was recorded as edible. In general, phasmids fed on leaves in a measurable way. If a leaf was left untouched while a nymph had moulted, the trial was discarded.

Four different groups of plants were offered to the four phasmid species maintained in the laboratory: (1) potential host plants, *i.e.* plant species where phasmids were found on in the field; (2) congeners and confamilials of the plant species that were proven edible; (3) a selection of 10 of the most abundant tree species on BCI (>1000 ind.*50 ha^{-1}; Condit *et al.* 1998); (4) a random selection of plant species from forest understory and forest edge. For the latter, I asked field botanists on BCI to bring leaves of a random plant species back to the lab.

2.2.3 Data analysis

2.2.3.1 Estimating phasmid community parameters

A list of phasmid species on BCI was achieved by means of all available field records (*i.e.* line-transect and collection records). Field records besides line-transect data have not been used for analysis of phasmid community structure (*e.g.*, niche breadth or niche overlap) for three reasons: 1) these data were biased towards collection on specific host plants and hence towards focal phasmid species; 2) rare phasmid species were underrepresented in these field records; and 3) species abundances would be underestimated because of the collection (*i.e.*, removal) of specimens from the community. To calculate relative abundances of phasmid species data from line-transects in the understory and at forest edges were pooled (for each habitat per monitor date) due to too many zero counts, especially in the understory.

This data also built the pool for the calculation of diversity, eveness and similarity of phasmid communities for the specific habitat. All measures of community structure were based on methods described by Krebs (1989). To facilitate comparison among studies, I decided to use the most common measures (applicability to the dataset presupposed).

Similarity in species composition between habitats was calculated with the index of Morisita C_λ:

Equation 2-1

$$C_\lambda = \frac{2\sum^n X_{ij}X_{ik}}{(\lambda_1+\lambda_2)N_jN_k}$$

where C_λ = Morisita's index of similarity between samples j and k

X_{ij}, X_{ik} = Number of individuals of species i in samples j and k

N_j, N_k = $\sum X_{ij}$, $\sum X_{ik}$ = Total number of individuals in sample j and in sample k respectively

$$\lambda_1 = \frac{\sum^n\left[X_{ij}\left(X_{ij}-1\right)\right]}{N_j\left(N_j-1\right)} \quad \text{and} \quad \lambda_2 = \frac{\sum^n\left[X_{ik}\left(X_{ik}-1\right)\right]}{N_k\left(N_k-1\right)}$$

The Morisita index of similarity is a quantitative measure for similarity and ranges from 0 for no similarity in species composition to 1 for identical species composition.

Species diversity and eveness were calculated on the base of the Shannon-Wiener Function:

Equation 2-2

$$H' = \sum_{i=1}^{s}(p_i)(\log_2 p_i)$$

where H' = Index of species diversity measured by the Shannon–Wiener function

s = Number of species

p_i = Proportion of total sample belonging to the species i

The Shannon–Wiener index of species diversity expresses the uncertainty of predicting the species of the next individual sampled. Thus if H' = 0 then all individuals in a sample belong to one species. H' is increasing with the number of species in a sample and with increasing eveness of these species. The most common approach to measure eveness is to scale the observed index of species diversity relative to its maximum value when each species in the sample is represented by the same number of individuals. In case of the Shannon Wiener Function maximum diversity occurs when $p = 1/S$ and can be calculated as:

Equation 2-3

$$H'_{MAX} = \log_2 S$$

where H'_{MAX} = Maximum possible value of the Shannon-Wiener Function

S = Number of species in the community

Eveness ranges from 0 to 1. Generally all eveness measures are overestimated (see Krebs 1989) as they assume that the total number of species in the community is known. However, in the presented study this problem may be of minor importance as the phasmid community in forest understory and at forest edges was sampled excessively.

On the base of line-transect data I calculated population density of the phasmid community and in particular population density of *M. diocles* for forest understory and forest edges separately. Generally population densities from line-transect data can be calculated as described by Krebs (1989):

Equation 2-4
$$D = \frac{n}{2La}$$

where D = Density of animals per unit area

n = Number of animals seen on transect

L = Length of transect

a = Constant that describes the probability of detecting an animal

The estimation of the detection probability a affords knowledge of sighting distance and angle for each animal measured from the transect line. These parameters could not be assessed because for detection of nymphs the undersides of leaves had to be searched and therefore I had to leave the central transect lines. Consequently, I simplified the calculation of density by assuming that detection probability was 1 (*i.e.*, all animals were found) and thereby that 'a' was negligible (density estimates based on numbers of observed animals solely are sometimes referred to as apparent density; Krebs 1989). This assumption may have lead to an underestimation of densities, as it was likely that animals were missed. Further, I accounted for the fact that in the understory line-transects were searched on both sides while at forest edges they were sampled one-sided facing the forest (width of 2 m). Accordingly, in transects of 80 m length, the corresponding searching area in the understory was 320 m^2 versus 160 m^2 at forest edges. These modifications led to the calculation of population densities by:

$D = \frac{n}{2Lw}$ for forest understory, and $D = \frac{n}{1Lw}$ for forest edges,

where w is the one-sided monitored width of the transect.

Resulting phasmid densities (ind.*m^{-2}) for transects were averaged on habitat level per monitor date (28 monitoring dates and three transects per habitat). To allow for comparison I extrapolated densities to the base of number of individuals per hectare searching area (ind.*ha^{-1}).

Under the assumption that phasmids would show a pronounced seasonal pattern in densities, I expected higher densities of phasmids (and particularly of nymphs) in the early rainy season. Seasonal variation in abundances for nymphs and adults was compared on the base of cumulative absolute abundances on a monthly scale. As data for the forest understory were few, I only analyzed abundances in forest edges. Data for November 2000 and July 2001 were excluded from the analysis, because only one monitor date was available for each of these months. I tested for temporal heterogeneity of absolute abundances by comparing observed and expected abundances in Chi-Square Goodness-of-Fit Tests (GOF). Although data for phasmid abundances may not be independent, *e.g.* because of contagiousness, GOF analysis is appropriate if the user is only interested in heterogeneity or homogeneity (Fowler *et al.* 1998).

2.2.3.2 Estimating niche dimensions and overlap

As one dimension of niche breadth, I assessed diet breadth using the potential food resources (*i.e.* plant families) registered along line-transects. Because none of the four tested phasmid species was specialized below the family level, all niche measures were expressed on the plant family level, *i.e.* each potential host plant family represented a resource state. As measure for uniformity of distribution of individuals among the resource states I used Levin's standardized measure of niche breadth B_A:

Equation 2-5
$$B_A = \frac{B-1}{n-1}$$

where B_A = Levin's standardized niche breadth

B = Levin's measure of niche breadth $B = \frac{1}{\sum p_j^2}$ with p_j = Proportion of individuals found in resource state j

n = Number of possible resource states

Levin's standardized measure of niche breadth ranges from 0 to 1. B_A is maximal when a species does not discriminate among resource states.

Overlap in the use of food niches among phasmid species was calculated by Pianka's measure of niche overlap O_{jk}:

Equation 2-6
$$O_{jk} = \frac{\sum^n p_{ij} p_{ik}}{\sqrt{\sum p_{ij}^2 \sum p_{ik}^2}}$$

where O_{jk} = Pianka's measure of niche overlap between species j and species k

p_{ij} = Proportion resource i is of the total resources used by species j

p_{ik} = Proportion resource i is of the total resources used by species k

Pianka's measure of niche overlap ranges from 0 (complete separation of niches) to 1 (complete overlap).

To describe overlap between realized food niche (*i.e.* potential host plant families from line-transects) and absolute food niche (*i.e.* plant families accepted in feeding-trials) I used Sørensen's coefficient of similarity S_S:

Equation 2-7
$$S_s = \frac{2a}{2a+b+c}$$

where S_S = Sørensen's similarity coefficient

a = Number of plant families recorded in line-transect and accepted in feeding-trials

b = Number of plant families only recorded in line-transect records but not accepted in feeding-trials

c = Number of plant families only accepted in feeding-trials but not recorded in line-transects

Sørensen's similarity coefficient is a qualitative measure usually used for matches in species composition between samples. As feeding-trials result in qualitative data (edible *vs.* inedible) this coefficient is also applicable to measure qualitative overlap between realized and absolute food niche. Sørensen's similarity coefficient weighs matches more heavily than mismatches and thereby accounts for the fact that mismatches were over represented both 1) in line-transect data, as all recorded plant species were considered as potential host plants and 2) in feeding trials, as not all tested plant species that were edible occurred in line-transects.

2.3 Results

2.3.1 Phasmid diversity and density

In 15 months of line-transect monitoring I recorded 307 specimens representing 11 phasmid species (Table 2-1). The species accumulation curve from line-transect records reached an asymptote after four months (Figure 2-1) suggesting that the phasmid fauna of forest edge and understory habitats was sampled adequately. This was supported by the fact that in field records other than along line-transects only one additional phasmid species was found (*Pseudophasma menius*; Table 2-1). One species (*Otocrania* sp.) has not been previously reported for Panamá (compare to Appendix 1).

The local phasmid fauna was dominated by four common species comprising more than 89 % of the sampled specimens (Figure 2-2). Seven phasmid species were found in low numbers of 10 or less individuals. One of these species was represented by a singleton (*Stratocles multilineatus*).

Line-transect data uncovered a clear spatial separation in the community structure. Diversity in phasmid species was higher in forest edges compared to the forest understory (Table 2-1). This higher diversity resulted from higher species richness and higher eveness along forest edges. The data indicate that phasmids were mainly restricted to forest edge habitats; ten out of eleven species and 93 % of the recorded individuals occurred here. In the forest understory phasmids were rare and singletons represented three out of four species. Only *Metriophasma diocles* was found regularly and comprised 86 % of the recorded specimens in the understory. As this habitat was poor in species and in individuals, similarity of phasmid communities between forest understory and forest edge was low (demonstrated by Morisita's similarity index $C_\lambda = 0.25$; Table 2-1). Phasmid density was 35 times higher in forest edge habitats than in the understory (Table 2-1). Analogous population density for *M. diocles* was lower in the understory than at forest edges. While absolute abundances doubled population density trebled from 6.7 ind.*ha^{-1} in the understory to 21.6 ind.*ha^{-1} at forest edges after adjusting for size of the sampled area (with a respective total area searched of 0.096 and 0.048 ha).

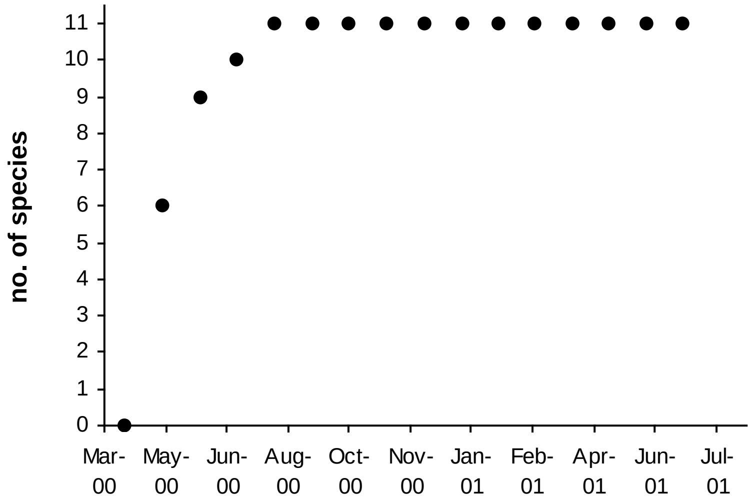

Figure 2-1: Species accumulation curve for phasmids recorded along line-transects on BCI (May 2000 to July 2001; $N = 307$).

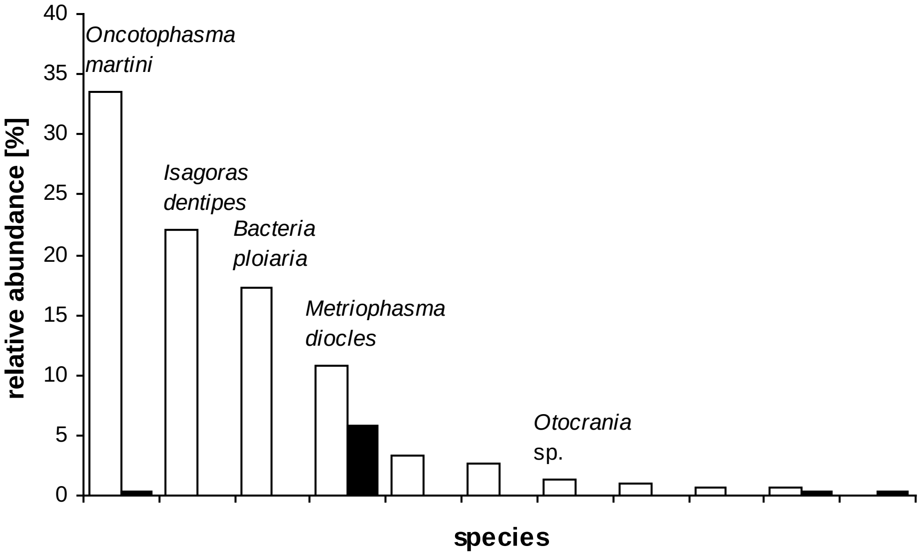

Figure 2-2: Relative abundances of phasmid species at forest edges (white bars) and in the forest understory (black bars) of BCI (line-transect data; $N = 307$).

2.3.2 Seasonality

Absolute abundances of phasmids along forest edges were temporally heterogeneous (Figure 2-3) as demonstrated by comparison of cumulative frequencies per month (GOF, $\chi^2 = 40.17$, df = 12, $p < 0.01$). This temporal heterogeneity in phasmid abundances held for phasmid adults (GOF, $\chi^2 = 40.25$, df = 12, $p < 0.01$) but not for nymphs (GOF, $\chi^2 = 18.45$, df = 14, $p > 0.05$). Heterogeneity largely resulted for adults from highest abundances in May and June 2000 (each with 18 ind.). Because such extremes in abundances covered seasonal effects, a potential increase in abundances from dry season (January to April) to the rainy season (from May on) was tested from for the year 2001: there was no detectable heterogeneity in phasmid abundances (for all specimens: GOF, $\chi^2 = 4.55$, df = 5, $p > 0.05$; for phasmid nymphs: GOF, $\chi^2 = 5.03$, df = 5, $p > 0.05$; for phasmid adults: GOF, $\chi^2 = 10.25$, df = 5, $p > 0.05$).

Table 2-1: Phasmid species and their community structure on BCI (absolute numbers of individuals in subscript; line transect data, *N* = 307).

Phasmid species	**Habitat**	
	Forest edge	**Understory**
Bacteria ploiaria (WESTWOOD)	$+_{53}$	
Bacteria sp.*	$+_{10}$	
Isagoras dentipes REDTENBACHER	$+_{68}$	
Metriophasma diocles WESTWOOD	$+_{33}$	$+_{18}$
Oncotophasma martini (GRIFFINI)	$+_{103}$	$+_{1}$
Otocrania sp.*	$+_{3}$	
Prisopus berosus WESTWOOD	$+_{4}$	
Pterinoxylus spinulosus REDTENBACHER	$+_{8}$	
Stratocles multilineatus REHN		$+_{1}$
Diapheromerini: Phanocles group*	$+_{2}$	
*Metriophasma iphicles** REDTENBACHER	$+_{2}$	$+_{1}$
Pseudophasma menius (WESTWOOD)	not found in line-transects	
Shannon Wiener Diversity H'		
Overall (forest edge & understory)	2.47	
Per habitat	2.40	0.78
Shannon Wiener Eveness J'		
	0.69	
	0.72	0.39
Morisita's Similarity C_λ	0.24	
Phasmid density [ind*ha^{-1}] ± SD		
All phasmid species	274.92 ± 211.48	7.81 ± 14.07
Metriophasma diocles.	21.58 ± 43.36	6.70 ± 11.42

* until submission no type material was available, therefore identification should be considered as preliminary

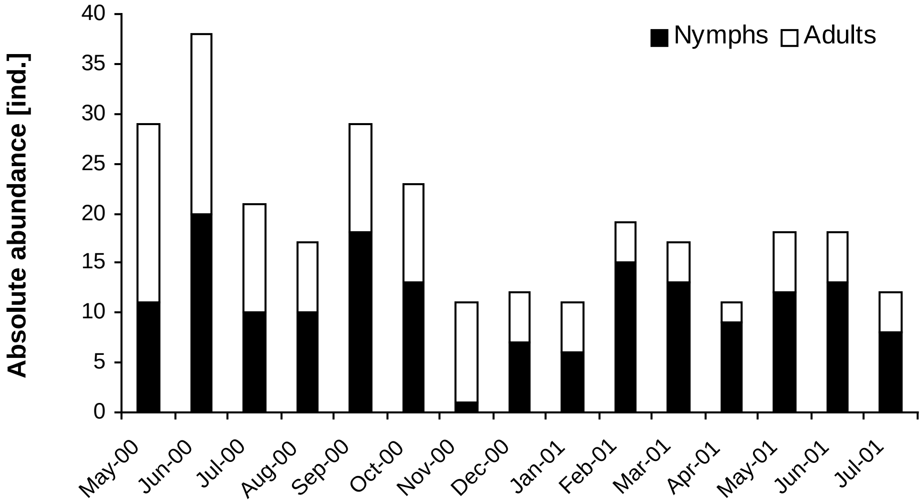

Figure 2-3: Temporal fluctuations of absolute phasmid abundances along forest edges on BCI for phasmid nymphs (black bars) and phasmid adults (white bars). Each bar represents the cumulative catch per month along three line-transects ($N = 286$). Please note that Oct. 2000 and Jul. 201 were excluded from GOF tests.

2.3.3 Food niche

Single phasmid species showed a highly aggregated distribution on particular host plant families resulting in narrow resource niches with values for Levin's standardized niche breadth B_A ranging from 0 (*Otocrania* sp.) to 0.40 (*Bacteria ploiaria*) (Table 2-2). For example, the low niche breadth of 0.10 for *I. dentipes* (the second most abundant species in forest edge habitats; *cf.* Figure 2-1) resulted from 68 specimens recorded on two plant families, and 91 % of these specimens were found on two individuals of *Vismia baccifera* (Clusiaceae). All three individuals of *Otocrania sp.* were found on Sapindaceae. Niche breadth was larger in the other three species because they were recorded on more plant families and individuals were less aggregated (major proportion of individuals per plant family: *O. martini*, 55 % on Clusiaceae, 18 % on Euphorbiaceae; *B. ploiaria*, 34 % on Rhamnaceae; *M. diocles*, 49 % on Piperaceae,and one third of these on *P. marginatum*, 25 % on Araceae).

Generally, resource niches of phasmid species were separated as demonstrated by Pianka's index of niche overlap O_{jk}, usually ranging from 0 to 0.19. In contrast, niche overlap between *O. martini* and *I. dentipes* was high ($O_{jk} = 0.91$) because 55 % of the *O. martini* and 91 % of the *I. dentipes* individuals shared 2 individuals of *Vismia baccifera* as food resource (*cf.* Table 2-2).

Table 2-2: Niche breadth and niche overlap of four common and one rare (*Otocrania* sp.) phasmid species on BCI based on individuals recorded on potential host plant families in line-transects ($N = 279$).

	Oncotophasma martini	*Bacteria ploiaria*	*Metriophasma diocles*	*Otocrania* sp.	*Isagoras dentipes*
Levin's standardized niche breadth B_A	0.31	0.40	0.33	0	0.10
No. of phasmid individuals recorded on plants	104	53	51	3	68
No. of potential host plant families	5	7	6	1	2
Pianka's niche overlap O_{jk}					
Oncotophasma martini		0.27	0.17	0.01	0.91
Bacteria ploiaria			0.18	0.19	0.03
Metriophasma diocles				0.17	0.08
Otocrania sp.					0

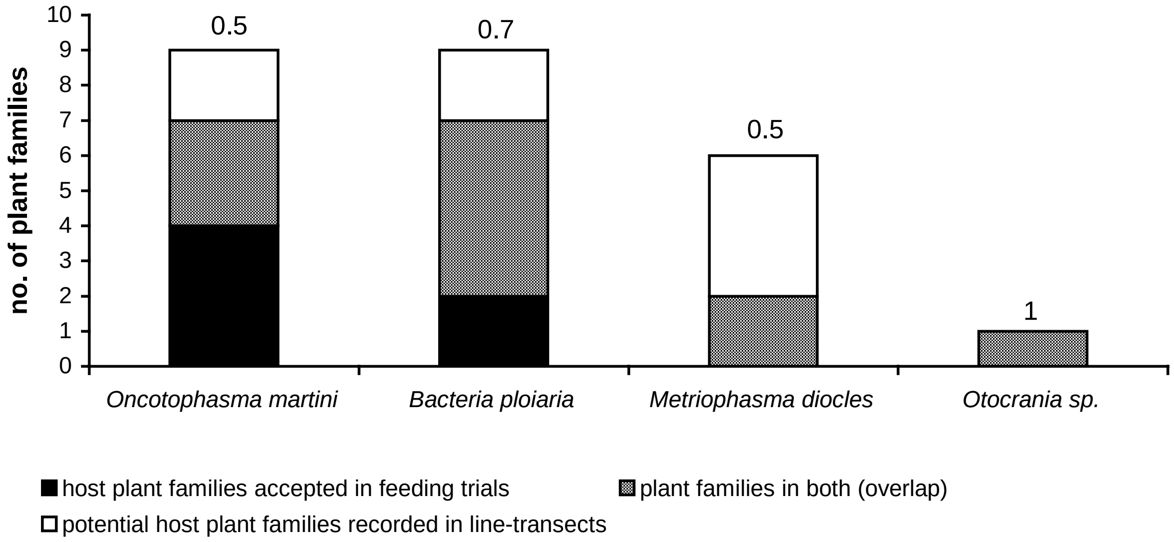

Figure 2-4: Number of plant families composing realized (potential host plant families recorded along line-transects) and absolute dimension of the food niche (host plant families accepted in feeding trials) of selected phasmid species. The overlap between realized and absolute food niche is expressed by Sørensen's index of Similarity S_S (values given above the bars). Note that this index does not weigh relative use of host plant families.

Observed resource niches only partly represented food niches, as potential food plant families from line-transect records (*i.e.* the realized niche) did not completely overlap with edible plant families from feeding trials (*i.e.* the absolute food niche; Figure 2-4). The overlap between the two niche types ranged from 0.50 to 1.00 (expressed as Sørensen index of similarity, Figure 2-4). None of the four focal phasmid species was specialized beyond the plant family level. However, *Otocrania* sp. exclusively fed on plant species of the Sapindaceae and *M. diocles* was clearly restricted to Araceae and Piperaceae (Table 2-3). Generally, plant families that were recorded as preferred resources along line-transects (see above) proved to be edible to the according phasmid species. But for the tested phasmid species the palatability of plants was not consistent in-between the taxonomic borders of plant families (*i.e.,* not all confamilial plant species tested were edible, *cf.* Table 2-3).

The focal phasmid species had distinct diets (Table 2-3). In contrast to the plant records from line-transects (see Table 2-2), the food niches resulting from feeding trials did not overlap (Table 2-3). Only *B. ploiaria* shared one host plant family each with *O. martini* and *Otocrania* sp.. Nonetheless, plant life form of palatable species corresponded with the preferred habitat of phasmid species. *O. martini*, *B. ploiaria* and *Otocrania* sp. fed exclusively on pioneer tree species and lianas. Whereas the diet of *M. diocles*, the only phasmid species with considerable occurrence in the forest understory (*cf.* Table 2-1), contained herbs, shrubs and hemiepiphytes, which are characteristic elements of this habitat. None of the four phasmid species fed neither on any of ten highly abundant tree species of BCI which represented eight plant families, nor did they feed on any of 13 randomly selected plant species out of 12 plant families (*cf.* Table 2-3).

Table 2-3: Dimensions of the food niche of four phasmids on BCI. Results from feeding trials represent the absolute food niche, while overlap with field records (*i.e.* the realized multidimensional niche) represents the realized food niche.

	Plant											
Family	**Order**	**Species**	lf [1]	ab [2]	*Metriophasma diocles*		*Oncotophasma martini*		*Bacteria ploiaria*		*Otocrania* sp.	
					ft [3]	lt [4]	ft	lt	ft	lt	ft	lt
Food plant species												
Araceae	*Philodendron*	spp.			+	+	-		-		-	
Araceae	*Dieffenbachia*	spp.	he		+	+	nt		nt		nt	
Araceae	*Homalomena*	*wendlandii*	he		+		nt		nt		nt	
Araceae	*Monstera*	sp.	h		+		nt		nt		nt	
Piperaceae	*Peperomyia*	spp.	he		+		nt		nt		nt	
Piperaceae	*Piper*	spp.			+	+	nt		nt		nt	
Piperaceae	*Piper*	*cordulatum*	ls	28	+		-		-		-	
Clusiaceae	*Vismia*	spp.	pi		-		+		-		-	
Clusiaceae	*Vismia*	*baccifera*	pi	164	-	+	+	+	-		-	
Dilleniaceae	*Tetracera*	*portobellensis*	l		-		+	+	-			
Euphorbiaceae	*Croton*	*billbergianus*	pi	48	-	+	+	+	-	+		
Malpighiaceae	*Stigmatophylon*	*ellipticum*	l		-		+		-		nt	
Melastomataceae	*Miconia*	*argentea*	pi	52	-		+		-		-	
Sterculiaceae	*Byttneria*	*aculeata*	l		-		+		+	+	nt	
Sterculiaceae	*Guazuma*	*ulmifolia*	pi	187	-		nt		+		nt	
Bignoniaceae	*Arrabidaea*	spp.	l		-	+	-		+	+	-	
Rhamnaceae	*Gouania*	*lupuloides*	l		-		-	+	+	+	-	
Tiliaceae	*Luehea*	*semanii*	pi	113	-		-		+	+	-	
Melastomataceae	*Mouriri*	*myrtilloides*	ls	6	nt		nt		+		nt	
Sapindaceae	*Paullinia*	spp.	l		-		-		+		+	
Sapindaceae	*Serjania*	spp.	l		-		-	+	+	+	+	+
Urticaceae	*Urera*	sp.			-	+	nt		nt		nt	
Vitaceae	*Vitis*	*tiliifolia*	l		nt		-		-	+	nt	
Abundant tree species on BCI (>1000 ind./50ha) [2]												
Violaceae	*Hybanthus*	*prunifolius*	ls	1	-		-		-		-	
Rubiaceae	*Alseis*	*blackiana*	ls	5	-		-		-		-	
Rubiaceae	*Psychotria*	*horizontalis*	ls	7	-		-		-		-	
Chrysobalanaceae	*Hirtella*	*triandra*	ls	8	-		-		-		-	
Burseraceae	*Tetragastris*	*panamensis*	ls	10	-		-		-		-	
Burseraceae	*Protium*	*tenuifolium*	ls	14	-		-		-		-	
Bombacaceae	*Quararibea*	*asterolepis*	ls	20	-		-		-		-	

Family	Plant Order	Species	lf[1]	ab[2]	*Metriophasma diocles*		*Oncotophasma martini*		*Bacteria ploiaria*		*Otocrania* sp.	
					ft[3]	lt[4]	ft	lt	ft	lt	ft	lt
Moraceae	*Poulsenia*	*armata*	ls	23	-		-		-		-	
Fabaceae	*Prioria*	*copaifera*	ls	36	-		-		-		-	
Apocynaceae	*Tabernaemontana*	*arborea*	pi	37	-		-		-		-	
			Random species									
Anacardiaceae	*Anacardium*	*excelsum*	ls	208	-		-		-		-	
Annonaceae	*Annona*	*spraguei*	pi	135	-		-		-		-	
Bignoniaceae	*Jacaranda*	*copaia*	pi	102	-		-		-		-	
Bombacaceae	*Ochroma*	*pyramidale*	pi	246	-		-		-		-	
Bombacaceae	*Pseudobombax*	*septenatum*	pi	221	-		-		-		-	
Euphorbiaceae	*Hyeronima*	*laxiflora*	pi		nt		-		nt		nt	
Flacourtiaceae	*Hasseltia*	*floribunda*	ls	56	-		-		-		-	
Gesneriaceae	*Codonanthe*	*crassifolia*	ls		-		nt		nt		nt	
Mimosoideae	*Inga*	*quaternata*	l	65	nt		nt		-		-	
Moraceae	*Cecropia*	*obtusifolia*	pi	189	-		-		-		-	
Myristicaceae	*Virola*	*surinamensis*	ls	109	-		-		-		nt	
Orchidaceae	*Catasetum*	*viridifloris*	e		-		nt		nt		nt	
Papilionoideae	*Dipteryx*	*panamensis*	ls	185	-		-		-		-	

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[1] lf = life-form: e = epiphyte, h = hemiepiphyte, he = herb, l = liana, ls = plant of late succession, pi = plant of early succession

[2] ab = abundance: species rank of absolute abundance in the 50 ha plot on BCI according to Condit *et al.* (1998)

[3] ft = feeding trial: + = edible, - = not edible, nt = not tested;

[4] lt = line-transect: + = phasmid species was recorded on plant species

2.4 Discussion

This study is the first to demonstrate diversity and distributional patterns of the phasmid community of a tropical rainforest. The phasmid community of BCI was poor in species and in density. Spatially phasmids were largely restricted to forest edge habitats resulting in higher diversity and density compared to the forest understory. While phasmid abundances varied throughout the study period, no seasonal effect on phasmid abundance was detectable. The distribution pattern of phasmid species was clearly linked to restrictions in their host range that seemed to reflect the successional status of host plant species.

2.4.1 Phasmid diversity, density, and distribution

With a total of 12 species, the phasmid community of Barro Colorado Island was expectedly low in species richness. Worldwide, the order Phasmatodea contains about 3000 described species that occur mainly in tropical regions (Whiting *et al.* 2003) and is species poor compared to other insect orders that are highly diverse in herbivores like Lepidopterans, Coleopterans, and - to a lower extent – Orthopterans. For example, on BCI there are approximately 274 species of true butterfly (Lepidoptera, Papilionoidea; DeVries 1994) and 250 species of katydids (Orthoptera, Tettigoniidae; A. Lang, unpublished data). I found reports of 42 phasmid species occurring in Panamá (see Appendix 1 for species and references) whereof BCI accommodates 26 %; plus one species not formerly reported from Panamá (*Otocrania* sp.). Analysis of collection records (Windsor, Aiello, Berger, unpublished data) indicates that Gamma-Diversity in Panamá is high with discrete regions giving shelter to different phasmid species. Together with the fact that I recorded all but one species previously reported from BCI (*Trychopeplus laciniatus*; Aiello, unpublished data) I am confident that I assessed the phasmid community adequately.

I showed that forest edges and gaps represented important habitats to the majority of phasmid species on BCI. In contrast, the forest understory seemed to provide a suitable habitat for *M. diocles* only. Higher abundance of insect herbivores in gaps is often inferred from higher herbivore damage on gap-adapted versus understory plant species (Coley 1980, 1982, 1983; Coley & Barone 1996). Smiley (1978) found that *Heliconius* butterflies were more abundant in young successional than in understory habitats (Table 2-3). My results support this pattern on the phasmid community and species level. In forest edges phasmid density was 35 times, and *M. diocles* density three times higher than in the understory (*cf.* Table 2-1). Low densities of phasmids in the BCI forest understory correspond with Elton (1973) who found low total arthropod densities of 0.92 ind.*m^{-3} in the same habitat (18400 ind.*ha^{-1}, extrapolated on two meter height; Table 2-4).

Table 2-4: Densities of phasmids, insects, and arthropods in the understory and in gaps/forest edges of neotropical rainforests and one temperate forest.

Region	Habitat	Density [ind.*ha^{-1}]	Studied organisms	Reference
Neotropical, Panamá	understory	7.8	Phasmatodea	presented study
	forest edge	274.9		
	understory	6.7	*Metriophasma diocles* (Phasmatodea)	
	forest edge	21.5		
	understory	18000	arthropods	Elton 1973[1]
Puerto Rico	gap	3520 – 6460	*Lamponius portoricensis* (Phasmatodea)	Willig *et al.* 1986[1&2]
	understory	2111.1		Willig *et al.* 1993[1]
	gap	351.7		Willig & Camilo 1991[1]
	gap	57.55	*Agamemnon iphemedeia* (Phasmatodea)	
Costa Rica	understory	0.5 - 1.5	*Heliconius cydno*	Smiley 1978
	gap	1.4 – 5.0	*H. hecale* & *H. erato* (Lepidoptera)	
	understory	160000	arthropods	Janzen & Schoener 1968[1]
		Density [ind*m^{-2}]		
Guyana	understory, seedling foliage	2.4	insects	Basset 1999[3]
Temperate, Switzerland	understory, foliage	19 - 78	insects	Basset & Burckhardt 1992[3]

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[1] to allow for better comparison density estimates per ha were extrapolated from published data

[2] the authors used different calculation methods on their dataset resulting in variation of density estimates

[3] densities in these studies are based on sampled foliage area and thus could not be extrapolated

Surprisingly, empirical studies assessing arthropod or insect densities of rainforest edge/gap and understory habitat types are rare (for references see Table 2-4), not to mention comparisons of densities between habitats (Smiley 1978). Largely, existing studies focus on the understory including several feeding guilds, or they refer to sampled leaf area and are therefore of restricted comparability (*cf.* Table 2-4). In addition, densities are site dependant and may enormously differ in magnitude over time (Table 2-4). For example, densities of the Puerto Rican phasmid *Lamponius portoricensis* in gaps varied tenfold between studies within the same forest (Willig *et al.* 1986; Willig & Camilo 1991; Table 2-4). In comparison, density of *L. portoricensis* in the understory was similar to higher estimates for gaps (Willig *et al.* 1993; Table 2-3). While *L. portoricensis* seems to be an abundant herbivore that is not clearly restricted in the use of different habitat types, my results showed that phasmids on BCI are comparably rare and preferably occur in forest edge habitats.

Are these results representative for the whole forest? BCI may harbor higher phasmid abundances and diversity than presented in this study because I may have missed species living in the upper canopy. Arthropod densities are generally known to be high in the forest canopy. For example, Leigh (1999, p. 165) lists data for insect densities in tropical forest canopies ranging from 32 to 161 ind.*m^{-2}, exceeding the numbers presented in Table 2-3 for understory and edge/gap habitats. Based on empirical studies, the forest canopy also is thought to give shelter to the majority of insect species in tropical rainforests (Erwin 1982; Ødegard 2000; Novotny *et al.* 2002a). This may well be true for phasmids on BCI. However, the forest canopy also is a largely hostile habitat to insect herbivores: it is hotter, drier and windier than the understory (Lowman 1985), and sun leaves are smaller, tougher, and have higher phenolic contents than do shade leaves (Lowman 1985; Coley & Barone 1996). Such climatic and nutritious constraints could negatively influence phasmid presence in the canopy. Furthermore, the studied phasmid species rejected all additionally tested late successional plant species in feeding trials (selection of most abundant and random species; *cf.* Table 2-3). Even though only part of these plants represented canopy trees, this suggests that including the upper canopy may not involve an increase in abundances of the focal phasmid species of this study.

2.4.2 Seasonality

Unlike for other arthropods, phasmid abundances were not higher at the beginning of the rainy season. Seasonal fluctuations in abundances have been shown for many tropical insects, with different insect groups showing different rhythms (Smythe 1982). At more seasonal tropical sites, arthropod abundance is higher during the wet season than the dry season (Janzen 1973; Wolda 1978, 1983; Windsor 1978; Smythe 1982) but rainfall may also negatively affect abundances of externally feeding folivores (Marquis & Braker 1994).

Many herbivorous insects seem to synchronize their hatching or emergence with the flush of young leaves (Coley & Barone 1996). Basset (2000) showed that the influence of leaf production on insect seasonality can be species-specific. In this study overall phasmid abundances were heterogeneous throughout the study period but abundances of nymphs seemed to remain on similar levels. This contradicted my expectation that particularly nymphs should show higher abundances at the beginning of the rainy season. The lack of seasonality especially in phasmid nymphs may indicate that they do not depend on the availability of young nutrient rich foliage.

However, in the presented study, seasonal differences in phasmid abundances may have been concealed by high variation of overall abundances throughout the study period (*cf.* Figure 2-3). The magnitude of variation could reflect deficiencies of the sampling method. For example, insect activity is often restricted to particular times of day (*e.g.*, Willig *et al.* 1993; Basset 2000) and phasmids, when resting, are highly camouflaged (Bedford 1978; pers. obs.). Thus alternating sampling times may have resulted in time-dependant sample size (*e.g.*, heavy rains often did not allow to follow a strict monitor plan) indicated by heterogeneity of phasmid adult abundances throughout the study period. In contrast, sample time may affect the sighting of nymphs to a lower extent. Phasmid nymphs have small home ranges and were shown to display patch fidelity (Willig *et al.* 1993). Consistent with these findings, high occurrence of *O. martini* on two *Vismia baccifera* individuals was mainly based on nymphs (data not shown).

2.4.3 Niche breadth and habitat choice

Phasmids on BCI were restricted in the use of their resources. Values for Levin's standardized niche breadth assessed from records of potential food plants along line-transects ranged from 0 to 0.4 (representing the realized niche). Niche breadths resulted both from a low number of potential host plant families (*Otocrania sp.*, *I. dentipes*) and from contagious distribution of phasmid individuals among resources (all focal phasmid species). For example, 55 % of all *O. martini* and 91 % of all *I. dentipes* specimens shared two plant individuals of *Vismia baccifera*. Such clumped distribution was shown before to partially reflect habitat characteristics like plant composition (Willig *et al.* 1993) and may result from preference for and/or availability of a resource (Bernays & Chapman 1994). Here contagiousness reflected 'true' food resources: In feeding trials all the plant families turned out to be edible, where phasmid individuals were aggregated on in he field. (Unfortunately *I. dentipes* could not be established in lab populations. But field observations confirmed *Vismia baccifera* as food plant.) In contrast, other potential host plant families, that were included in the realized niches were rejected in feeding trials. Partial overlaps of realized niches, assessed from phasmid presence on plants in the field, and absolute food niches, assessed in feeding trials (as depicted by Figure 2-4), underline that field data represent multidimensional niches, including resources such as mating, oviposition, transience and the realized food niche (Krebs 1989; Bernays & Chapman 1994).

When discussing the factors that may have led to the observed restriction in using food resources evolutionary and ecological processes have to be considered. A first insight into host range evolution can be gained by applying conventional definitions that refer to taxonomic relationships of host plant range on the results of feeding trials (representing the absolute food niche). Utilizing this concept, the user can conclude the most probable behavioral response (Bernays & Chapman 1994). To exemplify with my data, *Otocrania* sp. that fed on two plant species out of the Sapindaceae (and hence is monophagous) may use some characteristic of the plants they have in common in determining their acceptability. In contrast, *B. ploiaria* and *O. martini* that fed on plants from a number of different families (meaning they are polyphagous) most likely employ a number of different cues. The latter also applies for *M. diocles* whose diet included members of the Araceae and Piperaceae; two plant families that are not closely related (Judd *et al.* 2002). Such a feeding pattern of an insect that feeds on a small number of plants from different unrelated families is often described as disjunct oligophagous (Bernays & Chapman 1994).

With its relation to plant taxonomy, the above definitions of host range involve conclusions regarding evolutionary processes. Monophagous species like *Otocrania sp.* are thought to be driven to specialization by physiological adaptations to host plant chemistry (Futuyma 1991). This may not apply for the disjunct oligophagous *M. diocles*. This term does not include any information on the connection of used host plants and Bernays *et al.* (1989) suggested that specializing on distantly related hosts indicates that factors such as competition or predation may have selected for restriction in host range.

But what are the reasons that the realized food niches of phasmids (demonstrated by the overlap region in Figure 2-4) were smaller than their absolute food niches assessed in feeding trials? In other words: why did phasmids not use all available hosts that were confirmed edible in feeding trials? An explanation of these discrepancies between absolute and realized food niche may be found in biotic factors in the natural setting. For example, the observed distribution pattern of an herbivore among host plants can result from density dependent food selection, from food selection due to nutritional constraints, or from varying predation pressure on different hosts (Bernays & Chapman 1994). More specifically, Willig *et al.* (1993) found that phasmids modulated host selection to density of their host plants rather than to preference. The studied phasmid *L. portoricensis* occurred mainly on *Piper treleaseanum*, the least preferred plant species in feeding trials but the most abundant in the field. Sandoval (1994) proved that predation pressure can narrow down host use of phasmid nymphs to plant species where they suffered the least predation-related mortality. Such processes, for example, may explain why *O. martini* in line-transects never was found on *Byttneria aculeata* that was proved to be edible and was present in the habitat.

Nevertheless, careful interpretation is inevitable because feeding trials involve some methodological imponderability that partially may have contributed to differences between realized and absolute food niche (see *e.g.* Gangwere 1961; Holecheck *et al.* 1982; Capinera 1985; Barone 1998). It is well known that feeding trials under lab conditions tend to produce more positive responses from insect herbivores than they would under natural conditions (*e.g.*, Rowell-Rahier 1984). For instance, no-choice tests may overestimate the host range of an herbivore because there is no alternative to the offered food. On the other hand the number of offered plants is inevitably limited and may thus lead to an underestimation of host range.

On the species level, phasmids used different resources reflected by the comparison of both realized or absolute niche dimensions. Niche differentiation among species occupying the same habitat is often an effect of competition (Begon *et al.* 1996). One simple prediction arising from the assumption that the observed niche differentiation resulted from interspecific competition would be: while phasmid species use resources differentially in the field they should not discriminate among resources when they are released from competition, like in feeding trials. Thus, interspecific overlap in realized niches should be smaller than overlap of absolute niches. This prediction was not confirmed for phasmids on BCI.

Principally overlap of realized niches between phasmid species was marginal with overlap indices from 3 to 20% (Pianka's niche overlap). This cannot be generalized as being opposed by an almost complete niche overlap between *O. martini* and *I. dentipes*.

Results from feeding trials revealed that absolute niches of phasmid species were even more distinct (note that *I. dentipes* was not included in feeding trials). Overlap in absolute niches of four phasmid species was restricted to *B. ploiaria* sharing one host family each with *O. martini* and *Otocrania* sp.. There was no overlap of the absolute food niche of *M. diocles* and any of the other three species.

This can be partially explained by the fact that many plants in the diet of *M. diocles* (Araceae and Piperaceae) mirror the understory-habitat, the use of which was negligible for all the other species. Nevertheless, many *Piper* species are gap adapted (Croat 1978), and hence also available to other phasmid species. Possibly these phasmids are not able to handle particular chemical compounds of Piperaceae and Araceae. The phylogenetic separation of the Piperaceae from other dicots may involve differences in phytochemistry (Judd *et al.* 2002). In fact, *Piper* species are characterized by a wide array of aromatic compounds (Sengupta & Ray 1987; Baldwin & Schultz 1988; Parmar *et al.* 1997; Dyer *et al.* in press) many of them deterring insect herbivores (Parmar *et al.* 1997 and references therein). Likewise the Araceae as monocots are distinct from dicots in many features. Araceae contain calcium oxalate crystals, cyanogenic compounds, and sometimes alkaloids, all known to deter herbivores (Judd *et al.* 2002 and references therein). *M. diocles* seems to be able to detoxify such toxic or deterrent plant chemicals suggesting that its evolutionary history is closely linked to these plants. However, a coevolutionary scenario (*sensu* Ehrlich & Raven 1964) would demand that Araceae and Piperaceae were closely related. Recent phylogenetic analyses cannot support this imperative (Judd *et al.* 2002).

While this approach so far lacks an explanation for niche differentiation among *O. martini*, *B. ploiaria* and *Otocrania* sp., I suggest that their host ranges may reflect their evolution with less defended fast growing pioneer plants of high nutritious quality (Coley 1983; Coley *et al.* 1985). Both evolutionary hypotheses are supported by the refusal of foliage of late successional plant species in feeding trials.

The presented pattern in host range corresponded with the mobility of three of the focal phasmid species. Limits to the rate at which a suitable host can be found (like mobility) are clearly related to host range (Jaenike 1990). Small organisms perceive their environment in a coarse grained fashion (Levins 1968). For specialist herbivores, the resolution becomes worse because with decreasing host range, a decreasing availability of patches with suitable hosts is inherent (particularly in high diverse tropical rainforests). This may explain why both sexes of *M. diocles* dispose of cost intensive wings. Higher mobility as compared to wingless phasmids may allow them to search for scattered resources more efficiently. But finding suitable resource patches also represents a critical challenge for nonvolant phasmids like the generalistic *O. martini* and *B. ploiaria*. In *B. ploiaria* only the male is winged and able to fly short distances (personal observation) while in *O. martini* both sexes are wingless. Their restricted host range consisting of characteristic gap plant species involves the risk of depleting host plants when a light gap closes. Then the detection of a new gap becomes crucial; the more as nonvolant phasmids were shown to move at low velocities (a maximum of 6 m per day; Willig *et al.* 1986). I suggest that gap phasmid species may escape from being doomed because their diet contains liana species. I argue that the wingless habitus of the *Otocrania* species does not contradict its specialization, because *Otocrania* specialized on Sapindaceae, a plant family that contains many lianas such as *Paullinia* spp. and *Serjania* spp. (Croat 1978). Lianas may contribute up to 14 % of above ground biomass in tropical forests (Gerwing & Farias 2000). They are characteristic components of early successional habitats such as gaps, but they are also present all over the canopy (Putz 1984, DeWalt *et.al.* 2000; Schnitzer & Bongers 2002). Thus, phasmids that include lianas into their diet may find sufficient food in upper canopy. The generalistic *O. martini* and *B. ploiaria* that were abundant in forest edges may on their search for new light gaps be able to traverse the forest canopy and rely on lianas. In contrast, the forest canopy may represent the preferred habitat of the specialist *Otocrania* sp. and thereby explain its low abundance in my study.

2.4.4 Conclusions

Broadening the meaning of specialization, I presented evidence that phasmids are specialized to habitat specific food resources and that their distribution was clearly connected to the distribution of their host plants (as reflected by their successional status). The higher density and species richness of phasmid species in forest edges as compared to the understory may reflect their evolution with less defended fast growing pioneer plants of high nutritious quality (Coley 1983; Coley *et al.* 1985). The refusal of foliage of persistent late successional tree species gives support to this view.

Other than by resources, the distribution of an herbivore can be influenced by its natural enemies. Contagiousness could then be due to differential predation, *i.e.* an herbivore species suffers less predation related mortality on a particular host plant species (Sandoval 1994; Price *et al.* 1980; Bernays & Graham 1988). Such mechanisms could have led to narrow host range including unrelated plant species of *M. diocles*. Low abundances of *M. diocles* in the understory then would result from high predation pressure rather than from nutritious limitations through the host plants.

3 Life cycle, potential population growth, and egg hatching failure of *Metriophasma diocles*

3.1 Introduction

Great variation in patterns of population densities clearly exists in herbivorous insects, but generally most insect species in the tropics occur in low numbers (*e.g.*, Elton 1973; Basset *et al.* 1992; Basset 1997, 1999; Novotny & Basset 2000). Such low population densities reflect the biotic potential of a species in the limiting boundaries of habitat capacity, intraspecific competition and regulating mechanisms within trophic cascades.

A whole wealth of factors acts on individuals in a population thereby affecting net recruitment (*i.e.*, births minus deaths). These factors either do not respond to population density or are density-dependent and can be roughly categorized as: (1) intraspecific density-dependent factors, (2) environmental density-dependent factors, and (3) density-independent factors.

The upper limit of population size is defined by intraspecific competition. Intraspecific competition as density-dependent regulation factor increases with population size. Higher competition leads to increasing death and decreasing birth rates resulting in a population decline (and *vice versa*). At a certain density, birth and death rate are equal and there is no net change in population size. This density is known as carrying capacity (*K*). The carrying capacity represents the population size the resources of the environment can just maintain without a tendency to either increase or decrease (Begon *et al.* 1996). However, natural populations lack simple carrying capacities. Alongside with intraspecific competition, populations are regulated by multiple factors reducing the population size below the carrying capacity.

Most factors in the biotic setting of a species respond to population size. Density of prey translates into predator densities (Volterra 1926; Lotka 1925), and diseases spread faster in higher populated areas (*e.g.*, Stevenson 1959). Increasing population density also involves decreasing food availability and higher levels of interspecific competition (reviewed in Schoener 1983).

Other biotic factors like intrinsic food quality or breeding sites on the other hand do not relate to population density, but their limited nutritious value or availability may act as regulators (Andrewartha & Birch 1954, 1984). Likewise, abiotic factors such as climate and natural disasters are density independent and can cause drastic impact on populations (*e.g.*, Willig & Camilo 1991).

Releasing a population from regulating constraints can uncover the impact of the above described control factors. When there are no limits on its growth, the population of a species will increase infinitely according to its biotic potential, *i.e.* the inherent power of an organism to reproduce and survive (Chapman 1931). The intrinsic rate of natural increase (*r*) will then be at its maximum. That means each individual of the population will contribute to population growth with peak reproduction (Begon *et al.* 1996). In natural populations, most individuals are not capable of peak productivity because restrictions in the biotic and abiotic setting of an organism's environment almost always affect fecundity and survivorship (*e.g.*, Joern & Gaines 1990, and references therein). In practice, an approximation of the biotic potential can be obtained from laboratory populations where most of these restrictions can be cut off or minimized (*e.g.*, populations under laboratory conditions are not necessarily competition-free but experience the least competition, *cf.* Begon *et al.* 1996).

Data on demographic population parameters and life history traits assessed from laboratory populations can help in understanding an organism's realized density in its natural setting and provide the basis for estimating potential population growth. Under the assumption that density estimates obtained from field records are derived from a stable population oscillating below *K* (not being in colonization or in extinction), the difference between potential and realized population densities then reflects the impact of control.

Generally, phasmids on BCI occurred in low abundances in both the understory and at forest edges as I showed in Chapter 2. Although being rather common in comparison to the majority of phasmid species, *M. diocles* density was low in both habitats. Most other phasmid species are rare in tropical forests (Bedford 1978, Willig & Camilo 1991, Novotny & Basset 2000) while they occasionally reach pest status in temperate ecosystems (*e.g.*, Campbell 1961, 1974).

Low densities of a species may at least partially result from a low biotic potential, whereas low density opposed to high biotic potential is indicative of high levels of control. Among phasmid species biotic potentials seem to be highly variable as indicated by great differences in generation times and fecundity (available data do not include these parameters but report developmental times of eggs and nymphs as well as maximum adult lifetimes and maximum egg production; *cf.* Bedford 1978).

In this chapter, I present data on demographic population parameters and life history traits from a laboratory population of *Metriophasma diocles*. In particular, I assessed mean values for (1) individual fecundity (birthrate), sex ratio and generation time. Based on these parameters (2) I developed a model for the potential population growth of *M. diocles* (*i.e.*, the biotic potential). This model established the base for conclusions on the impact of population control factors, namely the effects of (3) egg mortality (included here), food quality (Chapter 4) and predation (Chapter 5).

Sex ratio and birth rate represent principle demographic population parameters. The sex ratio provides information on the proportion of females in following generations and approximates equality in most insects (Wrensch & Ebbert 1993). The birthrate describes the number of offspring produced per unit time, also referred to as mean individual fecundity (Begon *et al.* 1996). Individual fecundity of insects is known to be influenced by female body size (or weight) with smaller females laying fewer eggs (*e.g.*, Price 1984, Wiklund *et al.* 2001, Zanuncio *et al.* 2002). In the natural setting of an organism, female body size or weight may reflect impact from different factors, such as food quality and availability, competiton and predation pressure, and therefore may depend on their according intensities (Price 1984). If such a relation between body size and fecundity was also found in *M. diocles,* then population control factors like food quality and predation pressure (addressed to in Chapters 4 and 5) most likely will influence female fecundity.

The life span of an organism is one life history trait reflecting a part of its life cycle. In phasmids, life span can be separated into three distinct life stages: (1) developmental time of eggs, (2) developmental time of nymphs (maturation) and (3) adult lifetime. The mean durations of these life stages merge together to the mean generation time of insects that represents the average parental age at which all offspring are born (Pianka 1978).

The above described parameters are a reflection of an organism's life cycle (*i.e.*, patterns of birth, death and growth) and can serve as basis for a mathematical model of population growth. Population growth differs depending on the life cycle of an organism. Populations of organisms with discrete breeding seasons (*i.e.*, discrete generations) grow in discrete steps whereas populations with continuous breeding (*i.e.*, overlapping generations) grow continuously (Begon *et al.* 1996). Consequently, knowledge about the life cycle of an organism is necessary before deciding on a model. With the exception of social insects, generations do not overlap in most insect populations (*i.e.*, the parental reproductive period does not overlap with the offspring's reproductive phase) and models of discrete stepwise growth best describe their population growth (Begon *et al.* 1996).

Accordingly, generations in *M. diocles* populations were expected not to overlap: if mean developmental time of egg and nymphal stage together exceeded mean adult lifetime then potential population growth would be modeled by discrete stepwise growth.

3.2 Materials and methods

For details on study site, line-transect and field records, and for maintenance of lab populations please refer to the introductory chapter and to Chapter 2 respectively.

3.2.1 Assessing demographic population parameters and life history traits

For the estimation of the biotic potential of *M. diocles,* I gathered data on demographic population parameters and life history traits from a lab population from January 2000 to January 2002.

Individual fecundity was assessed as the mean number of offspring produced per day in a *M. diocles* female adult lifetime. For the estimation of mean individual fecundity, I observed the egg production of single females over the course of a 24-hour period. Observations usually started in the early afternoon and ended at the same time the following day. A female was collected from the lab colony and weighed before placing it in a plastic container with screened lids (to allow for ambient conditions). Individuals were provided with leaves of different food plants (*Philodendron inaequilaterum*, *Piper marginatum*, *P. hispidum*, *P. reticulatum*, *etc.*). At the end of an observation I carefully searched the container and the remainders of the leaves for eggs. In total, I yielded 500 observational days from 99 individual females. Multiple observation units per female individual were averaged.

Because individual fecundity of females may be influenced by female body size (weight) (*e.g.*, Price 1984) female weight was correlated to egg production in a Spearman Rank correlation. For this analysis I only included females that had laid at least one egg while being under observation (49 females). The elimination of zero counts for female oviposition (50 females) was justified by the fact that the disposition to lay eggs was independent from female weight (comparison of weight for females having laid eggs and females not having laid eggs: T = -0.54; df = 97; P = 0.59).

For the estimation of the proportion of females in *M. diocles* populations, I calculated sex ratio for the lab population on the base of the numbers of emerged adult males and females.

The mean generation time of *M. diocles* was measured on the basis of the mean duration its three distinct life stages: (1) developmental time of eggs, (2) developmental time of nymphs (maturation) and (3) adult lifetime.

Mean developmental time of eggs was derived from observations of the hatching of nymphs from eggs with known date of oviposition. Eggs were collected from cages at least every third day and stored in plastic food containers covered with mesh. Containers were labeled with the date of oviposition and stored in plastic boxes with 10 mm of sand on the bottom. Eggs were sprayed with water regularly to provide for ambient humidity. Hatching of nymphs was controlled daily.

If eggs fail to hatch, for example due to fungal infestation (Bedford 1978), then this reduced hatching success will negatively affect population growth. To describe the influence of failed hatching on population growth, I assessed hatching success. Infestation of eggs by fungi (notable by fungal hyphens covering the eggshell) was checked once per month. Infested eggs were collected and kept separately to observe hatching (no hatching of infested eggs occurred). In January 2002 I counted all eggs that were older than 120 days and had not hatched. I assumed that these eggs had failed to hatch because less than 0.31 % of almost 2000 eggs took longer than 120 days for development. I randomly selected 10 of these eggs and opened them: one contained a dead dry nymph, four were dried out, and in five the usually green liquid had turned black.

Mean developmental time of nymphs was assessed from individually raised nymphs. A nymph was set in a plastic container with screened lids. At least every third day individuals were provided with fresh leaves of Piperaceae or Araceae (see Chapter 2) and the containers were cleaned. Nymph development was controlled daily.

Average adult lifetime was calculated on the base of individuals with certified records of date of emergence and death from July 2000 to January 2002. The lab population of *M. diocles* was checked at least weekly for new emerged and dead adults. New adults were measured from head to terminal abdomen, marked individually with a four-color code (permanent ink) on the pronotum, and transferred to an extra cage only containing adults. Emergence date and time of death were recorded individually.

3.2.2 Modeling potential population growth and the effect of limited hatching success

The above described parameters are a reflection of the life cycle of *M. diocles* (*i.e.*, patterns of birth, death and growth) and can serve as the basis for a mathematical model of population growth.

For modeling population growth of *M. diocles,* I assumed (1) that generations do not overlap, (2) that the population increases competition-free, and (3) that all individuals complete their life cycle (*i.e.*, all individuals complete their mean expected lifetime and reproduce).

Generations in *M. diocles* populations will not overlap if mean developmental time of egg and nymphal stage together exceed mean adult lifetime. Population growth will then be best described by models of discrete stepwise growth (Begon *et al.* 1996):

Equation 3-1 $$N_T = N_0 R^T$$

where N_T = Population size N at generation T

R = Fundamental net per capita rate of increase

This model describes the exponential growth of a competition-free population with discrete generations and constant R. If $R > 1$ the population will grow exponentially.

R usually combines the birth of new individuals with the survival of existing individuals (like in organisms with overlapping generations). When generations are discrete R describes only the birth of new individuals and is equivalent to the basic reproductive rate R_0:

Equation 3-2 $$R_0 = \frac{\sum F_x}{a_0}$$

where R_0 = Basic reproductive rate

F_x = Total number of fertilized eggs produced during one generation

a_0 = Original number of individuals

The natural logarithm of R_0 describes the intrinsic rate of natural increase r. This is the change in population size per individual per unit time, which is one generation in the presented study.

R_0 is usually derived from cohort life tables that describe mortality and survivorship in particular life stages during the life cycle of organisms with discrete generations (*e.g.*, Begon *et al.* 1996). Therefore the initial number of individuals a_0 (here eggs) differs to some extent from individuals alive in the last life stage a_x, which is for insects the reproductive adult phase. In this study, I wanted to model potential population growth and as a consequence I assumed that all individuals survive until the reproductive phase; thus $a_0 = a_x$.

The total number of fertilized eggs produced during one generation (ΣF_x) of *M. diocles* can be calculated on the base of the assessed average values for life history parameters by:

Equation 3-3 $$\sum F_x = \overline{F}_d \overline{L}_f a_f$$

where $\overline{F}_d$ = Mean number of fertilized eggs produced per day and female

$\overline{L}_f$ = Mean adult lifetime of females

a_f = Number of females

For the calculation of ΣF_x knowledge about the sex ratio of a population is inevitable. Female-biased populations will result in higher values for the net per capita rate of increase R and hence in faster growth. Replacing ΣF_x in Equation 3-2 by $\overline{F}_d \overline{L}_f a_f$ shows that the proportion of females in the *M. diocles* lab population (estimated by the sex ratio) is included by the term a_f/a_0.

The outlined model in Equation 3-1 describes the infinite exponential population growth in the absence of limiting factors. Such hypothetical populations do not reflect biological facts. Factors affecting population growth can act on every life stage of an organism. Here, I assessed the effect of limiting factors in the first life stage. More precisely, I estimated egg mortality due to fungal infestation and failed hatching. A reduction in hatching will result in a lower net per capita rate of increase R (or basic reproductive rate R_0 respectively) because $a_0 > a_x$ and fewer females enter the reproductive adult stage. The population will still grow exponentially but the proportion of eggs that fail to hatch decelerates the increase. This model assumes that failed hatching is constant.

3.3 Results

3.3.1 Demographic population parameters and life history traits

In the *M. diocles* lab population sex ratio was balanced (females : males = 1.01; Table 3-1). Females and males showed a pronounced sexual dimorphism, differing significantly in size and weight. In average, females weighed more than three times more and were *ca.* 20 % longer than males (Table 3-1). Consistently, it took females approximately 8 days longer to develop from nymph to adult, resulting in a total developmental period of 108 days (Table 3-1).

Mean adult lifetime differed significantly between sexes, with females living approximately 13 days longer than males (*i.e.*, 60 *vs.* 47 days; *cf.* Table 3-1). Generally adult lifetimes varied enormously among individuals. At maximum an individual female tripled average adult lifetime and lived for 177 days. Likewise maximum lifetime for males was more than twice as long as male mean adult lifetime.

Females produced in average 0.66 eggs per day (Table 3-1). But individual females posed up to ten eggs in one day. Mean egg production was modestly related to female weight ($R_S = 0.43$; *cf.* Figure 3-1).

In average nymphs hatched after 71 days. Duration of egg development ranged considerably from 35 to 172 days but clearly peaked with 50 % of all nymphs emerging between days 68 and 74 after oviposition (Figure 3-2).

Table 3-1: Estimates of demographic population parameters and life history traits of *M. diocles*. All data were based on lab records. Differences were tested by Student's T-tests.

	Females	**Males**	**Difference**
Sex ratio	249	245	
Body weight ± StDev **[g]**	1.44 ± 0.29 $N = 79$	0.41 ± 0.04 $N = 28$	T = -18.4, df = 105, $P < 0.01$
Body length ± StDev **[mm]**	84.15 ± 2.85 $N = 122$	67.7 ± 2.47 $N = 126$	T = -48.6, df =246, $P < 0.01$
Mean female fecundity ± StDev **[eggs*day^{-1}*ind.$^{-1}$]**	0.66 ± 1.18 $N = 99$		
Egg developmental time ± StDev **[days]**	71.22 ± 9.69 $N = 1958$		
Nymph developmental time ± StDev **[days]**	108 ± 13.9 $N = 7$	99.8 ± 15.14 $N = 12$	
Mean adult lifetime ± StDev **[days]**	59.96 ± 40.77 $N = 69$	46.73 ± 27.84 $N = 83$	T = -2.4, df = 150, $P < 0.05$
Maximum adult lifetime [days]	177	122	
Mean generation time [days]	239	218	

Independently from sex, mean developmental time (egg and nymph development) was longer than mean adult lifetime. Development for females lasted in average 179 days; three times longer than mean adult lifetime of (60 days; *cf.* Table 3-1). Similarly, male developmental time took more than three times as long as male mean adult lifetime (171 *vs.* 47 days).

Mean generation time for *M. diocles* lasted approximately eight months (Table 3-1) with female life span taking three weeks longer than for males. Even under consideration of maximum values for adult lifetime developmental and mature periods in *M. diocles* populations overlap only marginally (4 days for males, no overlap for females; *cf.* Table 3-1). Consequently generations in *M. diocles* were considered as distinct.

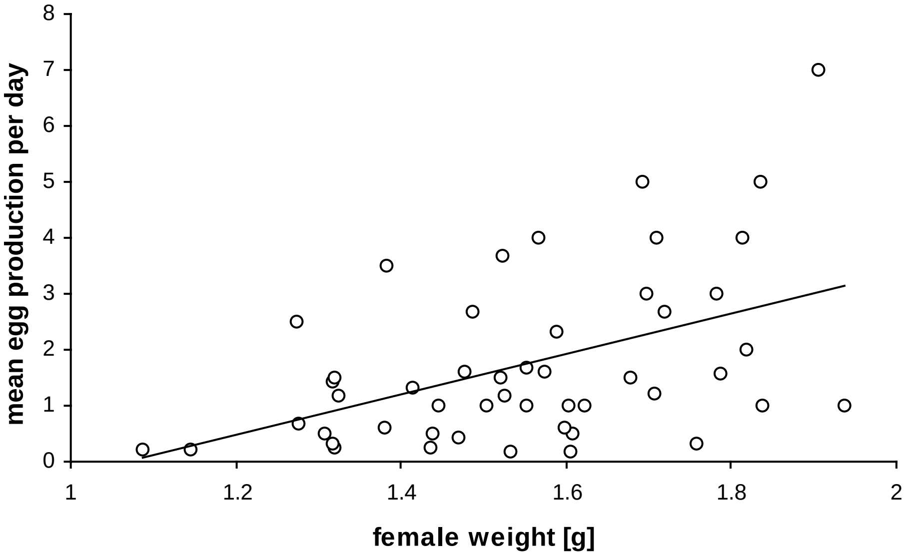

Figure 3-1: Mean daily egg production of *M. diocles* females in relation to body weight ($R_S = 0.43$; $N = 49$; $P < 0.01$). The solid line represents the trendline for $y = 3.6308\,x - 3.89444$. Zero counts were eliminated (see Methods).

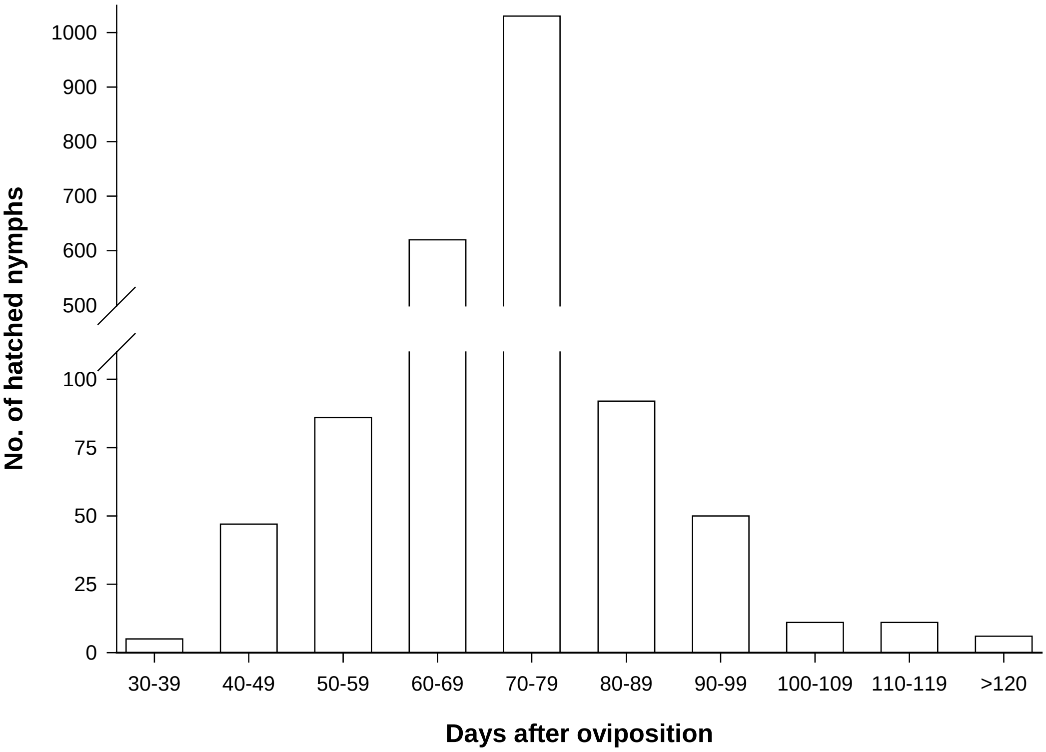

Figure 3-2: Hatching phenology of *M. diocles* nymphs ($N = 1958$).

3.3.2 Potential population growth and effects of limited hatching success

As generations in *M. diocles* were shown not to overlap potential population growth was calculated by the model of discrete stepwise growth described by Equation 3-1.

The fundamental net per capita rate of increase R (Equation 3-2) for *M. diocles* was 19.79 (intrinsic rate of natural increase per generation $r = 2.98$ and per day for females $r = 0.0125$). In other words: each individual of a generation contributed with a progeny of 20 individuals to the next generation.

The model resulted in exponential population growth (Figure 3-3). Starting with a hypothetical population of two individuals representing the founder generation T_0 (male and female) the population comprised 40 individuals in the first generation ($2 * 19.79 = 39.58$), multiplying to 783 individuals in the second, and 15494 individuals in the third generation (population size $T_4 \sim 306572$; $T_5 > 6*10^6$).

About 90 % of the eggs hatched; 2% of embryos were killed by fungal infestations and another 8% failed to hatch for unknown reasons. The reduction in hatching success of 10 % resulted in a lowered net per capita rate of increase of $R = 17.81$ ($r = 2.88$ per generation and $r = 0.0121$ for females per day). Accordingly population growth was decelerated with $T_1 \sim 36$, $T_2 \sim 634$, $T_3 \sim 11299$ and $T_4 \sim 201227$ individuals (Figure 3-3).

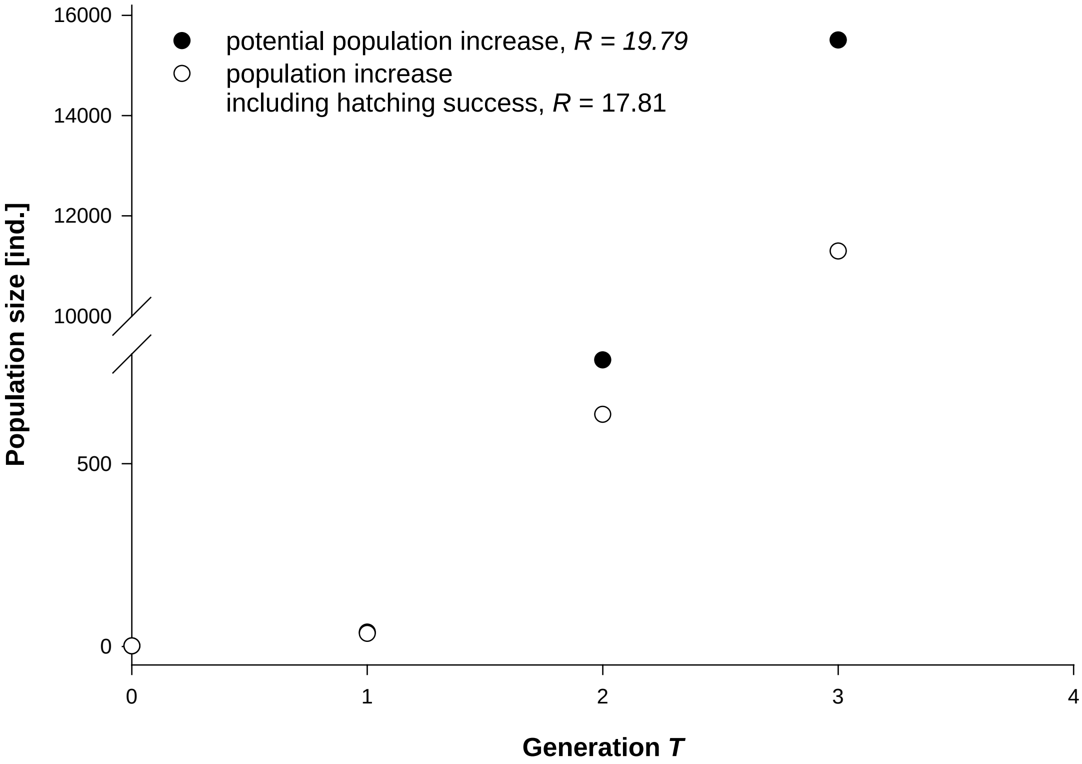

Figure 3-3: Model of discrete stepwise population growth (Equation 3-1) of *M. diocles* for potential population growth (filled dots) and population growth decreased by reduced hatching success (white dots). Models start from a hypothetical founder generation T_0 of two individuals. Net per capita rates of increase (R) are depicted in the figure legend.

3.4 Discussion

This study is the first to model potential population growth of a tropical phasmid and to give a first estimate of the impact of hatching failure of eggs on population increase. *M. diocles* disposed of a comparably low biotic potential that may partially explain the low density of this species documented on BCI (*cf.* Chapter 2). As expected, generations of this phasmid did not overlap and the according model of potential discrete stepwise population growth resulted in markedly higher population size than realized in the field. Hatching failure of eggs caused a marked reduction of population increase by 10 % suggesting that infertility and fungal infestations of eggs may be important but not prominent factors in the maintenance of low population density of *M. diocles*.

3.4.1 Demographic population parameters and life history traits

Presented data for *M. diocles* demographic and life history parameters largely corresponded with data compiled by Bedford (1978), who provided a helpful and capacious source of information on phasmid biology. Unless otherwise cited the following comparisons are based on Bedford (1978).

Sex ratio of the *M. diocles* lab population equaled 1:1 and is in accordance with sex ratio derived from many phasmid (Bedford 1978) and insect species (Wrensch & Ebbert 1993). Female to male ratios of sexually reproducing animals generally are expected to near equality when sons and daughters are equally costly to produce (Fisher 1930). For phasmids, sex ratio is sometimes female-biased, indicating that a species reproduces at least partially parthenogenetic. For instance, the sperm supply of a spermatophore may not last the whole adult life of a female. Some phasmid species then lay unfertilized eggs that develop into females. In contrast, *M. diocles* seemed to reproduce sexually resulting in a balanced sex ratio (even though copula could never be observed).

As for all phasmid species, sexes in *M. diocles* differed in size and weight with females being bigger and heavier than males. Sexual size dimorphism is widespread in animals with bigger females in most invertebrate species (Andersson 1994; Fairbairn 1997). Female-biased dimorphism is often attributed to a fecundity-advantage of large female size (Shine 1989). Larger female body size positively relates to longevity and to energy resources for egg production, both favoring higher reproductive success (Fairbairn 1997). Positive relationships of female body size to reproductive success of variable strengths were demonstrated for several insect taxa (*e.g.*, Fox *et al.* 1995a; Zanuncio *et al.* 2002) and proofed to hold proved to be true for *M. diocles* on a modest level. Fox *et al.* (1995a) found a similar weak relation of reproductive success to female weight in a seed beetle, but they provided evidence that larger females more often mated with larger males than their smaller conspecifics. Thereby larger females received more male-derived nutrients via extragametic substances of the spermatophores (that are bigger in bigger males; Fox *et al.* 1995b) and increased their egg production. Similarly larger *M. diocles* females may gain additional reproductive benefit via intrasexual competition for bigger sized males.

Size differences among females may reflect the effects of differing intensities of multiple control factors like food quality and availability, competition and predation pressure in the natural setting of an organism (Price 1984). Hence, in *M. diocles* control factors like food quality and predation pressure (addressed to in Chapters 4 and 5) most likely will influence female fecundity.

However, larger body size and higher weight can also be at the expense of mobility. Bedford (1978) reported that in most phasmid species females become gravid with eggs and cannot fly, although the wings may be large as in *M. diocles*. This may decrease female searching efficiency for new food patches. One deriving possible scenario for *M. diocles* would be that fertile females show a high fidelity to food patches resulting in clustered distributions as described in Chapter 2.

Generally, the variability in developmental times of phasmid eggs is enormous; hatching times range from 13 days up to three years. Species in temperate regions take longer times of egg development often linked to diapauses. For tropical phasmid species Bedford (1978) reported hatching times from 13 to 114 days, with the main hatching spreading over a time span of 20 to 30 days and only small numbers taking longer. By comparison *M. diocles* is situated in the middle of embryonic development time.

Developmental time of *M. diocles* nymphs and adult lifetime were consistent with other species, but mean female adult lifetime ranged on the lower end of published data on phasmids. Phasmid males in general have shorter larval development than females and are shorter lived as adults. Both was confirmed for *M. diocles*, although the difference in adult longevity was small compared to other phasmid species. *M. diocles* females in average lived 13 days longer than males. For example, adult females may live two (9 weeks, *Phyllium bioculatum* Gray) to eight times longer than males (8 months, *Phasma gigas* Amboine). Nonetheless, maximum lifetime of *M. diocles* adults almost reached 6 months for females and 4 months for males.

In terms of egg production *M. diocles* remained below the lowest values reported with 60 to 80 eggs per female for *Timema californica* Scudder. *P. bioculatum* produced in a comparable lifespan 80 to 100 eggs per female. In other species females can lay up to 1000 eggs.

3.4.2 The biotic potential of *Metriophasma diocles*

Both, low egg production and short-lived adult females converted into low reproductive potential of *M. diocles,* especially facing a relatively long generation time of approximately eight months. Prominent tropical herbivore groups, such as Lepidopterans and Coleopterans exhibit much shorter generation times and much higher reproductive potential (*e.g.*, Braker & Greene 1994, Caldas 1994, Chi & Yang 2003, Atluri *et al.* 2004). To give an example, Braker & Greene (1994) provide data on eight tropical butterfly species at La Selva, Costa Rica, with lifetime fecundity ranging from 217 to 521 eggs and maximum generation times of 74 to 227 days. While generation times of single species may be comparable to *M. diocles* (239 days for females) these species all possess much higher individual fecundities (40 eggs for *M. diocles* females). The low biotic potential of *M. diocles* becomes apparent compared to the intrinsic rate of increase of a predatory coccinelid beetle including stage specific mortality ($r = 0.113*d^{-1}$, Chi & Yang 2003), that exceeds maximum r of *M. diocles* almost tenfold ($0.0125*d^{-1}$; no mortality until mean generation time). Thus low abundances of *M. diocles* may at least partially result from its low biotic potential.

Despite large differences in biotic potentials among species, most insect herbivores in the tropics are rare (Basset 1996, 1999; Basset *et al.* 1992, 1996; Barone 1998; Novotny *et al.* 2002b). This is also true for phasmids (Bedford 1978, Willig & Camilo 1991, Novotny & Basset 2000) and was shown for *M. diocles* in Chapter 2. Regardless of the comparably low reproductive output, potential population growth of *M. diocles* described by a model of discrete stepwise growth resulted in exponential population growth. Already in the first generation, modeled potential population densities were higher (applying $T_1 = 40$ ind. on a ha base; *cf.* Figure 3-3) than realized in the forest edge or understory on BCI (21.5 and 6.7 ind.*ha^{-1} respectively; *cf.* Chapter 2). In following generations modeled population sizes by far exceeded *M. diocles* realized population densities (Figure 3-3). Consequently *M. diocles* cannot reach its potential population size in its natural setting and population control factors must act continuously to maintain its low realized density.

3.4.3 A first insight into *M. diocles* population control

Egg mortality (*sensu* hatching failure or fungal infestation) may be one factor reducing reproductive output of *M. diocles*. Mortality of insect eggs can be of major importance in limiting insect populations (*e.g.*, Parker & Wakeland 1957, Prior & Greathead 1989, Caldas 1994, Dyssart 1995, Phoofolo *et al.* 2001). Hatching failure may occur due to infertility of eggs (Caldas 1994) or because of fungal or bacterial infestation (Bedford 1978). However, the impact of hatching failure due to these factors has reached little attention. Data from Caldas (1994) indicate that infertility of eggs may play a minor role (0.5 % infertile eggs in a nymphalid butterfly). Here, I observed hatching failure of 10 % with 2 % of *M. diocles* embryos being killed by fungi. Hatching failure initially lowered population growth markedly (*cf.* Figure 3-3). Nevertheless, this may be of no impact after generation five when population density had reached legion (only being limited by resource availability). On the other hand, egg mortality may play an important role when population density is low as shown for *M. diocles* in Chapter 2 and when a number of other factors increases mortality levels. For eggs, predators (Parker & Wakeland 1957, Phoofolo *et al.* 2001) and parasitoids (Prior & Greathead 1989, Caldas 1994, Dyssart 1995) may play a much more prominent role than infertility or pathogens. For example, predators may reduce lepidopteran egg densities by 50 % (Phoofolo *et al.* 2001) and parasitoids were shown to kill 11 % of grasshopper (Dyssart 1995) and up to 38 % of butterfly eggs (Caldas 1994). Likewise it is known that wasps parasitize phasmid eggs (Chrysididae, Cleptidae ;Bedford 1978). Most likely *M. diocles* eggs face a wider array of mortality agents in its natural setting and egg mortality may be much higher than under lab conditions. Accordingly, egg mortality may be one important factor among a variety of population control factors acting on later life stages that explain low population densities of *M. diocles*.

Some minor restrictions of this study need comment. In general, the presented results are based on extensive data collection, and hence are considered safe from negative influence from lab conditions. Fecundity and adult lifetime of phasmids can be negatively affected by crowding (stress and epidemic diseases) and by poor quality of food plants (*cf.* Joern & Gaines 1990). I compensated for low quality food by providing animals with a range of fresh food plant species planted in pots. Diseases most likely played a role in the lab population because two major die-offs of nymphs were observed during the study period. As stated in the introduction, negative density effects due to intraspecific competition cannot be avoided and epidemic diseases as competition-free situations are almost non-existent (Begon *et al.* 1996). Still, if these factors influenced the presented results, then this would weaken only my conclusion regarding the role of the 'low' biotic potential as one factor partially explaining low *M. diocles* density.

3.4.4 Conclusions

Clearly the presented model of population growth represents a synthetic situation while natural populations are exposed to an interplay between biotic and abiotic factors leading to fluctuating population densities as suggested for grasshoppers (Berryman *et al.* 1987, Joern & Gaines 1990). The comparably low biotic potential and the reduction of population growth by failed hatching of eggs are the first steps leading towards the understanding of why natural *M. diocles* population densities are low. Clearly, this approach lacks any explanation for population fluctuations. Reproductive output of females and hatching success vary depending on factors like the females physiological state, pathogens and egg predators, humidity and temperature (*e.g.*, Bedford 1978; Chapman & Joern 1990; Bernays & Chapman 1994). Nevertheless, I demonstrated that *M. diocles*, despite its comparably low reproductive output, has the potential to reach much higher densities than found in its natural setting (*cf.* Chapter 1).

4 Preference of female adults and performance of larval stages of *M. diocles* on single food plant species

4.1 Introduction

In tropical forests, herbivores consume up to 11 % of annual leaf production (Coley & Barone 1996). Yet, despite high availability of plant tissue most herbivorous insects in the tropics are rare (Basset 1996, 1999; Basset *et al.* 1992, 1996; Barone 1998; Novotny *et al.* 2002b). In the initial debate about the factors that keep insect herbivores at low densities, Hairston *et al.* (1960) suggested that natural enemies were responsible for the state of affairs. Alternatively it was suggested that plants themselves keep herbivores rare because they are poor food (McNeill & Southwood 1978; Janzen 1988), and that evolution has favored selection of plant defenses and high plant biodiversity as response to herbivore pressure (Ehrlich & Raven 1964; Janzen 1970; Connell 1971; Rhoades & Cates 1976; Coley *et al.* 1985). In the meantime there is consensus that both, bottom-up and top-down regulation mechanisms maintain tropical herbivores at low densities and attention has shifted towards their relative roles (Pace *et al.* 1999; Persson 1999; Polis 1999; Dyer & Coley 2001).

There is no doubt that most plants are poor food for particular herbivores, either because they are nutritionally inadequate or because they are poisonous (Southwood 1973; Lawton & McNeill 1979; Strong *et al.* 1984; Janzen 1988; White 1993). Low suitability of a plant is not necessarily a consequence of herbivore selection, as for example high fiber and lignin contents are inevitable for plant architecture (Hartley & Jones 1995). Regardless whether the evolution of poor quality of plant tissue reflects herbivore pressure or functional traits, nutritional quality has been implicated as a major factor in the debate about the roles in population regulation of herbivorous insects (House 1967; Onuf *et al.* 1977; Onuf 1978; McClure 1980). Limitations in the value of plant tissue as food reflect bottom-up forces that influence herbivore performance determining larval growth rates, survival, adult size, and fecundity (e.g., Häggstrom & Larsson 1995; Burghardt & Fiedler 1996; Barker & Maczka 1996; Awmack & Leather 2002).

Plants are inadequate food because plant tissues contain low amounts of nitrogen and protein (McNeill & Southwood 1978; Mattson 1980), sometimes they have lower water contents than animals (Scriber & Slansky 1981), and they have tough leaves (Feeny 1976; Lowman & Box 1983; Nichols-Orians & Schultz 1989). Plants also contain toxins, repellents, growth-inhibitors and digestibility reducing compounds that can be present all the time, i.e. constitutive secondary metabolites (Rosenthal & Berenbaum 1992). In addition, secondary metabolites may be induced in response to herbivore attack including phenols (Niemelä *et al.* 1979; Rossiter *et al.* 1988; Haukioja 1990; Kogan & Fischer 1991;

Tscharntke *et al.* 2001), alkaloids and protease inhibitors (Baldwin & Ohnmeiss 1993; Baldwin 1996; Karban & Baldwin 1997; Baldwin *et al.* 1998; van Dam *et al.* 2001a).

The defensive character of these plant traits has been documented in numerous studies. For instance, low water contents of leaves have been associated with reduced preference and performance of insects (Moran & Hamilton 1980; Mattson & Scriber 1987; Stamp & Casey 1993; Joern & Behmer 1997; Schädler *et al.* 2003). Leaf toughness is discussed to be the most effective physical defense (for review see Coley & Barone 1996 and references therein) and was shown to be negatively correlated with herbivory (Cherret 1968; Coley 1983; Lowman & Box 1983; Nichols-Orians & Schultz 1989; Reich *et al.* 1991; but see Landa & Rabinowitz 1983). Tannins can act in a dosage-dependent manner (Feeny 1970, Rhoades 1977; Coley 1986) and can be toxic or deterrent to particular herbivores (reviewed in Bernays *et al.* 1989). Phenolic compounds may be induced in response to insect damage and increased phenolic contents then may negatively affect herbivores (e.g., Niemelä *et al.* 1979; Rossiter *et al.* 1988; Haukioja 1990; Kogan & Fischer 1991; Tscharntke *et al.* 2001). Such chemical defenses are compounds that affect either the digestion of biomass or have toxic effects after being absorbed. Physical leaf properties, such as toughness and water content, reduce the digestion of biomass. Compared with studies on chemical defenses, there are fewer studies that have demonstrated that the physical structure of plants can prevent or influence herbivory (Sanson *et al.* 2001).

Confronted with these factors lowering the value of foods herbivores must assess more variables to determine food selection than do carnivores (Stephens & Krebs 1986). Thus herbivores forage within higher nutritional constraints than carnivores (Southwood 1973), the more as plant tissue is lower in nutrients and higher in non-digestible structural materials than the body tissues that must be built from these (e.g., Mattson 1980). A single food type of such poor or even toxic quality rarely will provide all essential nutrients for survival. As a consequence herbivores may feed selectively on a mixture of plants (Joern 1979; Cates 1980; Bernays *et al.* 1994) or on plant parts of differing quality (Bernays & Chapman 1994).

Nutritional requirements of herbivores are not consistent within a population (Cates 1980; Karowe 1989). Individual variation in dietary constraints may exist as a function of age, sex or morphology (Boys 1978; Sandlin & Willig 1993; Bernays & Chapman 1994; van Dam *et al.* 2001b). During developmental stages nutritional requirements may differ (Bernays & Chapman 1994) and particular plant defenses may influence early larval instars stronger than later instars (van Dam *et al.* 2001b). Feeding habits of adult herbivores may reflect previous feeding experience as a consequence of induced preferences or physiological specialization throughout an individual's lifetime (Cassidy 1978; Papaj & Prokopy 1988; Redfearn & Pimm 1988; Karowe 1989; Sandlin & Willig 1993) and female adult feeding behavior may be linked to preferential oviposition (Bernays & Chapman 1994). Above that adult insects experience additional reproductive constraints. Especially females may be more nutrient limited than males considering the relatively higher reproductive investment (Boys 1978; Bernays & Chapman 1994).

Explaining the effects of food quality on herbivores one has to differentiate between generalists and specialists. According to biochemical coevolution, herbivore generalists should be more susceptible to qualitative toxic chemical plant defenses than specialists (Ehrlich and Raven 1964; Feeny 1976; Cornell & Hawkins 2003). Thus generalist herbivores may have complex diets to meet nutritional requirements while avoiding plant defenses (Belovsky 1984). Specialized herbivores that overcome such plant defenses by detoxifying or even sequestering plant compounds should be less affected. Consequently specialists such as mono- or oligophagous herbivores may particularly respond to physical defenses such as leaf toughness and water content.

The study presented here assessed the impact of interspecific variation in food quality due to physical and chemical defensive leaf traits on preference and performance of the walking stick Metriophasma diocles.

As shown before (see Chapter 2 and Berger & Wirth 2001) *M. diocles* feeds exclusively on plants from two different plant families, the Piperaceae and Araceae. According to recent phylogenetic analyses these plant families are not closely related (Judd *et al.* 2002). The Piperaceae are phylogenetically separated from other dicots, which may involve differences in phytochemistry (Judd *et al.* 2002). In fact, Piper species are characterized by a wide array of phenolic compounds (Sengupta & Ray 1987; Baldwin & Schultz 1988; Parmar *et al.* 1997; Dyer *et al.* in press), many of them deterring insect herbivores (Parmar *et al.* 1997 and references therein). Likewise the Araceae as monocots are distinct from dicots in many features. Araceae were characterized by the (probably) universal presence of tannins (Grayum 1990), they contain calcium oxalate crystals, cyanogenic compounds, and sometimes alkaloids, all known to deter herbivores (Judd *et al.* 2002 and references therein). Although *M. diocles* seems to be able to detoxify such toxic or deterrent plant chemicals (cf. Chapter 2), the phylogenetic distance of Araceae and Piperaceae does not allow to conclude on a strict coevolutionary scenario (sensu Ehrlich & Raven 1964; Strong *et al.* 1984). Such specialization on distantly related hosts is best described as disjunct oligophagous (Chapter 2; Bernays & Chapman 1994). Considering *M. diocles* as herbivore specialist, I hypothesized that preference and performance of this species would rather be explained by physical leaf characters than by content of chemical compounds.

In particular, (1) I assessed interspecific differences in food quality of *M. diocles* host plant species on the base of leaf toughness, water content, specific leaf weight (SLW), tannin and total phenol contents. By the use of feeding trials, (2) I ranked host-plant species according to female adult preference and (3) surveyed nymph performance (sensu survival and growth) on single plant species. (4) By correlation analysis I examined the relation of defensive leaf traits to adult preference and nymph performance. In addition (5) I tested whether nymphs were sensitive towards increasing levels of leaf tannin or total phenol contents reflecting induced responses of the host to herbivory.

Defensive leaf traits were either measured from leaves that were fed to adult females in feeding trials (water content, SLW, tannin, phenol) or from leaves sampled from the *M. diocles* habitat (toughness). Specific leaf weight (sometimes referred to as leaf mass per area – LMA) is the product of leaf thickness and dry matter concentration (Witkowski & Lamont 1991) and reflects structural reinforcement of leaves. SLW shows a strong positive relationship with leaf lifespan across species (Wright & Cannon 2001) reflecting the important role of structural reinforcement of leaves in determining their lifespan presumably by rendering them less susceptible to herbivory (e.g., Bernays & Chapman 1970; Schädler *et al.* 2003).

To assess feeding preference I conducted dual-choice feeding trials with 15 host plant species of *M. diocles*. I concentrated on adult females because they are more nutrient limited than males, they are larger than males (see Chapter 3) - a pattern found consistently in phasmids (Bedford 1978) - and they invest more in reproduction (Boys 1978; Bernays & Chapman 1994). In addition female longevity directly affects population increase as demonstrated in Chapter 3.

I surveyed nymph growth and survival on 13 single host plant species over the course of six weeks. Nymphs should be affected stronger by low food quality and it was argued that shortage in suitable food may be the main mortality factor in early lifestages of insect herbivores (Joern & Gaines 1990).

Because biochemical differences in leaves may increase with taxonomic distance of plant species (Ehrlich & Raven 1964; Rhoades & Cates 1976) differences in phytochmistry between Araceae and Piperaceae may cover effects of other defensive traits. To account for taxonomical dissimilarities in plant chemistry correlation analysis of prefernce and performance with leaf traits was repeated under exclusion of the Araceae (2 species).

Finally, I expected adult preference to relate to nymph performance because phasmid adults were shown to display preferences based on previous feeding experience as nymphs (Cassidy 1978; Sandlin & Willig 1993).

4.2 Materials and methods

For details on study site, identification of plant species and maintenance of the *M. diocles* lab population please refer to Chapter 1.

Feeding trials on preference of *M. diocles* adult females and performance of nymphs were conducted from March 2000 to January 2002 on Barro Colorado Island, Panama.

Feeding trials on nymph preference under varying phenol and tannin leaf contents took place from April 2002 to June 2002 at the Technical University of Kaiserslautern, Germany.

Phenol and tannin leaf contents were assessed from freeze-dried leaves in February and March 2003 at the Technical University of Kaiserslautern, Germany.

4.2.1 Measuring leaf traits

Relative leaf toughness, water content, specific leaf weight, total phenol and tannin content were assessed for mature leaves of 15 host plant species of *M. diocles*.

Leaves were collected in the vicinity of the BCI laboratories at the forest edge and in the understory. No more than three leaves were collected per plant individual to cover intraspecific variation in leaf traits. Leaves were stored in zip log bags in a cooler until measurements were taken.

Data for leaf water content and specific leaf weight derived from leaves used in dual choice feeding trials and are expressed as species-specific means (for sample size see Table 4-1). For details see Chapter 4.2.2.

4.2.1.1 Measuring leaf toughness

Coley and Barone (1996) considered leaf toughness as the most effective plant defense. For this reason I decided to use the *M. diocles* host plant species with lowest leaf toughness as reference in dual-choice feeding trials (see Chapter 4.2.2). Consequently I had to measure leaf toughness before starting preference tests. This initial screening included nine *Piper* and two *Philodendron* species. From these species I collected six leaves from six individuals each in the nearby vicinity of the BCI laboratory and took toughness measurements as described below. Later, when preference tests were already running, I added four more *Piper* species. Here, I measured toughness of all leaves that were used in dual-choice feeding trials. For these reasons sample sizes for toughness measurements vary among species (for sample size see Table 4-1).

Leaf toughness was measured using a Chatillon penetrometer (Chatillon Co. Ltd., New York; rod diameter = 3.25 mm; one unit represents a force of 1 g * 8.3 mm^{-2}) (alternative methods are described by Choong 1996). As I wanted to measure the strength an herbivore needs to cut leaf tissue I had to assure to minimize tissue flexibility. Therefore each leaf was positioned between two Plexiglas plates seated on four stands. The Plexiglas plates were perforated with holes of 5mm diameter both. Upraising the plates prevented from flawed measurements as the punching rod may jump back up if it hits a hard surface directly after having pierced the leaf tissue. From each leaf I took 10 measurements. Mayor leaf veins were avoided. Toughness was calculated on a relative scale as species-specific mean toughness of mature leaves.

4.2.1.2 Leaf phenol and tannin content

To evaluate leaf foliar chemistry freeze-dried leaves from dual-choice feeding trials were ground and extracted in 70 % aqeuous acetone (Hagerman 1988).

Total phenolics were assayed using the technique of Price & Butler (1977) modified by Mole & Waterman (1994). The reaction is an oxidation-reduction in which the phenolate ion is oxidized and ferric irons are reduced to the ferrous state forming the Prussian Blue complex. Phenolic content is then measured spectrophotometrically.

Tannins were measured with the radial diffusion method (RDM; Hagerman 1987). The RDM is a protein-binding assay that can be used to determine the amount of both condensed and hydrolysable tannin albeit the method is more sensible to hydrolysable tannins (Hagerman 1987). The tannic solution is placed in a petry dish with a bovine serum albumine (BSA) containing agar. As tannin diffuses into the gel it reacts with BSA and protein precipitation becomes visible as a ring. The area of the ring is proportional to the tannin content of the extract.

Both total phenols and tannins were measured from 5 leaves of 14 plant species each, and from 20 leaves for *P. marginatum* (see Table 4-2). For extractions I used approximately 50 mg dry leaf material. Price and Butler phenolics and tannins are reported as percent Tannic Acid Equivalent (% TAE) per g dry leaf weight derived from standard curves using tannic acid as a standard.

4.2.2 Dual-choice feeding trials with *M. diocles* adult females

The synchronous offer of food alternatives is a way to reveal the relative preference for a given plant (*e.g.*, Krebs 1989). To assess preference of adult *M. diocles* females I performed dual-choice feeding trials. In such a design preference for a plant species (tested species) is expressed as relative preference in comparison to a fixed reference plant species (Richardson & Whittaker 1982). In varying test plant species dual-choice tests offer the possibility to assess a food rank order.

M. diocles females were presented with leaves of two different plant species: *Piper marginatum* always served as reference, while the test plant species varied. I decided for *P. marginatum* for two reasons: (1) In field records *M. diocles* proofed to be highly associated with plants of the genus *Piper*, in particular with *P. marginatum* (*cf.* Chapter 2). And (2) *P. marginatum* proofed to have the leaves with lowest toughness (see Figure 4-2). According to Coley & Barone (1996) leaf toughness may be the strongest herbivore defense and consequently the plant species with lowest toughness should be preferred. In a pre-study I measured leaf toughness of 11 host-plant species (see Chapter 4.2.1): *P. marginatum* had the lowest leaf toughness (Table 4-1).

Test animals were caught in the field or reared in a laboratory population. As feeding behavior of adult phasmids may reflect prior feeding experience (Cassidy 1978; Sandlin & Willig 1993), animals in the lab were fed with all according food plant species simultaneously.

I tested preference of *M. diocles* females for 12 *Piper* and two *Philodendron* test plant species. Leaves for feeding trials were collected in the vicinity of the BCI laboratory. No more than three leaves were collected per plant individual to cover intraspecific variation in leaf characters. Leaves were put in a plastic zip log bag, which then was kept on ice in a cooler to keep the leaf material fresh. Between every following step in handling the leaves were stored coolly.

Leaves first were marked individually using a waterproof marker. Then every leaf was cut in two along the midrib. The half attached to the midrib was used for the feeding trial. Its area was measured using a Leaf Area Meter LI-3100 (LI-COR inc., Lincoln, USA) on 1 mm^2 area resolution. Both the share of the reference and the test leaf were put together with their petioles sticking in a water filled vial being closed with cotton batting. The vial then was attached to a wire clamp in a single feeding arena so that leaves stood upright. When all arenas were equipped, I collected the animals from the lab colony and started the trial by setting one adult female in each arena. A trial usually started around noon and then lasted 24 hours. To quantify consumption, leaf area was measured again after a trial was finished.

The second half of each leaf was used for measuring water content. For assessing water content I used a punch to get a leaf disc of 15 mm diameter, which was weighed and then freeze-dried for at least three days. Afterwards dry mass was weighed. By that I could calculate water content for each leaf used in feeding trials. The leaf remnants also were freeze-dried for analysis of leaf phenol and tannin contents.

Each *M. diocles* female was used only once per test plant species. I conducted 30 plus individual choice tests for each of 14 test plant species (for sample size see Table 4-1). Differences in numbers of replicates resulted from females that did not feed in trials and from females having died while the choice-test was running. For *Piper arboreum* only 20 replicates could be accomplished, as no more females were available.

4.2.3 Problems inherent with dual-choice feeding trials

To assure the accuracy of the results from choice tests I addressed two major problems inherent in the use of feeding trials: Food choice of an animal may be based on prior feeding experience (*e.g.*, Sandlin & Willig 1993) and the selection of the reference test plant may affect the preference ranking (see *e.g.*, Richardson & Whittaker 1982; Capinera 1985).

4.2.3.1 Conditioning due to prior feeding experience

The number of females in the lab colony generally restricted the number of replicates. As a basic principle no individual was used twice with the same test plant species. But individuals had to be deployed in feeding trials with different test plant species, *i.e.* preference data in different choice-tests (e.g. *P. grande* and *P. hispidum* as test plant species) origin from the same female. As a consequence prior experience and conditioning on a particular food source of these females could have influenced the outcome of succeeding choice tests. The resulting question was whether females of *M. diocles* showed such conditioning behavior or whether they acted naively in every new choice situation. Only in the latter case data gathered in feeding trials would be independent as every female in every new choice situation differentiated among offered food sources.

To test for female naivety I designed a sequence of two dual-choice tests with different test plant species (*P. grande* and *P. hispidum*) and *P. marginatum* as reference. In both dual-choice tests identical females were used. The procedure of the feeding trials followed the protocol as described above (Chapter 4.2.2). In case food selection was influenced by prior experience, I assumed females to display similar preference patterns in succeeding feeding trials. In contrast to this expectation food preference of females differed significantly (Figure 4-1). The preference shift from *P. hispidum* in test 1 towards *P. marginatum* in test 2 clearly demonstrated that in the following choice-test females decided in a naïve manner and favored the formerly disregarded plant species. This result was supported by pair wise comparisons of choice-tests that shared female individuals. The exclusion of females that were not shared between these dual-choice feeding trials always resulted in a pattern consistent with the preference pattern for all females (data not shown).

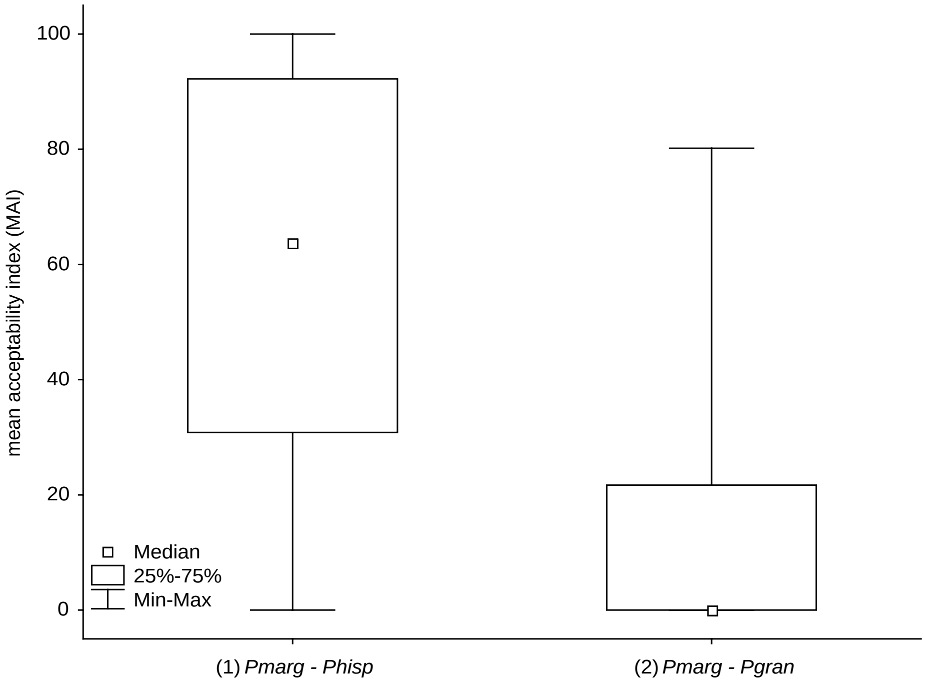

Figure 4-1: Comparison of paired dual-choice preference tests of *M. diocles* adult females. Identical individuals were used in both tests to control for influence of prior feeding experience (for details see text; Wilcoxon paired signed rank test, T = 21, $N = 21$, $P < 0.01$).

4.2.3.2 Choice of reference plant species

A further problem inherent with dual-choice feeding trials lies within the choice of the reference plant species. The observed preference pattern could result from discrimination towards a particular plant species and change if an alternative reference was offered (Richardson & Whittaker 1982). Hence the preference pattern may not be consistent if observed with different plant species serving as reference.

To account for this problem I conducted a series of dual-choice feeding trials using *Piper hispidum* as reference plant species and compared the resulting MAI with preference assessed against *P. marginatum* in pair wise Mann-Whitney U-tests (test species: *P. aequale*, *P. dilatatum*, *Phil.* sp., *P. reticulatum*). Changing the reference plant species did not change the overall preference pattern of *M. diocles* adult females. MAI resulting from dual-choice feeding trials with different reference plant species (*P. marginatum* and *P. hispidum*) did not differ (all p > 0.35).

4.2.4 Long term feeding trials with *M. diocles* nymphs

To document growth and mortality of *M. diocles* nymphs I conducted long term feeding trials on single host plant species. At least 30 nymphs were raised on each of 13 food plant species. All performance trials lasted 6 weeks (performance *sensu* survival and growth).

The selection of food plant species resulted from diet assessment from field records and no-choice feeding trials of *M. diocles* (see Chapter 2). Because *Piper* spp. were the main potential food sources in the field I tested 10 *Piper* species to evaluate interspecific differences in diet suitability of congeners. I included three species of Araceae: two *Philodendron* species were also recorded as potential food in the field whereas no *M. diocles* individual was found on *Dieffenbacchia longispatha* (but *D. longispatha* was accepted in no-choice feeding trials). Due to partial lack of nymphs and to restrictions in labor potential I could not include all *Piper* species tested in adult female preference tests.

Newly hatched nymphs were weighed and then separately kept in plastic food containers (10cm wide, 5cm deep, 5cm high). Adult leaves were harvested in the forest, placed in a sealed plastic zip log bag and brought back to the laboratory. Within 2 hours of being collected leaf discs (punch of 13 mm diameter) were cut and presented to nymphs. Leaf discs were stuck into a piece of cardboard to assure free access for the nymph. A piece of humid paper towel helped minimizing desiccation. If multiple nymphs hatched on the same day they were allocated to different food plant species. Nymphs then were provided with new food daily and controlled for survival. To document growth, nymphs were weighed in weekly periods.

4.2.5 Dual-choice feeding trials with *M. diocles* nymphs

This experiment was conducted at the Technical University of Kaiserslautern, Germany.

P. hispidum plants were grown in greenhouses from cuttings I imported from Panamá. *M. diocles* nymphs had hatched from eggs I previously had collected from a lab population at Barro Colorado Island, Panamá.

As defensive or deterring effects of phenolic or tannic compounds may be dose-dependent (Feeny 1970; Rhoades 1977; Coley 1986), I designed a test series of dual-choice feeding trials where nymphs were offered leaf discs with artificially increased natural phenol and tannin contents by use of a method described by Beyschlag & Pfanz (1990) and modified by Herz (unpublished). Beyschlag & Pfanz (1990) used pressure infiltraton of water into a leaf via the stomatal pores to determine stomatal aperture. Herz (unpublished) used this method to infiltrate sugar solutions in leaves and offered these to leaf cutting ants. I modified the method as I used leaf discs (15 mm punch) of *Piper hispidum* and infiltrated aqueous solutions of tannic acid and of total phenol extract from *P. hispidum* leaves.

Phenolic compounds were extracted with 70 % aqueous aceton from *P. hispidum* leaves following the same procedure as described by Waterman & Mole (1994). Aceton and water of the phenolic solution evaporated within 48 hours under room temperature and the remaining phenolic aliquot was resolved in distilled water. The phenolic content of the resulting solution was assessed by the Price and Butler method (1977) as described above. In preliminary infiltration trials I assessed the amount of water that could be infiltrated into leaves (27.4 ± 4.3 % fresh weight). In combination with dry weight and natural total phenol contents of *P. hispidum* leaves I could then calculate the concentration of solution needed to yield particular increases of % TAE in leaf discs.

Infiltration was carried out with a 50 ml syringe filled with approximately 25 ml of aqueous phenol or tannin solution (or water for the control). Immediately after punching the disc was weighed and put in the syringe. All remaining air was removed through the outlet. Then the outlet was closed and the piston was forcefully pulled outward the depression leading to an evacuation of the internal air and the intercellular space. Simultaneously the syringe was shaken to remove gas bubbles from the leaf surface and prevent them from entering the leaf disk during the following infiltration process. By slowly pushing the piston back into the syringe the solution (or water) column was set under pressure and entered the leaf via open stomatal pores and via intercellular space at the cutting edges. After infiltritation the leaf disc surface was dried carefully with a peace of paper towel and weighed again. Weight gain was considered as infiltrated amount of solution (water). In combination with natural total phenol contents of leaves and their dry weight infiltrated percent TAE in the leaf disc was calculated. Until the start of the feeding trials leaf discs were kept on ice.

First instar nymphs of *M. diocles* were presented with two leaf discs: 1) infiltrated with water (control) and 2) infiltrated with the according solution (treatment).

Nymphs were set in plastic food containers (10 cm wide, 5 cm deep, 5 cm high). Leaf discs were stuck into a piece of cardboard to assure free access for the nymph. A piece of humid paper towel helped minimizing desiccation. Climate chambers allowed for conditions similar to the tropics (27°C average temperature, 70 to 90 % relative humidity). Dual-choice feeding trials lasted 24 hours. This was a sufficient time span to assess preference because a preliminary test on dual-choice feeding trials over three days showed no differences in preference among days (Friedmans ANOVA $\chi^2_{(N=38,\ FG=2)} = 1.28$, $P < 0.53$). After finishing the trial consumed leaf area was measured with a transparent grid. Preference was then calculated on the base of consumed dry weight (see Chapter 4.3.2).

To assure that infiltration did not alter feeding behavior I tested normal leaf discs against water infiltrated leaf discs. Both, control and treatment were fed equally resulting in a relative preference of 0.5 indicating that infiltration did not affect feeding behavior (compare to control in Figure 4-12 & Figure 4-13).

4.3 Data analysis

Multiple groups where compared in Analysis of Variance. ANOVA assumes normality of data and homogeneity of variances. If not stated differently data transformation resulted in normally distributed data and allowed minimizing differences in variances whereas homogeneity of variances could not be attained in all cases. However, failure to meet the assumption of homogeneity of variances is not fatal to ANOVA. Analysis of Variance still remains robust, even when groups are of different sample sizes (Zar 1999, p. 185 and references therein).

Data on leaf toughness, specific leaf weight and total phenol content were log-transformed before statistical analysis. As water content and MAI were expressed on a relative scale data were arcsine transformed. Interspecific variation in leaf characters was then analyzed in ANOVA.

4.3.1 Analysis of preference

In relation to *P. marginatum* preference of *M. diocles* females for 12 *Piper* and two *Philodendron* species was tested. On the base of consumed leaf dry material I calculated a mean acceptability index (MAI) (Richardson & Whittaker 1982) from the ratios of consumed dry weight (dw) of test material to the total of consumed dry weight in each replicate:

(Equation 4-1)

$$MAI = \frac{[\text{consumed dw}]_{\text{test}}}{[\text{consumed dw}]_{\text{test}} + [\text{consumed dw}]_{\text{reference}}}$$

The MAI allows to group plant species to their ranking as acceptable food source of a distinct phasmid species and ranges from cero to one. MAI-values below 0.5 and down to cero thereby indicate preference for *P. marginatum* whereas values above 0.5 represent preference towards the test plant species.

Consumption ratios from feeding trials were not normally distributed. The distribution of MAI values was two-tailed as 0 and 1 ratios derived frequently resulting from females eating only one food source. Consequently differences in preference among food plant species were tested by nonparametric Kruskal-Wallis ANOVA. Preference is expressed as median MAI.

The median of the MAI corresponded significantly with its mean (Product Moment Correlation $r = 0.98$, $df = 12$, $P < 0.01$) and median MAI values were normally distributed. Thus the relationships between each leaf trait and the established preference pattern were analyzed in parametric Product Moment Correlation Analysis.

A test series of dual-choice feeding trials was conducted where nymphs were offered leaf discs with artificially increased phenol and tannin contents. Resulting preferences, expressed as MAI, were compared in Mann-Whitney U-tests. The calculation of the MAI is described above.

4.3.2 Analysis of performance

Survival of nymphs was analyzed by the Kaplan-Meier estimate (also known as the product-limit estimate). The survival function gives the probability that an individual survives past a given time. Differences in survival times were analyzed by the Mantel-Cox log-rank test (Mantel 1966; Cox 1972). Under the assumption that deaths of nymphs were independent the Mantel-Cox test proved suitable for the dataset as it gives equal weight to all events.

For reasons of sample size resulting from partially high nymph mortality (in *D. longispatha* and *P. grande* no nymphs had survived beyond week three) relative growth rates of nymphs were calculated after approximately 14 days. For the same reasons weight measures spread from day eight to day 17 with 73 % of values referring to days 11 to 16. To account for temporal differences growth was calculated on a daily base as growth rate [$mg*d^{-1}$]. Initial weight of nymphs was significantly different among groups (*i.e.*, nymphs fed on different plant species) (ANOVA, $F = 31.9$, $df = 12$, $P < 0.01$). Calculation of relative growth rates (RGR) accounted for initial weight of nymphs and incorporated possible covariate effects:

Equation 4-2 $$RGR = \frac{\text{final weight - initial weight}}{\text{days}} \times \frac{1}{\text{initial weight}}$$

The relative growth rate expresses the weight gain relative to the initial weight at the start of an experiment per unit time (relative weight gain per day).

Plant species-specific relative growth rates of nymphs were then compared in Analysis of variance (ANOVA).

The relationships between each leaf trait and survival and growth of nymphs were analyzed in parametric Product Moment Correlation Analysis. *D. longispatha* was not included in correlation analysis, because representative measurements of leaf traits could not be taken before the end of the study period.

4.4 Results

4.4.1 Interspecific differences in physical and chemical leaf properties

All measured leaf characters showed significant interspecific differences indicating that host plant species of *M. diocles* varied in nutritional quality. Mean values are listed in Table 4-1 including the short names for plant species that are used in the Figures.

M. diocles food plants showed expressed heterogeneity in leaf toughness (Figure 4-2). Relative leaf toughness varied significantly among host plant species with leaves of *Philodendron sp.* possessing a fivefold higher relative toughness (505.47 ± 41.68) than *Piper marginatum* (102.40 ± 14.82). Although *P. marginatum* had lowest leaf toughness there was no significant difference to *P. arboreum* and *P. hispidum*. All tested *Piper* species showed significantly lower relative toughness in leaf tissue than the tested Araceae of the genus *Philodendron*. However, variation in toughness among *Piper* congeners was considerably high: *P. cordulatum* and *P. grande* (296.37 ± 29.98 and 323.36 ± 31.38) had threefold tougher leaves than *P. marginatum*. The pattern of interspecific differences in relative leaf toughness seemed to be expressed stronger than patterns assessed from other leaf characters (see below). No more than three plant species had similar toughness values.

Table 4-1: Physical leaf traits of 15 *M. diocles* host plant species. Measurements for water content and specific leaf weight were taken in dual-choice feeding trials with female adults, accordingly the number of leaves per species (*N*) equals the number of preference tests.

Plant species (short name)	*N*	**Relative toughness***	**Water content*** [%]	**SLW*** [mg*cm^{-2}]	*N**
Piper marginatum (Pmarg)	36	102.40 ± 14.83	77.98 ± 4.94	2.86 ± 0.80	422
Piper aequale (Paequ)	36	234.61 ± 33.26	78.13 ± 3.23	3.74 ± 0.77	31
Piper arboreum (Parbo)	20	105.92 ± 8.30	72.45 ± 2.44	5.73 ± 0.82	20
Piper cordulatum (Pcord)	36	323.36 ± 31.38	83.83 ± 1.92	5.49 ± 0.68	31
Piper culebranum (Pcule)	36	175.53 ± 18.82	75.20 ± 6.86	4.31 ± 1.17	32
Piper dariense (Pdari)	30	171.19 ±25.55	76.90 ± 2.13	3.77 ± 0.38	30
Piper dilatatum (Pdila)	36	121.42 ± 24.56	76.20 ± 3.44	4.04 ± 1.07	32
Piper grande (Pgran)	24	296.38 ± 29.98	78.49 ± 3.05	4.19 ± 0.66	30
Philodendron ineaquilaterum (Philinae)	36	471.72 ± 18.87	77.68 ± 1.52	3.55 ± 0.27	30
Philodendron sp. (Philsp)	36	505.47 ± 41.68	85.77 ± 1.52	4.69 ± 0.49	31
Piper peltatum (Ppelt)	36	108.36 ± 18.68	76.90 ± 3.86	3.60 ± 0.90	31
Piper perlascense (Pperl)	30	148.22 ± 21.70	72.00 ± 5.41	3.97 ± 0.77	30
*Piper reticulatum (*Preti)	36	163.17 ± 19.79	80.68 ± 3.83	2.76 ± 0.77	31
Piper hispidum (Phisp)	30	134.61 ± 14.19	70.23 ± 6.03	3.82 ± 0.61	30
Piper imperiale (Pimpe)	36	279.08 ± 31.28	64.65 ± 5.94	6.02 ± 1.30	32

* Mean ± StDev

** the same as number of dual-choice feeding trials

Leaf toughness was thought to be the primary defensive structural leaf trait. Consequently, I used *Piper marginatum* (with lowest leaf toughness) as reference plant species in dual-choice feeding trials. In the following figures *P. marginatum* is shown as the first species opposed to test plant species in alphabetical order.

Leaf water content was not as heterogeneous among species as leaf toughness (Figure 4-3). The leaves of eight species shared similar water contents between 72 % (mean wc of *P. imperiale*) and 78 % (mean wc of *P. marginatum*). And average leaf water contents of 12 *M. diocles* host plant species ranged from approximately 70 to 80 %. However, *P. cordulatum* and *Philodendron sp.* had significantly higher water contents (83.83 ± 1.91 % and 85.77 ± 1.51 % respectively) and differed from the 13 other species. Notably *Philodendron sp.* also differed from all species in toughness and *P. cordulatum* had tougher leaves than its congeners. Both species have thick and fleshy (almost succulent) leaves. With 64.65 ± 5.94 % the leaves of *P. reticulatum* had significantly lower water than all other plant species.

In general the pattern of variation in structural reinforcement of leaves (expressed as specific leaf weight) was opposed to the interspecific pattern of water content (Figure 4-4). This is an indication that most species shared similar thickness of leaves (specific leaf area - the reciprocal of SLW - is a measure of leaf thickness). However *P. cordulatum* and *Philodendron sp.* the species that possessed maximum water content both had significantly higher SLW than most other plant species reflecting their thick fleshy leaves. Highest biomass accumulation per unit leaf area of *P. reticulatum* (6.11 mg*cm^{-2}) in combination with lowest water content on the other hand reflected a comparatively thin and dense leaf matrix. Only *P. cordulatum* showed similarly high SLW.

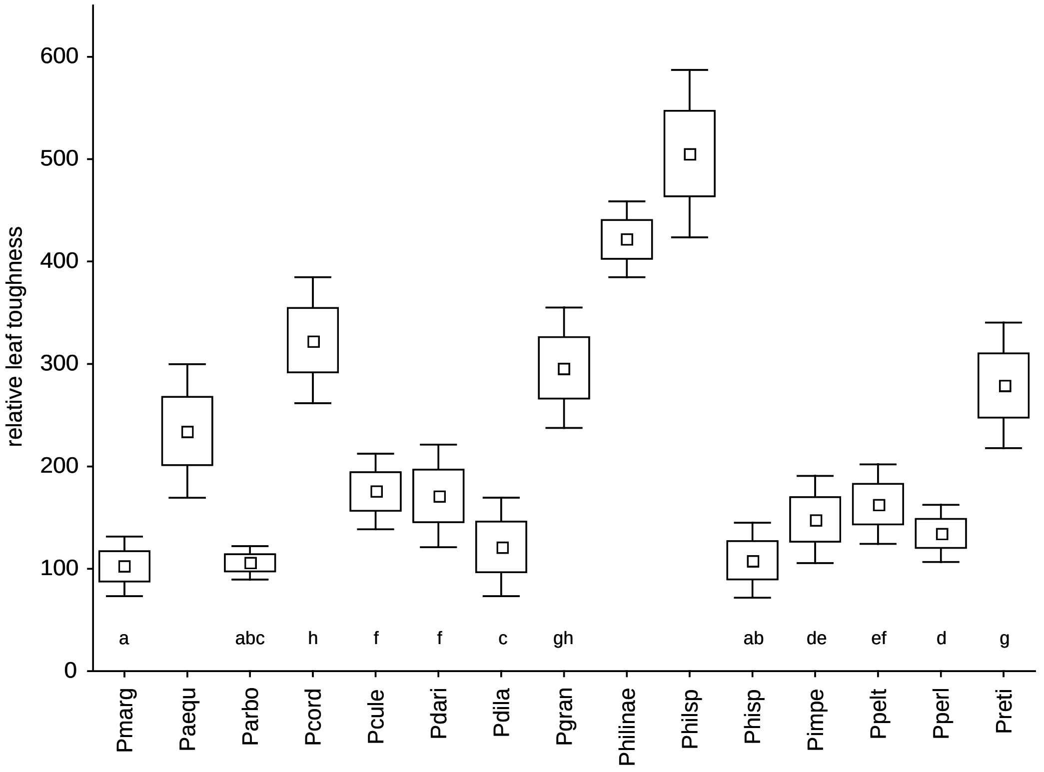

Figure 4-2: Relative leaf toughness of 15 selected *M. diocles* food plant species. Leaf toughness differed significantly among species (ANOVA, F = 534.8, df = 14, $P < 0.01$). Identical letters below boxes indicate no difference (Tukeys HSD unequal *N*, $P > 0.05$). Box-Whiskers show mean, standard deviation and 95 % confidence intervals of standard deviation.

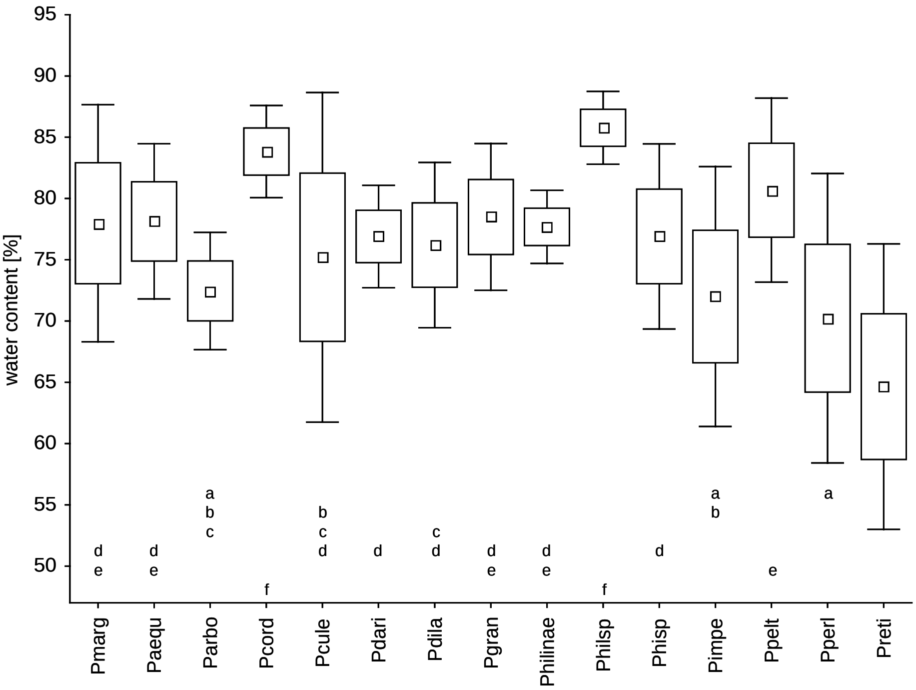

Figure 4-3: Water content of leaves from 15 food plant species of *M. diocles*. Water content differed significantly among species (ANOVA, F = 42.94, df = 14, $P < 0.01$). Identical letters below boxes indicate no difference (Tukeys HSD unequal N, $P > 0.05$). Box-Whiskers show mean, standard deviation and 95 % confidence intervals of standard deviation.

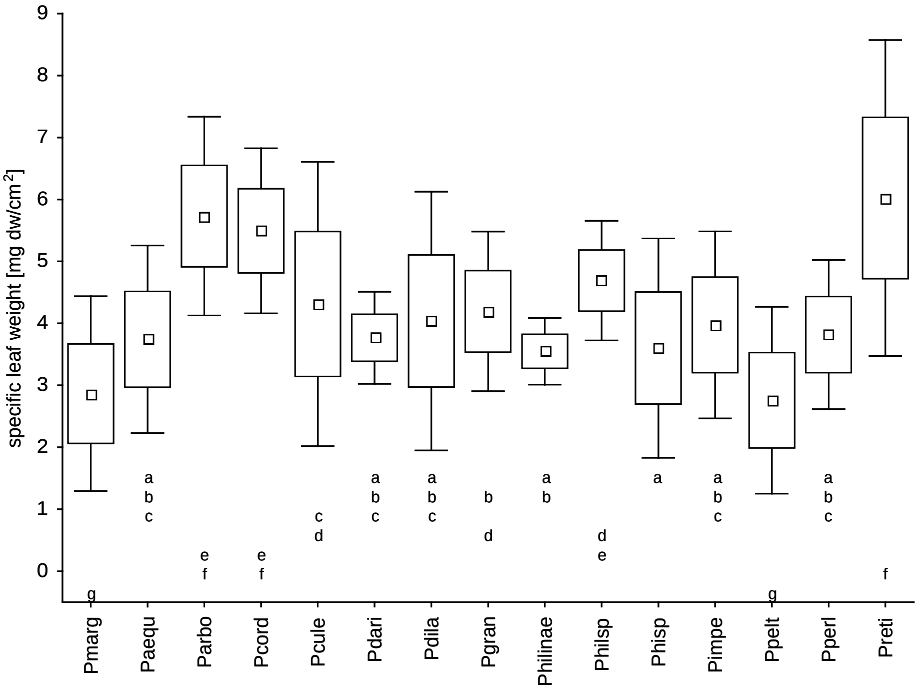

Figure 4-4: Specific leaf weight (SLW) from 15 food plant species of *M. diocles*. SLW differed significantly among species (ANOVA, F = 63.91, df = 14, $P < 0.01$). *P. marginatum* (Pmarg) served as reference plant in dual-choice feeding trials. Identical letters below boxes indicate no difference (Tukeys HSD unequal N, $P > 0.05$). Box-Whiskers show mean, standard deviation and 95 % confidence intervals of standard deviation.

Interspecific differences in total phenol contents ranged from lowest values for *P. dariense* with 0.64 % TAE to 8.34 % TAE for *Philodendron* sp. (Figure 4-5 & Table 4-2). Despite this contrast eight of the tested species shared similar phenol levels (1.68 ± 0.58 % TAE to 2.37 ± 5.81 % TAE). *P. marginatum* phenol contents ranged in the upper scale only being exceeded by *P. cordulatum* and *Philodendron* sp..

Tannins were not common in *M. diocles* host plant species. Four *Piper* species contained with 1.49 to 1.79 % TAE similar amounts of Tannin (Table 4-2). In *P. hispidum* tannins seemed to account for the major part of phenolic compounds (71 %). Only part of tested leaves of *P. marginatum* and *P. hispidum* showed tannic activity. For the remaining 10 species no BSA precipitation was detectable.

Table 4-2: Total phenol and tannin contents of *M. diocles* host plant species resulting from Price & Butler (1977) method modified by Mole & Waterman (1994) for phenols and radial diffusion assays (RDM) (Hagermann 1987) for tannins. For tannic content 3 samples per leaf were tested.. Note that detection of tannins wasn't consistent intraspecifically. The RDM assay mainly detects hydrolysable tannins (Hagermann 1987).

Plant species	**Total phenol content** [% TAE]*	**Tannin content** [% TAE]*	**No. of leaves**	
			tested	**containing tannin**
Piper aequale	1.21 ± 0.17	nd*	5	0
Piper arboreum	4.32 ± 0.37	1.49 ± 0.42	5	5
Piper cordulatum	5.79 ± 0.91	1.79 ± 0.25	5	5
Piper culebranum	3.87 ± 0.40	nd	5	0
Piper dariense	0.64 ± 0.09	nd	5	0
Piper dilatatum	2.26 ± 0.42	nd	5	0
Piper grande	1.72 ± 0.61	nd	5	0
Piper hispidum	2.45 ± 0.60	1.75 ± 0.68	5	2
Piper imperiale	2.33 ± 0.59	nd	5	0
Piper marginatum	5.71 ± 1.73	1.58 ± 0.44	20	14
Piper peltatum	1.87 ± 0.75	nd	5	0
Piper perlascense	1.95 ± 0.30	nd	5	0
Piper reticulatum	1.84 ± 0.56	nd	5	0
Philodendron ineaquilaterum	1.91 ± 0.27	nd	5	0
Philodendron sp.	8.33 ± 0.99	nd	5	0

* Mean ± StDev

** nd = not detected

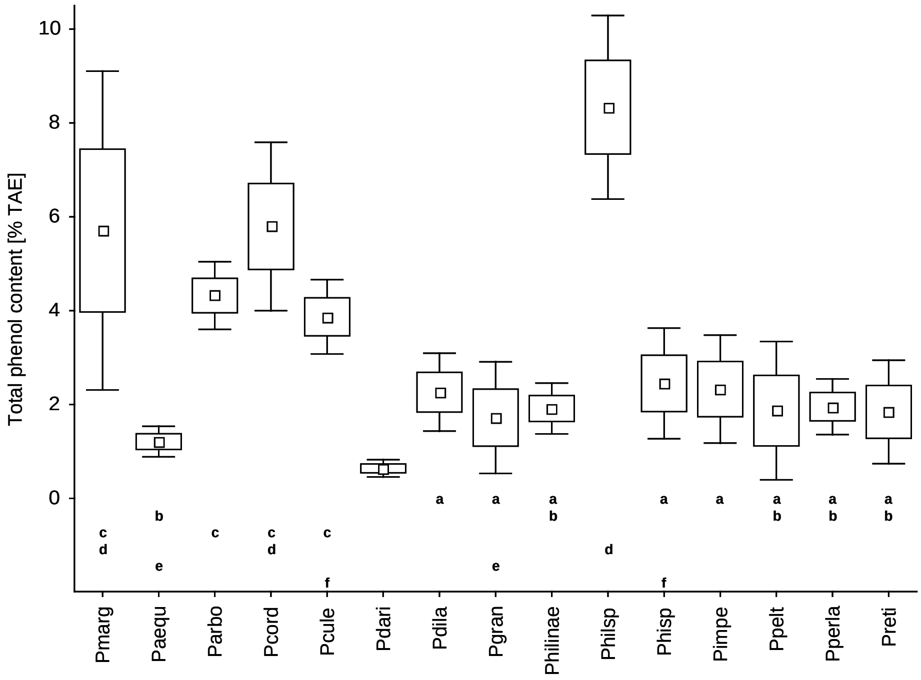

Figure 4-5: Total phenol content of leaves from 15 food plant species of *M. diocles*. Total phenol content differed significantly among species (ANOVA, F = 44.60, df = 14, *P* < 0.01). Identical letters below boxes indicate no difference (Tukeys HSD unequal *N*, *P* > 0.05). Box-Whiskers show mean, standard deviation and 95 % confidence intervals of standard deviation.

4.4.2 Preference of *M. diocles* adult females

Feeding preference of adult *M. diocles* females differed significantly indicating that they differentiated among food plant species (Figure 4-6). The assessed preference pattern at the same time revealed broad interspecific similarities. *P. marginatum* seemed to represent a food source of high value within the range of species tested. *P. peltatum* was most similarly accepted as food compared to *P. marginatum* as its median MAI was closest to 0.5. As preferences for six more plant species were not different to *P. peltatum* they also seemed to range on a similar preference level with *P. marginatum* as *M. diocles* foods. Only four out of 14 species were preferred to *P. marginatum*, *i.e.* the MAI resulting from dual-choice feeding trials was significantly above 0.5 (as compared to MAI of *P. peltatum*). Three plant species were clearly avoided by adult females: presented with *P. aequale*, *P. grande* and *P. perlascense* the insects strongly preferred the reference *P. marginatum*.

The measured leaf characters seemed not to function as defenses against herbivory by *M. diocles*. Preference for host species neither correlated with structural and physical parameters tested nor with total phenol contents of leaves (Figure 4-7). Likewise tannin contents of leaves seemed not to account for the observed preference pattern of *M. diocles* adult females (correlation analysis was dismissed due to rare presence of tannins). Assuming a defensive function of tannins the animals should have preferred the leaves of plant species missing tannins. This could not be confirmed (compare Figure 4-6 and

Table 4-2). In addition *P. arboreum* was preferred to *P. marginatum* despite the fact that they shared similar tannin contents (while no preference for the tannin containing *P. hispidum* and *P. cordulatum* was detected in dual-choice trials).

Particularly biochemical properties of leaves could be differing stronger with increasing taxonomic distance of plants (Ehrlich & Raven 1964). Under the assumption that leaf biochemistry influences feeding habits such differences could cover factors favoring or deterring feeding among closely related host plant species. To account for taxonomically related biochemical leaf properties correlation analysis was repeated among *Piper* congeners under exclusion of the two species of the Araceae. Within *Piper* host plants the preference pattern of adult females did not relate to phenol content (Product Moment Correlation MAI to phenol content $r = 0.10$, $df = 10$, $P > 0.05$).

Likewise particular biochemical properties of Araceae leaves could have influenced preference of females, thereby covering relationships between physical leaf traits and female preference considering *Piper* congeners. Exclusion of Araceae from correlation analysis did not reveal any significant relationship between any of these leaf traits and the MAI (Product Moment Correlation, $df = 10$, MAI to: leaf toughness $r = 0.14$, water content $r = -0.57$, specific leaf weight $r = 0.45$, all $P > 0.05$). Nevertheless, all resulting correlation coefficients increased after excluding Araceae host plants.

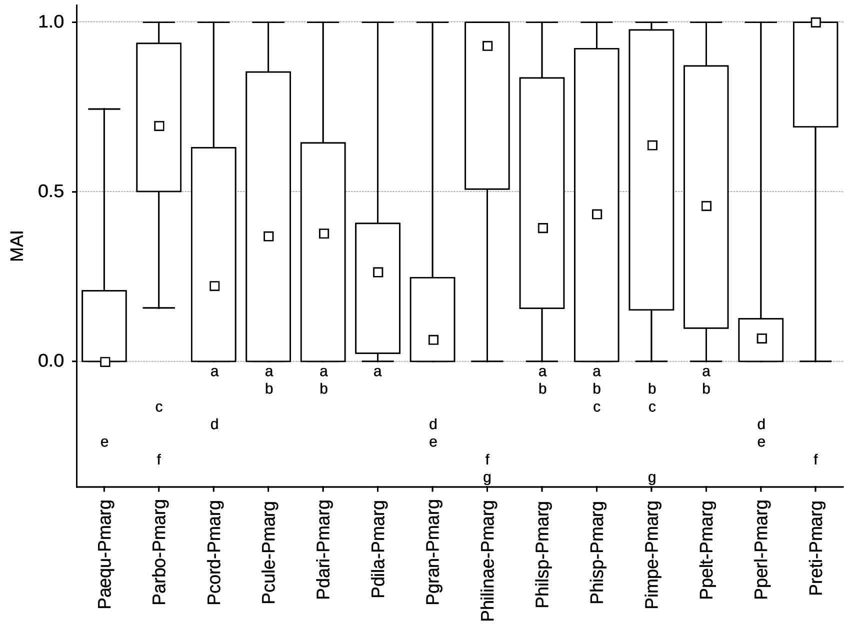

Figure 4-6: Feeding preference of *M. diocles* females resulting from dual-choice feeding trials. Preference is expressed as median of the mean acceptability index (MAI). MAI among feeding trials differed significantly (Kruskall Wallis $H_{(13, N=422)} = 101.90$, $P < 0.01$). Identical letters indicate no significant difference (Mann-Whitney U test, $P < 0.05$). Box plots show median, 25 to 75 % quartiles and minimum/maximum values.

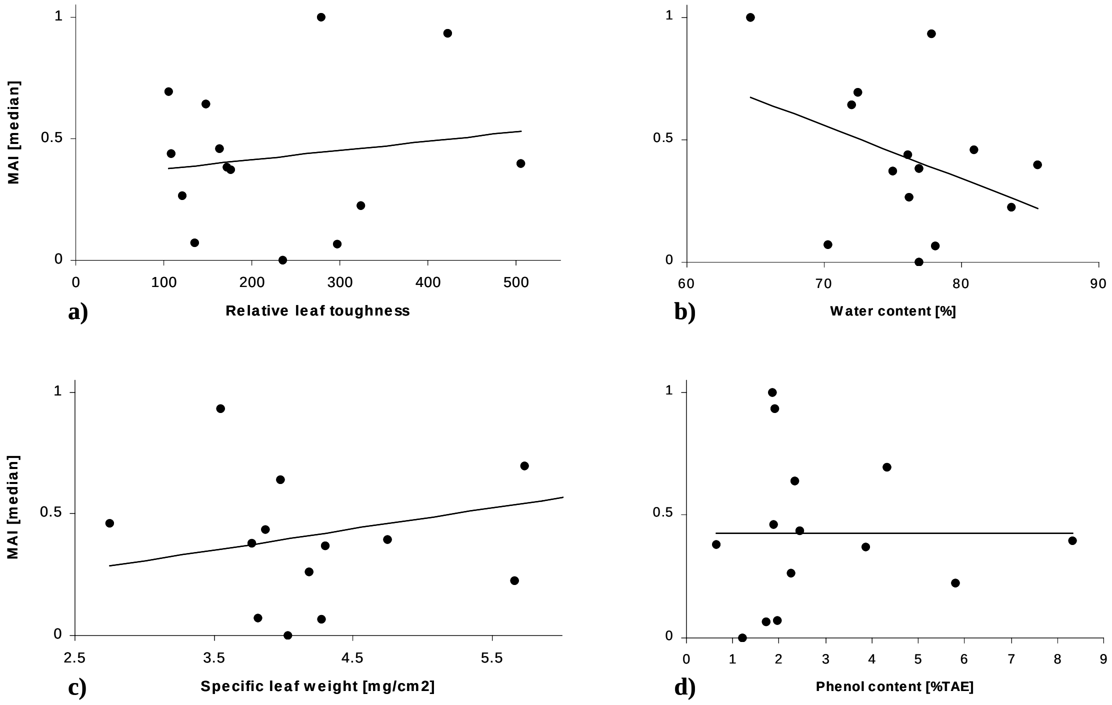

Figure 4-7: Preference of *M. diocles* adult females (expressed as Mean Acceptability Index; MAI) in relation to structural and chemical leaf characters of 14 food plant species. MAI showed no significant relation to any of the measured leaf traits (Product moment correlation, df = 12, all $P > 0.05$): a) leaf toughness ($r = 0.15$), b) water content of leaves ($r = -0.38$), c) specific leaf weight ($r = 0.26$), d) leaf phenol contents ($r < 0.01$).

4.4.3 Performance of *M. diocles* nymphs

Survival and growth seemed to be highly depending on the plant species nymphs fed on.

A comparison of survival probabilities of nymphs under different food sources revealed substantial and significant differences (Figure 4-8 and Table 4-3). The effects of food source on nymph survival were temporarily heterogeneous. Median survival and quartiles differed species specifically (Table 4-3). For instance within the first day 25 % of nymphs on *P. cordulatum* had died while initial mortality rate was lower on all other food sources. This effect changed over time. When mortality on *P. cordulatum* decelerated (and more than 25 % of nymphs had survived at the end) it accelerated on other plant species (*D. longispatha*, *P. grande*, *P. dariense*) leading to comparatively higher mortality in the long run. Median survival time was lowest for *P. grande*: after six days 50 % of nymphs feeding on *P. grande* had died. After day 15 no nymphs had survived. Likewise median survival time on *D. longispatha* was 9 days and no nymph survived beyond day 17. Opposed to these low quality foods, feeding on *Philodendron inaequilaterum* allowed high nymph survival. After six weeks 76 % of nymphs were still alive while on all other host plant species from 50 % (*P. dilatatum*) up to 100 % of nymphs had died.

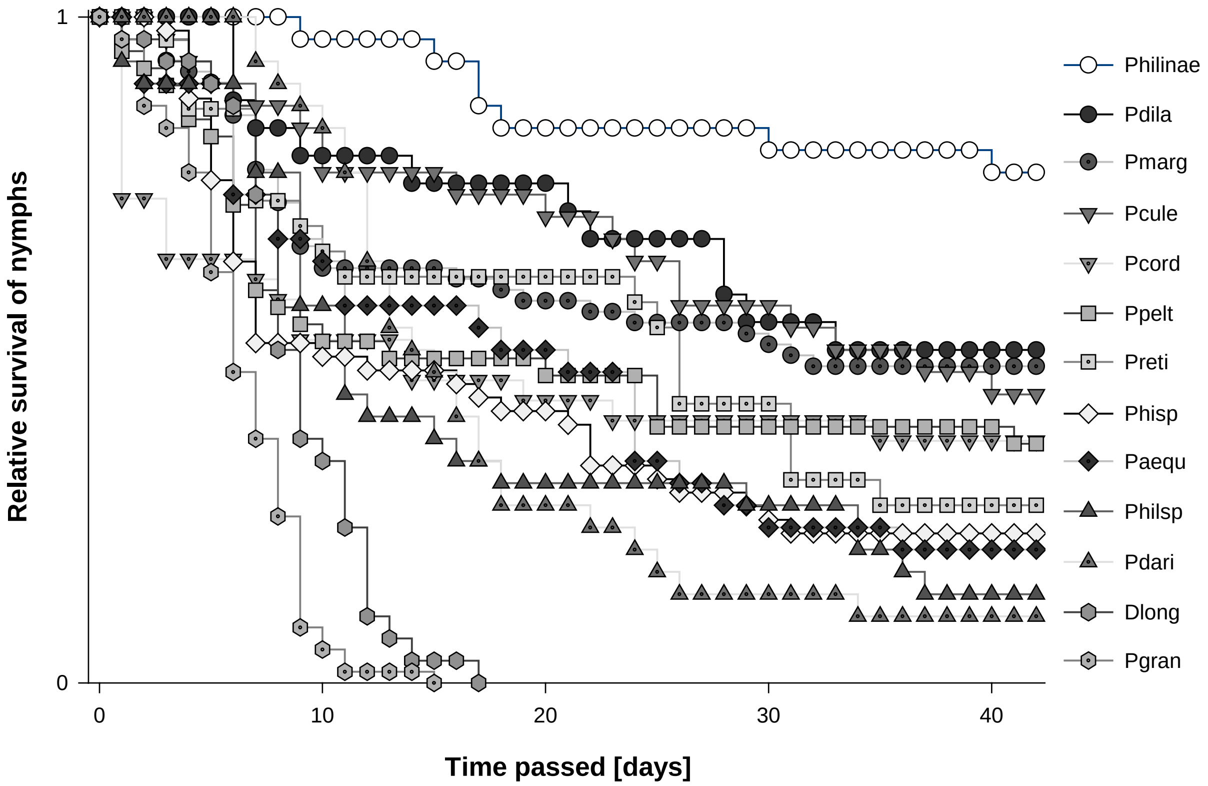

Figure 4-8: Time series of the survival of *M. diocles* neonate nymphs on single food plant species. A pronounced pattern in differential mortality of nymphs builds after two weeks of feeding and persists over time. Survival probabilities of nymphs calculated by the product limit estimate differed significantly depending on the food source (Log Rank Analysis, $X^2 = 178.32$, $df = 12$, $P < 0.01$). Species names on the right are in the order of relative survival at the end of the experiment. For further details and explanation of species name shortcuts refer to the text and Table 4-3.

Analysis of variance revealed that relative growth rates of nymphs differed significantly depending on food sources (Figure 4-9) and relative growth rates modestly corresponded with survival of nymphs (Product Moment Correlation, $r = 0.59$, $df = 11$, $P < 0.05$). Accordingly lowest relative growth rates matched high nymphal mortality on *P. grande* (RGR = 0.6 %) and *D. longispatha* (2.4 %), followed by *P. dariense* (4.3 %) and *Philodendron sp.* (5 %). Highest growth rates were yielded with 16.8 % weight gain in a two week period on *P. peltatum* insignificantly different from *P. marginatum*, *P. hispidum* and *P. cordulatum*. Nevertheless, the relative growth rate on *Philodendron inaequilaterum* - the species with highest survival - ranged on an intermediate level (9 %).

Table 4-3: Survival of neonate *M. diocles* nymphs resulting from long term feeding trials on 13 potential host plant species. Nymphs were fed on a single food plant species for 42 days. Plant species are in descending order of relative survival at the end of the experiment. The short names are used in Figure 8.

plant species	short name	number of replicates	nymphs died	nymphs survived	percent nymphs survived	days to median survival*	25% Quartile**	75% Quartile
Philodendron ineaquilaterum	Philinae	30	7	23	76.7	.	.	.
Piper dilatatum	Pdila	24	12	12	50	.	21	.
Piper marginatum	Pmarg	61	32	29	47.5	31	8	.
Piper culebranum	Pcule	30	17	13	43.3	37	16	.
Piper cordulatum	Pcord	33	21	12	36.4	14	1	.
Piper peltatum	Ppelt	39	25	14	35.9	13	6	.
Piper reticulatum	Preti	29	20	9	31	26	7	.
Piper hispidum	Phisp	49	38	11	22.4	10	6	30
Piper aequale	Paequ	30	24	6	20	21	6	30
Philodendron sp.	Philsp	30	26	4	13.3	11	9	34
Piper dariense	Pdari	30	27	3	10	15	12	22
Dieffenbacchia longispatha	Dlong	30	30	0	0	9	7	11
Piper grande	Pgran	60	60	0	0	6	5	9

* median survival time = the time when half of the nymphs had died

** 25% and 75% Quartiles = the time when 25% and 75% of nymphs had died

Survival and growth of nymphs as key demographic traits in insect herbivores were expected to decrease with increasing defensive leaf characters. Correlation analysis of performance with leaf toughness, specific leaf weight, water and total phenol content showed no relationship to nymph growth (Figure 4-10) and survival (Figure 4-11). Considering tannin contents of leaves their effects on nymph performance remained unclear. (Leaf tannin content could not be correlated to nymph performance due to rare presence of tannins.) If tannins influenced survival plant species containing tannins should have ranged on the upper end considering nymph mortality. In contrast to this prediction 47.5 % of nymphs survived on *P. marginatum* (1.58 % TAE) only exceeded by survival on two other tannin-free plant species (*P. dilatatum*, *Phil. inaequilaterum*; *cf.* Table 4-2 and Table 4-3). Similarly survival of nymphs feeding on tannin containing leaves of *P. cordulatum* and *P. hispidum* ranged on mid levels. While tannins seemed not to be related to nymph survival there is a clear pattern in terms of growth rates of nymphs. Growth rates on all three *Piper* species containing tannins and fed to nymphs (*P. cordulatum*, *P. hispidum*, *P. marginatum*; *cf.* Figure 4-9 and Table 4-2) were only exceeded by nymph growth on *P. peltatum* (tannin-free).

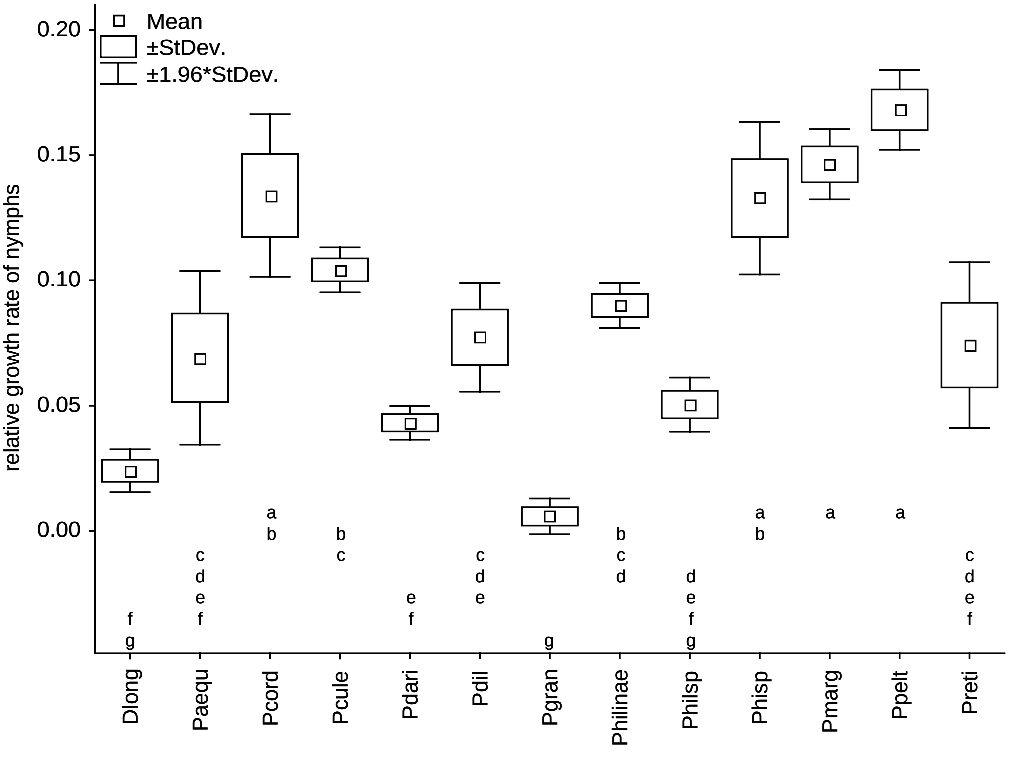

Figure 4-9: Relative growth rates of *M. diocles* nymphs on single food plant species (measurements after two weeks, one week later too many nymphs had died). Growth rates differed significantly depending on food source (ANOVA, F = 34.2, df = 12, $P < 0.01$). Identical letters indicate no significant difference (Tukeys HSD unequal *N*, $P > 0.05$).

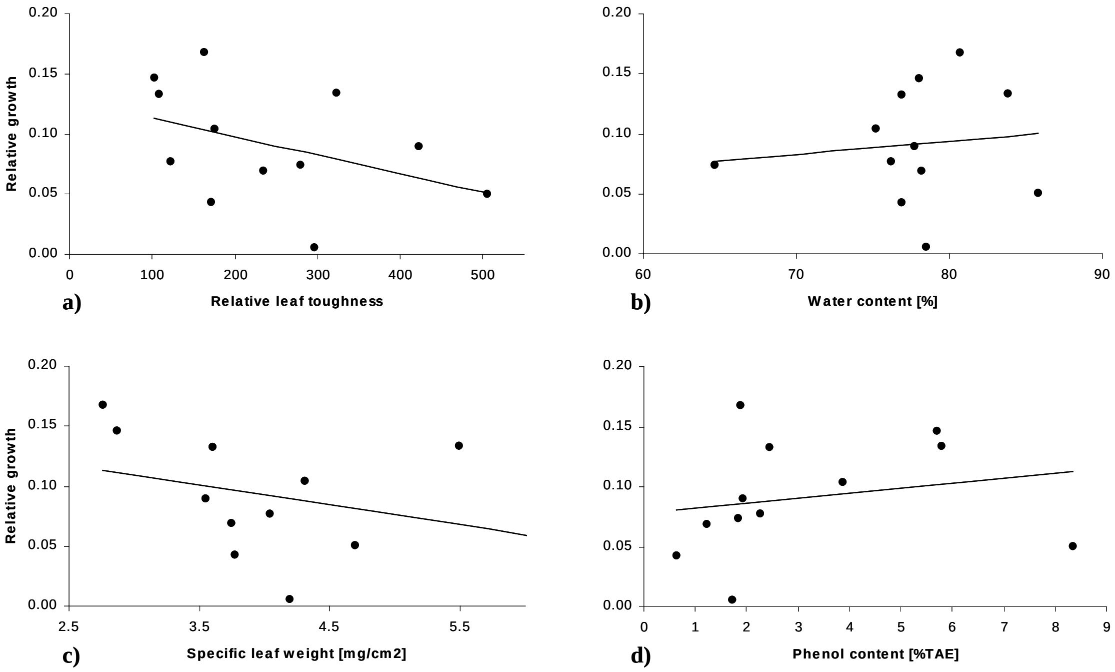

Figure 4-10: Relative growth rates of *M. diocles* neonate nymphs in relation to structural and chemical leaf characters of 12 food plant species. Relative growth showed no significant relation to any of the measured leaf traits (Product moment correlation, df = 10, all $P > 0.05$): a) leaf toughness (r = -0.41), b) water content of leaves (r = 0.12), c) specific leaf weight (r = -0.34), d) leaf phenol contents (r = 0.20).

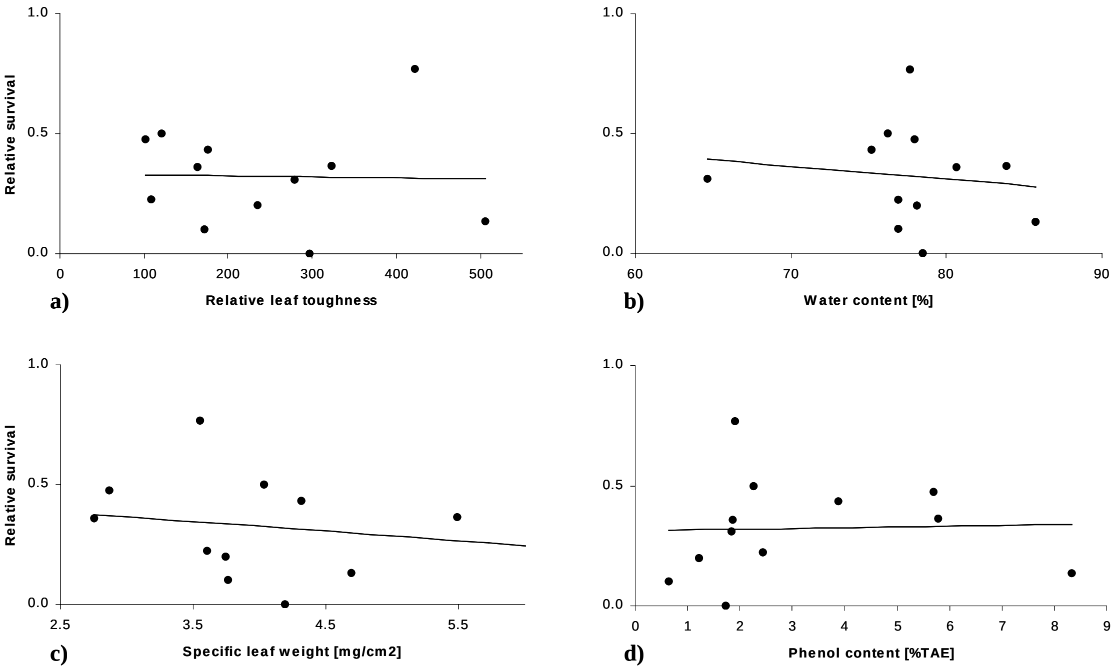

Figure 4-11: Relative survival of *M. diocles* neonate nymphs (after 42 days) in relation to structural and chemical leaf characters of 12 food plant species. Relative survival showed no significant relation to any of the measured leaf traits (Product moment correlation, df = 10, all $P > 0.05$): a) leaf toughness (r = -0.03), b) water content of leaves (r = -0.13), c) specific leaf weight (r = -0.19), d) leaf phenol contents (r = 0.03).

To account for taxonomically related biochemical leaf properties correlation analysis was repeated among *Piper* congeners under exclusion of the two species of the Araceae. Within *Piper* host plants performance of nymphs did not relate to phenol content, but exclusion of Araceae host plants led to marked increases in correlation coefficients (Product Moment Correlation, df = 8: survival to phenol content r = 0.58, relative growth rate to phenol content r = 0.61, both *P* > 0.05). In contrast to the expectation of a defensive function of phenolic compounds correlation coefficients demonstrated a positive trend in the relationship of performance and phenol contents.

Particular biochemical properties of Araceae leaves could also have influenced performance of nymphs, thereby covering relationships between physical leaf traits and nymph performance considering *Piper* congeners. Exclusion of Araceae from correlation analysis did not reveal any significant relationship between any of these leaf traits and nymph performance (Product Moment Correlation, df = 8, relative growth rate to: leaf toughness r = -0.40, water content r = 0.30, specific leaf weight r = 0.31; survival to: leaf toughness r = -0.41, water content r = -0.03, specific leaf weight r = -0.04; all *P* > 0.05). The exclusion of Araceae host plants had no effect on the resulting correlation coefficients except for the relation of survival and leaf toughness that decreased from r = -0.03 (*cf.* Figure 4-11) to r = -0.41.

Performance of nymphs seemed not to relate to female adult preference, indicating that prior feeding experience did not affect preference of females. In correlation analysis on MAI of females to survival and relative growth rates of nymphs no significant relation was detected (Product Moment Correlation, df = 9, MAI to: survival r = 0.50, relative growth rate r = 0.25, all *P* > 0.05). However, low mortality of nymphs on *Phil. inaequilaterum* corresponded with significant preference of adult females (compare to Figure 4-6), but growth of nymphs was intermediate on this food source. Opposed to that, 69 % of nymphs had died on *P. reticulatum*, a highly preferred food of adult females.

4.4.4 Preference of *M. diocles* nymphs under varying total phenol and tannin contents

The results of an infiltration experiment indicated that increased total phenolic contents as well as increased tannin levels in leaves might act against herbivory by phasmid nymphs. Both the increase of total phenol contents with phenol extract and tannin solution resulted in significantly reduced feeding preference (*i.e.*, water infiltrated control discs were preferred; Figure 4-12 & Figure 4-13). Yet, tannins may play a minor role in defense against *M. diocles* herbivory compared to other phenolic compounds. Increased contents of total phenols (by infiltration of phenol extract) proofed to be more effective than an increase of phenols by tannin solution alone. A rise of 0.5 % of total phenols by leaf phenol solution showed a significant effect in preference shift (Figure 4-12) whereas infiltration of 1.2% tannin was not different from the control (Figure 4-13). An increase of tannin concentrations up to 11.7 % above natural total phenol contents resulted in total rejection of tannin infiltrated leaf disks. Natural phenolic content in *P. hispidum* leaves from greenhouse plants was 2.86 ± 0.1 % TAE (similar to natural phenol content from field samples with 2.45 ± 0.6 % TAE).

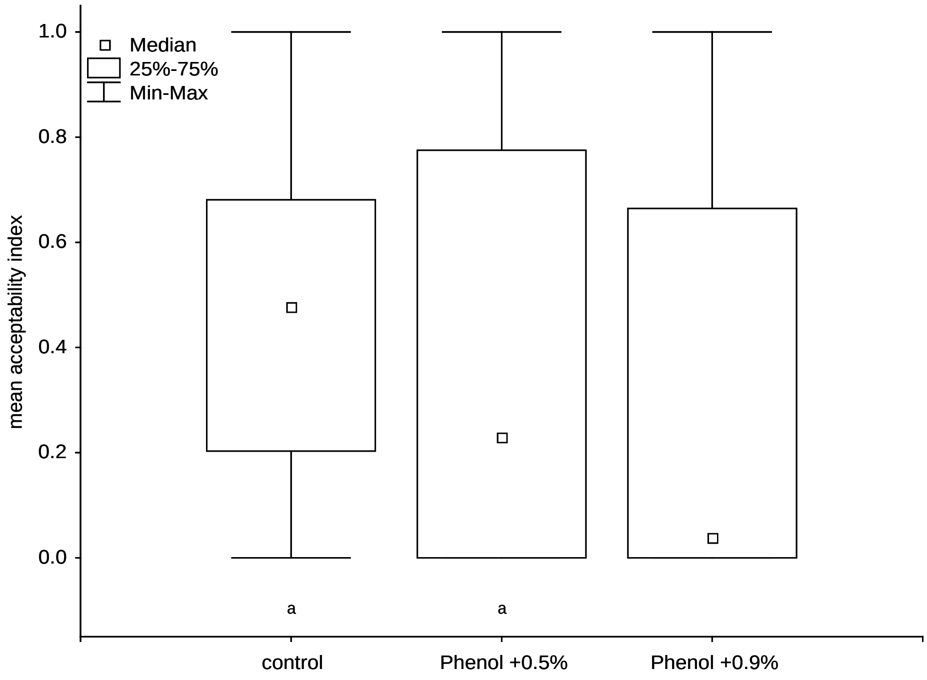

Figure 4-12: Feeding preference of *M. diocles* nymphs under varying levels of leaf total phenol content (expressed as increase of percent TAE compared to natural total phenol content of *P. hispidum*; total phenol content was increased by infiltration of leaf phenol extract solution). An increase of 0.9% TAE of total phenols resulted in a significant preference shift (Mann-Whitney U = 579, $P < 0.05$, control *vs.* Phenol$_{+0.9\%}$, N_{control}=37, $N_{\text{Phenol+0.9\%}}$=43).

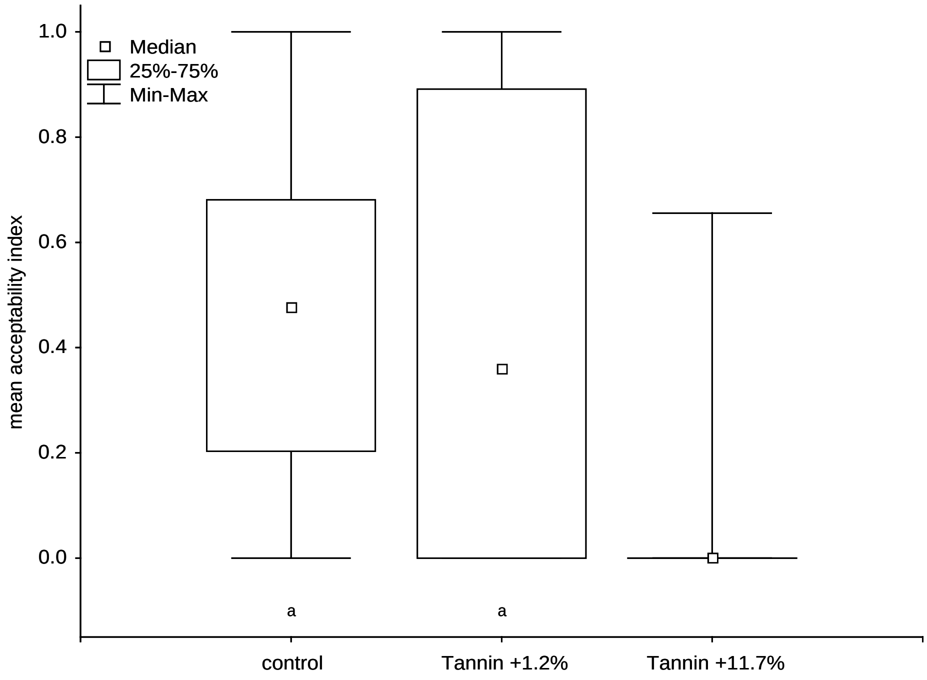

Figure 4 -13: Feeding preference of *M. diocles* nymphs under varying levels of leaf tannin content (expressed as increase of % TAE compared to natural total phenol content of *P. hispidum*; tannin content was increased by infiltration of tannin solution). An increase of 1.2 % TAE tannin had no effect on feeding preference whereas nymphs refused to feed on leaf discs with 11.7 % TAE more tannin (Mann-Whitney U = 401, $P < 0.01$, control *vs.* Tannin$_{+11.7\%}$, N_{control}=37, $N_{\text{Tannin+11.7\%}}$=24).

4.5 Discussion

The initially formulated hypothesis that *M. diocles* preference and performance would be particularly influenced by physical leaf properties of host plant species could not be supported in this study. Food plant species of *M. diocles* differed markedly in quality considering leaf toughness, water content, specific leaf weight, total phenol and tannin contents. Adult females clearly differentiated among host plant species and nymph performance differed significantly depending on their food source. However, nor adult female preference neither differential performance of nymphs (*sensu* survival and growth) did relate to any of the assessed leaf traits over the whole host range (Araceae and Piperaceae) and among *Piper* congeners. These results indicate that the measured leaf characters may be of minor importance for *M. diocles* feeding behavior. However, an intraspecific increase of total phenols via infiltration of phenol extract in leaves negatively influenced preference of nymphs while increased tannin contents were effective only in substantially higher concentrations. The deterring effect of increased total phenol on nymphs suggests that phenolic compounds may play an important role as qualitative defenses especially considering induced increases and intraspecific variation in total phenol contents of leaves.

Adult preference for plant species did not consistently reflect best nymph performance suggesting that adult preference does not relate to experience in early life stages. Survival and growth of nymphs on a particular diet corresponded significantly, albeit highest survival was not linked to highest growth rate. Consequently host plant choice in early life stages of *M. diocles* may crucially determine survival and growth and hence could have a substantial impact on population density.

(In the following discussion I will use the term preference-performance describing adult female preference and nymph performance. In another context preference-performance describes oviposition preference and larval performance.)

4.5.1 The pattern of feeding preference and performance: an attempt of an explanation

Adult *M. diocles* females displayed significant feeding preferences and diet source differentially influenced survival and growth of early life stages of *M. diocles*. However, in the presented study interspecific differences in physical and chemical leaf characters seemed not to relate to nymph performance or adult preference. These results do not support the initially stated hypotheses that in particular physical leaf traits should affect preference and performance of a specialist herbivore.

Both, high leaf toughness (Cherret 1968; Coley 1983; Lowman & Box 1983; Reich *et al.* 1991; but see Landa & Rabinowitz 1983) and low water contents (*e.g.*, Moran & Hamilton 1980; Coley 1983; Schädler *et al.* 2003) have been shown to negatively affect insect herbivores. Coley (1983) demonstrated that toughness and water contents had the highest negative correlations (among a multitude of other leaf traits) with herbivory rates on mature leaves of 46 canopy tree species. In their review on plant defenses in tropical forests, Coley & Barone (1996) considered leaf toughness as the most effective herbivore defense. Likewise, high structural reinforcement expressed as SLW (Wright & Cannon 2001) may negatively affect herbivore feeding behavior. Although direct evidence for this supposition is missing, SLW may be indicative of defense at least when high SLW represents a thick leaf matrix (SLW is the product of leaf thickness and density) (*e.g.*, Bernays & Chapman 1970; Schädler *et al.* 2003). For example, Bernays & Chapman (1970) showed that grasshopper nymphs were unable to feed on thick leaves because they could not open the mandibles wide enough to bite. Here, thick leaves of *P. cordulatum* and *Phil.* sp., combining high values for SLW and water content, seemed not to affect nymph performance. Likewise, my results did not support the defensive view of high leaf toughness and low water contents. For instance, despite the fact that *Philodendron inaequilaterum* had the second toughest leaves it ranged on second position as preferred food for females and most nymphs survived on this food source. *Piper reticulatum* ranged on top of female preference, and growth and survival of nymphs was intermediate while its leaves had significantly lower water contents and significantly higher SLW than all other species (reflecting a thin and dense leaf matrix).

The insensitivity of *M. diocles* adults towards low water contents may be explained by the fact that herbivores themselves amplify their food intake depending on their physiological state of hydration. Roessingh *et al.* (1985) showed that locusts will prefer dry leaf matrices after having fed on fully hydrated leaf material. While *M. diocles* nymphs had no food alternative, different adult females may have fed variably hydrated leaves before a feeding trial. In such a scenario female preference then simply would reflect their prior feeding.

Similar to physical leaf traits, chemical leaf contents did not explain the described patterns of *M. diocles* adult preference and nymph performance. Tannins are known to act in a dosage-dependent manner (Feeny 1970, Rhoades 1977; Coley 1986) and can be toxic or deterrent to particular herbivores (Bernays *et al.* 1980; Berenbaum 1984). Likewise phenolic compounds may negatively affect herbivores (Bernays & Chapman 1994), but in particular when phenol contents are increased following herbivore damage (*e.g.*, Niemelä *et al.* 1979; Rossiter *et al.* 1988; Haukioja 1990; Kogan & Fischer 1991; Tscharntke *et al.* 2001). For example, Rossiter *et al.* (1988) showed that pupal mass and fecundity of gypsy moth were negatively correlated with hydrolizable tannin content and with constitutive and induced total phenolic of oak. In contrast, in Coley's (1983) study on 46 tree species, natural phenol contents explained least of the variation in herbivore damage. This is consistent with my results considering phenols. Phenol contents varied significantly among tested plant species but did not relate to preference-performance of *M. diocles*. The difference between *P. dariense* (0.64 % TAE) and *Phil.* sp. (8.33 % TAE) was almost twelve fold while eight species shared similar and comparably low levels of leaf phenols. Despite their highest total phenol contents in *Piper* species, *P. marginatum* and

P. cordulatum ranged on third and fifth position considering survival of nymphs and shared highest relative growth rates with two other species.

Tannin containing leaf tissue was not discriminated against other foods by adults or by nymphs. Detectable tannins by the RDM assay (Hagermann 1987) were absent in the two Araceae detected (and thus tannins seem not to be characteristic for Araceae as was suggested by Grayum, 1990). Similarly, tannins were rare in tested Pipers (four out of 13 species contained tannins) and ranged on similar levels from 1.49 to 1.79 % TAE. For example, *P. marginatum* leaves contained 1.58 % TAE but adult females displayed no significant preference when tested against five tannin free plant species (as compared to the MAI of *P. peltatum*, that was closest to 0.5; *cf.* Figure 4-6). Above that *P. marginatum* was clearly preferred to three plant species missing tannins (*P. aequale*, *P. grande*, *P. perlascense*; *cf.* Figure 4-6 & Table 4-2). These results suggest that natural levels of tannins and phenols in *M. diocles* host plants do not affect its feeding behavior.

Biochemical differences between the two host plant families of *M. diocles* seemed not to cover defensive functions of leaf traits within *Piper* congeners. According to the theory of biochemical coevolutiondifferences in plant biochemistry will increase with increasing taxonomic distance of plants (Ehrlich & Raven 1964). Araceae and Piperaceae are not closely related (Judd *et al.* 2002) and both families are rich in secondary compounds (Sengupta & Ray 1987; Baldwin & Schultz 1988; Parmar *et al.* 1997; Judd *et al.* 2002; Dyer *et al.* in press). At least for Pipers it is known that many species contain exclusive and highly variable compounds (Dyer *et al.* 2003). However, an exclusion from correlation analysis did not reveal any significant relation between defensive leaf traits and preference-performance of *M. diocles*. Albeit, within *Piper* host plants physical leaf traits may involve a minor defensive function: the exclusion of Araceae strengthened all relationships to nymph performance markedly. In contrast, nymph performance tended to positively correlate with total phenols. This again may indicate that natural levels of secondary phenolic leaf compounds do not negatively affect this phasmid.

Nevertheless, a negative effect of phenolic and tannic leaf compounds on *M. diocles* nymphs must not be neglected. At least considering intraspecifically varying levels of these compounds. As noted above, both phenolic and tannic contents may negatively affect herbivores and many authors showed that phenolic compounds may be induced in response to insect damage (*e.g.*, Niemelä *et al.* 1979; Rossiter *et al.* 1988; Haukioja 1990; Kogan & Fischer 1991; Baldwin 1994; Tscharntke *et al.* 2001; but see Lempa *et al.* 2004). *M. diocles* nymphs reacted with a significant preference shift when phenol contents of *P. hispidum* leaf discs were increased above natural phenol levels and preferred the according control. Likewise, but in much higher concentrations increased tannin contents deterred nymphs. Hence *M. diocles* nymphs may be negatively affected at least by intraspecific variation of phenolic compounds in a particular host plant. This may apply to all host plant species presented in this study as all species contained notable phenolic concentrations. The deterring effect by a comparably small increase of phenolic content denotes the qualitative character of this defensive pathway. In contrast, tannin may only function against *M. diocles* in some *Piper* host species and even only in particular individuals (as represented by intraspecific absence of tannin in some leaves) if they had higher tannin contents as found here. Coley (1986) suggested that such intraspecific tannin variation could be governed genetically as she found between-plant differences in *Cecropia* tannin content persisted through time. Undoubtedly, tannin can deter herbivores: low tannin *Cecropia* plants suffered five times higher herbivory by caterpillars than plants with high tannin contents (Coley 1986). This difference in herbivory was based on substantial differences in tannin concentrations among of *Cecropia* individuals (13 to 58 mg*g^{-1} dw, Coley 1986). High tannin concentrations involve high costs as they have large initial construction costs and cannot be reclaimed upon leaf senescence (McKey 1979, Coley *et al.* 1985, Coley 1988).

Considering that tannins had to be accumulated in much higher concentrations compared to other phenolic compounds to deter *M. diocles* nymphs, evolution in *Piper* biochemistry may have favored qualitative defenses, particularly as many *Piper* species are gap adapted (Croat 1978). Coley *et al.* (1985) suggested that gap adapted plant species (pioneers, high light) should invest carbon in growth and less in carbon-based defense such as tannins. The rare presence of tannins in *Pipers* as demonstrated here and earlier (Baldwin & Schultz 1988) gives support to this view.

However, as all phenolic compounds are based on carbon, the resource availability theory of Coley *et al.* (1985) also predicts low levels of total phenols in pioneer species. Baldwin & Schultz (1988) showed that independent from habitat *Piper* species shared low levels of total phenols while *Miconia* forest species (persistent, low light) had almost 17 times more phenols than *Miconia* gap species. In this study, the prediction of similarly low phenol contents among *Piper* species cannot be supported as total phenols varied significantly among species (independent from habitat as *P. marginatum*, *P. culebranum*; *P. dilatatum*, *P. hispidum*, *P. peltatum* and *P. reticulatum* represent pioneer species).

Nevertheless, there is more support for a qualitative defensive pathway in *Pipers*. The genus *Piper* is enormously rich in secondary compounds (reviewed in Sengupta & Ray 1987; Parmar *et al.* 1997; Dyer *et al.* in press). So far in 112 out of more than 1000 species that have been investigated 661 different compounds have been found (Dyer *et al.* in press). Several studies have presented effects of all classes of *Piper* compounds against herbivores with Amides being particular strong (Bernard *et al.* 1995; Parmar *et al.* 1997 and references therein; Siddiqui *et al.* 2000; Dyer *et al.* 2003). For example, Dyer *et al.* (2003) showed that three Amides of *Piper cenocladum* all negatively influenced larval performance of lepidopterans with stronger synergistic effects of a mixture of the Amides. Given the large variety in secondary compounds in *Piper*, the presented preference-performance pattern of *M. diocles* may have resulted from interspecific qualitative differences in phenolic composition, with plant species that were disliked by adults or where nymphs suffered reduced performance containing particularly toxic compounds. This suggestion is supported by the fact that secondary compounds in *Piper* differ qualitatively among species (Parmar *et al.* 1997). Many species contain exclusive compounds and have evolved high varieties in particular compound classes (Dyer *et al.* 2003). To exemplify, from *Piper* *amalgo* 31 amides have been isolated while *P. reticulatum* seems not to have any amides (Dodson, unpublished in Dyer *et al.* 2003). Supposedly the lack of amides in *P. reticulatum* may explain its high preference by *M. diocles* adult females. Likewise some *Pipers* have very diverse herbivore faunas (95 species in *P. arieianum*; Marquis 1990) indicating that these species may be poor in defensive compounds.

Secondary plant compounds need not necessarily act as deterrents to herbivores, they also can stimulate feeding (Bernays & Chapman1994). Particularly interesting examples of such phagostimulants are phenolics and other compounds such as alkaloids, terpenes and flavonoids that can be taxon specific (listed in Bernays & Chapman 1994, p. 133) and that are thought to play a major role in defining host-ranges of associated insects. Nevertheless, phagostimulatory effects are only observed when the compounds are present in low concentrations and loose their stimulating effect when deterrent chemicals increase (Bernays & Chapman 1978). The presence of particular secondary phenolic compounds in *Piper* possibly acting as phagostimulants to *M. diocles* may explain the positive trend between leaf phenol content and nymph performance. When phenol content then increases, for example by induction, then deterrence outweighs stimulation.

Above that some insects use secondary compounds for pheromone production or for defensive sequestration (*i.e.* pharmacophagy, *e.g.*, Boppré *et al.* 1984; Bernays & Chapman 1994). For example, highly polyphagous *Zonocerus* grasshoppers feed preferentially on plants or flowers containing pyrrolizidine alkaloids and sequester the chemicals for defense against predators (Boppré *et al.* 1984). Most phasmids dispose of defensive glands at their prothorax (Bedford 1978). Their secretions have been shown to deter predators can be already functional in nymphs (Eisner 1968; Eisner *et al.* 1997). Likewise, *M. diocles* uses these glands and when threatened the smell of its spray becomes apparent (pers. obs.). Probably, this secretion is the result of sequestration of secondary compounds from *M. diocles* host plants. Thereby *M. diocles* would turn the potentially defensive biochemical diversity in its host range into its own protection against natural enemies. This would be of particular importance for nymphs as they are supposed to be most vulnerable to predation (Cornell & Hawkins 1995; Cornell *et al.* 1998).

Yet, the principle phagostimulants are nutrients. Many authors previously showed positive effects of nutritious plant quality (*e.g.* Nitrogen and carbohydrates) on survivorship and growth of insect herbivores (*e.g.*, Joern & Gaines 1990; Joern & Behmer 1997). And insect herbivores are known to discriminate among food sources of different nutritional quality (Behmer & Joern 1993, 1994; Simpson & Simpson 1990). For example, growth and survival of grasshopper nymphs is often protein dependent (Behmer & Joern 1993, 1994) and host plant and tissue selection of grasshoppers has repeatedly been related to N-content reflecting protein (Behmer & Joern 1993, 1994). Nymphs of *Locusta migratoria* can regulate food intake based on protein (Abisgold & Simpson 1987). Other studies supported the importance of N to reproduction and showed that insects can perceive differences in N-contents. Adult females may modulate their reproductive output depending on N-content of their foods as they decrease egg production with decreasing N-levels and vice versa (McCaffery 1975). Similarly, host selection (*i.e.* preference) and performance of *M. diocles* is likely to depend on nutritious quality, the more as I showed that defensive leaf traits may be of minor importance for this herbivore. I therefore suggest that analysis of leaf nutritional quality should be subject of further research on *M. diocles* diets.

4.5.2 Ecological and evolutionary consequences

The described differential performance of *M. diocles* nymphs and the discriminative preference of adults are of significance in several aspects of the biology and ecology of this herbivore.

Slow growth may involve a higher susceptibility to natural enemy attack (Feeny 1976). This slow-growth high-mortality hypothesis (Feeny 1976; Clancy & Price 1987) suggests that slow growing herbivores suffer greater mortality from natural enemies, because they remain longer in the more vulnerable early life stages of development than faster growing herbivores. Häggström & Larsson (1995) provided one of the few empirical tests for this hypothesis. They showed that predation on larva of the leaf beetle *Galerucella lineola* was positively related with longer development that depended on food quality. Accordingly, in their natural setting differential growth of *M. diocles* nymphs may translate into differential predation-related mortality. Thereby predation may outbalance high host plant related mortality of fast growing nymphs (*e.g,. Piper peltatum*) with high host plant related survival of slow growing nymphs (*e.g,. Phil. inaequilaterum*). As a consequence, selection should favor some intermediate host, *i.e.* with comparably high growth rate and low host plant related mortality. In the case of *M. diocles*, the gap associated *Piper dilatatum* and *P. marginatum* represent such hosts. Over evolutionary time one would predict that low suitable plants such as *P. grande* will be abandoned as hosts. Together with the fact that *M. diocles* densities were higher in gaps than in the understory (Chapter 2) the outlined evolutionary scenario may at least partly explain the distribution of this herbivore.

Still, nymph mortality in a six week period was 50 % or higher for all species but *Phil. inaequilaterum* (*cf.* Table 4-3). This time period reflects less than half the time of phasmid nymph development (approx. 100 days, *cf.* Chapter 3). Such strong constraints in tissue quality (regardless whether related to defensive or nutritional quality) may motivate an herbivore to leave its host and search for more adequate food. Leaving the host, the herbivore runs risk not to find a new suitable host. To cite an example, van Dam *et al.* (2000) showed that very young caterpillars often have trouble to find even neighbouring plants. In addition, when leaving its host the herbivore exposes itself to predation particularly by arthropods (Haccou & Hemerik 1985). Both risks probably decrease with increasing size of the herbivore, because its action range increases and thereby its searching efficiency (Damman 1991), while its susceptibility to predators may decrease (Dyer 1995). In addition, the herbivore is deprived of food while searching (Schultz 1983). Again all these factors favour selection pressure towards hosts where nymphs suffer littlest mortality resulting from food quality or from predation, mediated via extended larval development. However, a single food type of poor or even toxic quality rarely will provide all essential nutrients for survival. As a consequence herbivores may have to leave a host to feed on a mixture of plants (Joern 1979; Cates 1980; Bernays *et al.* 1994).

From the plants perspective it is most favorable to motivate the herbivore to leave as soon as possible. Early instars cause little damage but food consumption increases with development (*e.g.*, van Dam *et al.* 2001) and thereby may increase a plants fitness loss. To cite an example, Marquis (1992) showed that a leaf loss of 10 % on single reproductive branches in *Piper arieianum* caused a local seed loss of 80 %. However, in a competitive environment the production of lethal herbivore defenses may also involve high fitness costs (*e.g.*, Baldwin 1998). Thus many plants may use defenses on less cost-intense sublethal levels (Price *et al.* 1980) that already affect early larval instars and may motivate them to change hosts (van Dam *et al.* 2001). In this hypothetical framework *M. diocles* nymph performance then would reflect variation in sublethal reduction of leaf quality, and the observed mortality patterns would rather result from the experimental setup ‘forcing’ nymphs to stay on such low quality food. The positive relation of nymph survival and growth gives support to this thought.

As demonstrated, selection pressure on early lifestages of *M. diocles* may be high depending on food source. This pressure could be reduced if adult female oviposition was related to best nymph performance. Feeding habits of adult herbivores often reflect previous feeding experience throughout an individual’s lifetime (Cassidy 1978; Papaj & Prokopy 1988; Redfearn & Pimm 1988; Karowe 1989; Sandlin & Willig 1993). For example, adult feeding behavior of the neotropical phasmid Lamponius portoricensis depended on sex and prior experience and persistence of feeding preference (as proportion of a species in its diet) was stronger in females than in males (Sandlin & Willig 1993). Concluding from this, one could hypothesize that if adult feeding preference reflected prior feeding experience as nymphs, selection would favor evolution of oviposition on preferred host plants of females and nymphs would perform best on this food. In the many examples of insect females that lay their eggs on plants for larval development, the observed relationship between oviposition preference and offspring performance ranges from good to poor correspondance (for review see Thompson & Pellmyr 1991). Clearly, a preferential oviposition on high quality foods would involve immediate consequences for plant herbivore interactions and for herbivore population dynamics (Price *et al.* 1995). In the ‘Phylogenetic Constraints Hypothesis’, Price *et al.* 1990 predicted that larvae of such species would exhibit higher survival, that females would recognize and compete for rare high quality resources, and that they would disperse large distance to spread spacing of eggs preventing larval competition and overexploitation. In contrast to my expectations performance of nymphs seemed not to relate to female adult preference, indicating that prior feeding experience did not affect preference of females. Albeit, adult preference tended to relate modestly with nymph survival ($r = 0.50$; $P > 0.05$) and low mortality of nymphs corresponded with significant preference of adult females on *Phil. inaequilaterum* (compare to Figure 4-6 and Table 4-3). This Araceae is a typical and abundant plant in the understory of the BCI forest (Croat 1978). Considering it’s high acceptance by *M. diocles* this plant species may account for the presence of *M. diocles* in the understory (*cf.* Chapter 2). In addition, while many phasmids flick and drop their eggs (Bedford 1978), *M. diocles* attaches them to a substrate and indicating a selective oviposition behavior.

4.5.3 Conclusions

The study presented here, demonstrated that particularly *M. diocles* nymphs may be exposed to severe selection pressure depending on its food source. Differential mortality of nymphs depending on the host plant may directly (survival) or indirectly (slow-growth high-mortality) contribute to population control of *M. diocles*. Further studies on *M. diocles* should particularly focus on oviposition behavior for one striking reason: If an evolved life history trait, such as female choice of oviposition, is strongly linked to offspring performance then the reproductive response of females to host plant quality is the critical factor regulating population densities (Craig *et al.* 1989).

Now, does the fact that *M. diocles* seemed not to be affected by physical plant defenses contradict its specialization? I tend to say no. *M. diocles* was not affected by natural contents of leaf tannins or phenols of its host plants indicating that this phasmid can handle the large variability in secondary compounds in its host range. This supports the consideration of *M. diocles* as herbivore specialist, simply because host range is ultimately defined by the occurrence of deterrent compounds in non-hosts (Bernays & Chapman 1994).

5 Predation pressure and its effects on survival and off-plant migration of *M. diocles* nymphs

5.1 Introduction

Predation is considered an important determinant of the abundance and distribution of prey organisms (Sih *et al.* 1985; Begon *et al.* 1996). To date, numerous studies have confirmed that predators and parasitoids can have significant impact on insect herbivore populations (*e.g.* Chang 1991; Gomez & Zamora 1994; Floyd 1996; Moran & Hurd 1998). Extensive analyses of life table data found natural enemies as the most important source of mortality for holometabolous herbivorous insects (Cornell & Hawkins 1995; Cornell *et al.* 1998; Walker & Jones 2001). Cornell *et al.* (1998) concluded that this applies in particular for insects feeding externally on plants, starting out that the importance of natural enemies of holometabolous herbivores increases from the early larval stage to the pupal stage. These results emphasize the role of predation as a key factor in determining herbivore survival (Hairston *et al.* 1960; Walker and Jones 2001). Data on predation pressure on exophytic feeding hemimetabolous herbivores mainly concentrate on Orthopterans. In general, natural enemies exert a significant impact on populations of grasshoppers and population dynamics are most affected by nymphal mortality (Joern & Gaines 1990 and references therein). Oedekoven and Joern (1998) showed that the youngest instars experience the greatest losses and that predation from wandering spiders contributed significantly to nymphal mortality. The majority of larval mortality however reflected other undefined sources. According to Belovsky and Slade (1995) grasshopper nymphs may be more likely to suffer death from starvation, while adults may be at greater risk from other factors such as avian predation. Thus specific mortality causes of different stages remain uncertain and to my knowledge there is no data available that assess the impact of predation on early life stages of phasmids.

Herbivorous insects face predation from a variety of natural enemies (Abrahamson & Weis 1997). These undoubtedly influence how and where an herbivore feeds (Hawkins & Lawton 1987). In response, prey organisms have developed a variety of mechanisms to escape from top-down pressure, for example morphological mimicry, or behavioral and phenological escape (Sillen-Tullberg *et al.* 1982, Kinsmann & Platt 1984, Damman 1987, Witz 1990). Migration patterns often reflect such predation avoidance, either in prey moving within (Hopkins and Dixon 1997, Magalhães *et al.* 2002) or off (Roitberg & Myers 1979) their host plants. Such behavior usually involves costs such as reduced fecundity (Roitberg & Myers 1979) or larval growth (Heads 1986, Gotthard 2000). The latter may lead to prolonged development times, increasing exposure to natural enemies during vulnerable immature stages (Feeny 1976, Benrey & Denno 1997). In consideration of these costs, predator avoidance via migration should be displayed under accordingly high predation pressure (Snyder & Wise 2000, Venzon *et al.* 2000, Magalhães *et al.* 2002).

However, whether an observed behavior does reflect predator avoidance often remains speculative as many studies assume but do not test an anti-predator function (Witz 1990). Predation avoidance may not be the ultimate motivation of small-scale migratory movement patterns of herbivores. Most ecologists now agree that both bottom-up and top-down forces directly or indirectly influence populations and communities of herbivores (e.g. Price *et al.* 1980, Hunter & Price 1992, Hunter *et al.* 1997, Walker & Jones 2001, but see Hassell *et al.* 1998, Hunter *et al.* 2000 and references therein). Resource limitations, such as the depletion of a food source or reduced digestibility of plant tissue, are bottom-up forces that influence herbivore performance and distribution (e.g. Häggstrom & Larsson 1995, Burghardt & Fiedler 1996, Barker & Maczka 1996). Larval performance of insect herbivores can also be affected by plant size (Price 1991, Teder & Tammaru 2002) and according to Price (1991) herbivores should prefer bigger and more vigorous plants or plant modules. Depletion or low suitability of a host plant can then result in larvae moving more frequently between feeding sites (Bergelson & Lawton 1988, Loader & Damman 1991, but see Häggstrom & Larsson 1995). Another reason why herbivores move away from their host plants is that many herbivores perform best with a mixture of food plants, either to complete their diet or to dilute toxins (Bernays & Chapman 1994, Hägele & RoIll-Rahier 1999).

When explaining herbivore migration by bottom-up forces one has to differentiate between generalists and specialists. Bottom-up forces, such as chemical plant defenses, should be more effective against generalist herbivores (Feeny 1976). Specialized herbivores (mono- or oligophagous) that overcome such plant defenses by detoxifying or even sequestering plant compounds should be less affected (Ehrlich & Raven 1964).

Concluding from the above, I hypothesized that nymphs of an exophytic specialist herbivore should be particularly regulated by the third trophic level. If these nymphs show migration behavior, I expected predation avoidance to cause these movement patterns as bottom-up forces were expected to be of minor importance for this specialized herbivore (see Chapter 4).

The stick insect *Metriophasma diocles* (Phasmatodea) is an exophytic herbivore that occurs in low abundances in the understory of neotropical rainforest and feeds exclusively on plants from two different plant families, the Piperaceae and Araceae (see Chapter 2; Berger & Wirth 2001). As these families are not closely related, the degree of specialization of *M. diocles* is best described as disjunct oligophagous (see Bernays & Chapman 1994).

I quantified the predation-related mortality of *M. diocles* nymphs of by (1) exposing nymphs to natural levels of predation on uncovered host plants (controls), and simultaneously (2) estimating the proportion of intrinsic mortality (not related to natural enemies) through the exclusion of predators by covering host plants with mesh cages (treatment). For the description of natural enemies and the temporal pattern of their impact I included observations on (3) the identity of potential predators, and (4) on diurnal patterns of nymph survival.

To account for predation-related migration of nymphs I recorded the migratory activity of individual nymphs (1) in both exclosures and controls, and (2) under predator-free conditions in greenhouses. The study was complemented by an analysis of host plant size based on the number of healthy leaves to determine if bottom-up forces might instead have controlled migratory activity.

A general shortcoming of such field studies of survival is that they cannot distinguish between death versus emigration as the cause of disappearance. In the presented study this paucity of potential emigration of *M. diocles* nymphs out of control plots was acknowledged by following nymphs individually. Further I accounted for potential effects of the exclosures on intrinsic mortality and migratory activity of nymphs by assessing these parameters in a greenhouse experiment.

5.2 Materials and Methods

5.2.1 Study site and organism

The study was conducted in the forest understory of Barro Colorado Island. A detailed description on vegetation and climatic conditions of BCI is given in the Chapter 1 and in Leigh *et al.* (1990). The experiments took place from September to December 2001 during the rainy season.

Metriophasma diocles (Orthoptera, Phasmatodea) is a winged stick insect occurring on BCI in low abundances (see Chapter 2). In performing feeding trials and analyzing field observations I found that *M. diocles* feeds on leaves of Piperaceae and Araceae exclusively (Chapter 2; Berger & Wirth 2001). I selected *Piper marginatum* JACQ (Piperaceae) as a suitable and well-documented host plant in this experiment (*cf.* Chapters 2 & 4).

5.2.2 Field experiment

To assess the impact of predators on first instar nymphs of *M. diocles* I designed a field experiment excluding predators by covering an individual *P. marginatum* host plant with mesh cages (diameter 50 cm, height 60 cm, mesh diameter 0.1 cm). As controls, I exposed nymphs to natural levels of predation on uncovered host plants. Hereafter I will use the term ‘survival’ to refer to all nymphs present in control plots (on or off host) and in exclosures at a given time.

I selected sites based on field records indicating that they were representative of *M. diocles* habitat. This species was shown to occur at forest edges and in moist areas of the understory where several species of Araceae, especially *Philodendron* spp. and Piperaceae are abundant (see Chapter 2).

At each of three sites in the forest understory I installed 10 experimental plots. Each plot included both an exclosure and a control plant (1 m apart from each other).The experimental plots were at least 5 m apart. In each exclosure I planted one individual of *P. marginatum* that had a minimum of three adult leaves (*P. marginatum* saplings were grown in the greenhouse to keep them free from herbivores). The diameter of the exclosures was sufficiently large so that leaves of the enclosed plant did not touch the mesh sides. Control plants were positioned 1 m next to an exclosure.

The experiment was repeated four times (run 1 to 4) during three months in 2001 (September 18 to October 2, October 3 to October 17, October 25 to November 6, November 23 to December 5), each time with a new set of plants. Before starting an experimental runexclosures and the plants therein were checked for herbivores and predators such as frogs, ants, spiders and bugs, which were then removed. I also checked the surrounding vegetation of each control plant (in a circle of 1 m radius, 2 m height) to ensure that no individuals of *M. diocles* were present (no *M. diocles* specimens were found).

First instar nymphs of *M. diocles* were set in the experimental plots (one nymph per plant). In average nymphs weighed 14.82 mg (StDev 7.10 mg, $N = 219$) and were 12 to 15 mm long. The nymphs were reared from eggs collected in a laboratory colony. All individuals had hatched at least three days before the beginning of the experiment. After three days the cuticle and mandibles have hardened and the intestines are fully functional. All individuals were only used once.

In each run I recorded survival and migratory activity of the nymphs for 14 days. At the beginning of an experiment in the morning (before 10 am) of day 1, I set one nymph on each plant in exclosures and controls. I then checked each plant whether the nymph was still present: daily in the morning and the evening for run 1 to 3 and daily in the morning for run 4 (by then the difference between nocturnal and daytime events was supported by sufficient data).

Nymphs in the exclosures that were not found on the host plant either fell dead to the ground, or moved (*i.e.*, ‘migrated’) up to the mesh of the exclosures.

I thoroughly searched for nymphs that disappeared from control plants in the surrounding vegetation (in a circle of 1 m radius, 2 m height, see above). Nymphs found alive off their host were recorded as migrating (only two dead nymphs were found off-host). The location was marked and the distance traveled measured. Migrating nymphs were followed during the experiment to record remigration to their hosts, further emigration or loss. If a nymph could not be found, it was recorded as disappeared. In total, I obtained data for 238 nymphs in both exclosures and controls.

To allow for an analysis of potential bottom-up effects triggering nymph migration, I estimated the size and condition of the host plant by counting healthy young and mature leaves at the beginning of a trial and after a nymph had disappeared or left the plant. I discarded senescent leaves. Plant size was then expressed as the total number of healthy leaves per host plant individual.

5.2.3 Greenhouse experiment

To account for intrinsic mortality and migratory potential that is not predation related, I documented migratory activity and residence (survival) of nymphs for 14 days in seven greenhouse experimental plots (these open greenhouses are protected against intruders by mesh). Mortality comparisons of nymphs in screened greenhouses and in exclosures can also help to uncover microclimatic influences by exclosure design.

I installed plots of 1 m^2 size. An individual of *P. marginatum* was planted in the center of a plot and eight individuals at the outer borders. Nymphs were prevented from leaving a plot by an adhesive barrier (Tanglefoot – Insect Trap Coating, The Tanglefoot Co., USA). A single nymph was then set on the central plant and checked twice daily. Residence, migration and remigration were checked accordingly. I used the same criteria for nymphs and host plants as described above. In total, I followed 53 nymphs in this experiment. Occasionally, workers of *Ectatomma ruidum* ROGER, a ponerine ant, passed the adhesive barrier and entered the plots. As these were rare events, predation events can largely be ruled out.

5.2.4 Data analysis

Survival times were analyzed by the Kaplan-Meier estimate (also known as the product-limit estimate; Kaplan & Meier 1958). The survival function gives the probability that an individual will survive past a given time. Differences in residence times were analyzed by the Mantel-Cox log-rank test (Mantel 1966; Cox 1972). Under the assumption that deaths or disappearances of nymphs were independent, the Mantel-Cox test proved suitable for the dataset as it gives equal weight to all events.

Migration distances were calculated as mean migration distance per nymph because some nymphs moved multiple times.

Plant size was expressed as total number of healthy leaves per host plant after death or disappearance of a nymph or at day 14 in case the nymph was still present. As data were not normally distributed, I compared median plant size *via* Mann-Whitney U-tests.

5.3 Results

5.3.1 Predation-mediated patterns in *M. diocles* nymph mortality

Survival times of *M. diocles* nymphs differed significantly between exclosures and controls (Figure 5-1). In total, nymph survival was three times higher on caged plants than on exposed plants (81% *vs* 27%; Table 5-1). In the exclosures, survival times varied neither spatially among the three forest sites, nor temporally throughout the study period. In contrast, spatio-temporal variation increased in the controls where nymphs were exposed to natural levels of predation. Most notably, survival times differed significantly among runs in the controls (Mantel-Cox Test; $X^2 = 23.62$, df = 3, $P < 0.0001$).

In controls, significantly more nymphs disappeared at night as compared to the daytime (Figure 5-2). In contrast, there was no diurnal variation in mortality in the exclosures, where predation was absent (Mantel-Cox Test; $X^2 = 1.96$, df = 1, $P < 0.16$).

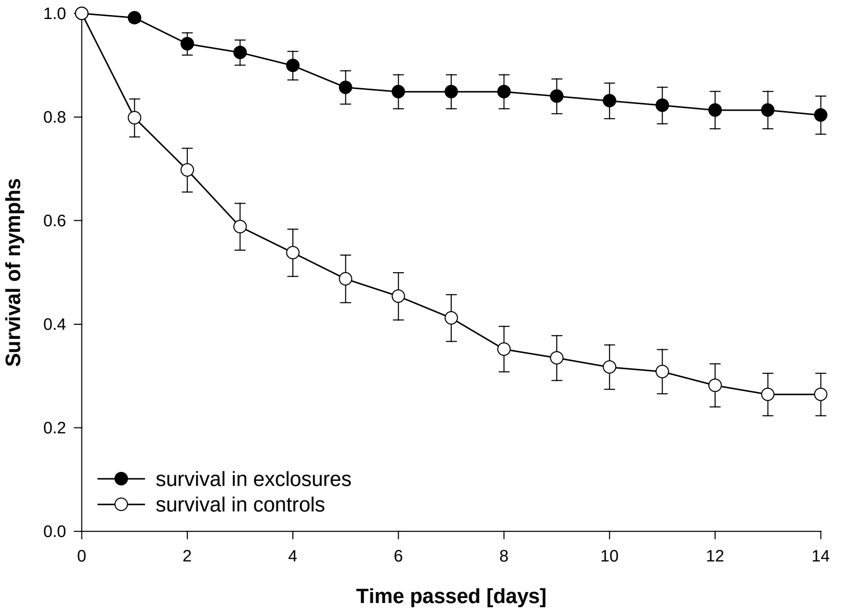

Figure 5-1: Cumulative survival of phasmid nymphs in exclosures *versus* controls. Survival is expressed as the probability that an individual survives past a given time (Kaplan-Meier estimate). Survival times of *M. diocles* nymphs differed significantly between exclosures and controls (Mantel-Cox Test; $X^2 = 74.08$, df = 1, $P < 0.0001$). Data were pooled from four experimental runs each lasting 14 days ($N = 119$ nymphs per treatment). Error bars show ±1 SE.

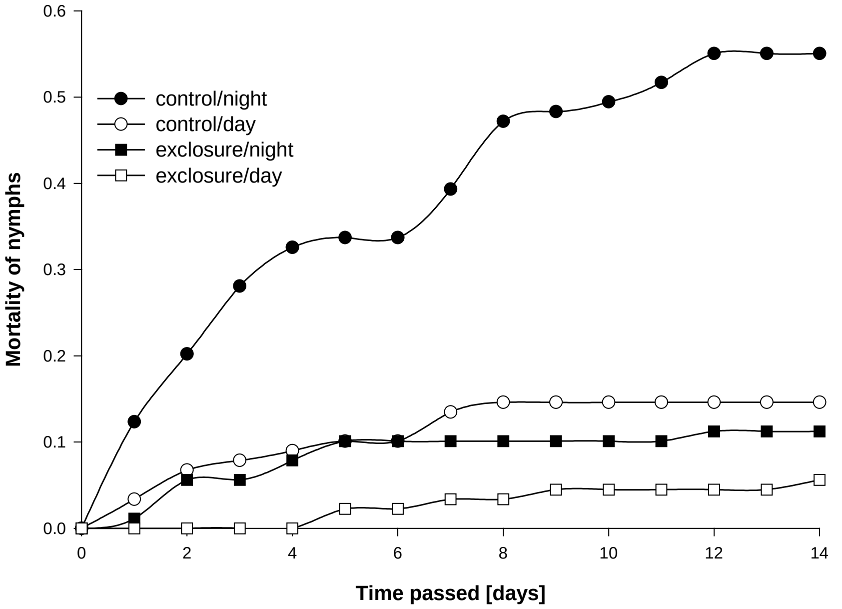

Figure 5-2: Cumulative mortality (*i.e.*, death or disappearance) of nymphs in exclosures *versus* controls at night and in the day. Mortality is expressed as the probability that an individual dies past a given time (Kaplan-Meier estimate). In controls, significantly more nymphs disappeared at night as compared to the daytime (Mantel-Cox Test; $X^2 = 31.13$, df = 1, $P < 0.0001$). Data were pooled from experimental runs 1, 2 and 3 ($N = 89$; evening checks were omitted in run 4).

Table 5-1: Disappearance or death and migratory activity of *M. diocles* nymphs in a field experiment with predation exclusion.

No. of *M. diocles* nymphs	**Treatment** Exclosure	 Control	Chi-Square test
Total	119	119	
Disappeared or dead after 14 days	23	87	$X^2 = 67.09$, $p < 0.01$
Migrated	30	37	$X^2 = 0.75$, $p = 0.39$

Various invertebrate predators were recorded (Table 5-2). Spiders (2 cases) and bugs (*Reduviidae*) (1 case) were seen preying upon nymphs, and *Ectatomma* ants were repeatedly found on control plants prior to the disappearance of nymphs.

The fate of any lost nymph in the control plots is either a result of 1) predation, 2) intrinsic mortality with subsequent removal by scavengers, or 3) emigration beyond the searched area. Intrinsic mortality was assumed to be the same in exclosures and controls and was therefore approximated as the proportion of nymphs having died on caged plants (19 %, i.e. 23 out of 119 nymphs; Table 5-1). This estimate proved legitimate as mortality levels in the greenhouse experiment did not differ significantly (32 %, *i.e.* 17 out of 53 nymphs; $X^2 = 2.66$, df = 1, $P = 0.10$). The latter correspondence also suggests that mortality levels were not considerably influenced by the exclosures. I consider disappearance due to emigration to have been negligible for two reasons. First, the search area around control plants (1 m radius) was well above the mean range nymphs moved: 47.53 cm (SD 39.13 cm, $N = 29$) between subsequent checks, with the 75 percent quartile lying below 72 cm. Second, overall migratory activity in controls appeared to be adequately estimated, as the proportion of moving nymphs corresponded well with exclosures (Table 5-1). This was further confirmed by the fact that the proportion of migrating nymphs in the predation-free greenhouse experiment (8 from 53 nymphs) was not different from the exclosures (30 from 119 nymphs; $X^2 = 1.49$, df = 1, $P = 0.22$).

The proportion of nymphs dying on control plants due to predation was therefore estimated as follows: disappearance in controls (73%) – intrinsic mortality in exclosures (19%) – emigration from control plots (negligible) = 54%.

Table 5-2: Potential predators of *M. diocles* nymphs observed on 119 control plants. Each plant was surveyed 29 times in runs 1 to 3 and 15 times in run 4 (including one night check per run).

Predator group	No. of observations
Ants (Formicidae); *Ectatomma* spp.	38
Army ants (*Eciton* spp.)	3
Others	7
Spiders (Araneae)	44
Reduvid bugs (Hemiptera)	3
Frogs (Anura)	2
Katydids* (Orthoptera); adults	35

* Katydids are often omnivorous

5.3.2 Predation and off-plant migration

The initially stated hypothesis that migration may be a response to predation pressure (*i.e.*, a mechanism for predator avoidance) was not supported by the observed pattern of migratory activity in the field experiment, because proportions of migrating nymphs between treatments were similar (25 % in exclosures, 31 % in controls; Table 5-1). Nevertheless, migratory activity in the field was markedly increased compared to the greenhouse experiment (15 %), and the proportion of migrating nymphs in the control plots was significantly higher than in the greenhouse plots ($X^2 = 4.07$, df = 1, $P < 0.05$).

In contrast nymph migration was related to the number of healthy leaves per host plant (as measure of resource availability). Plants from where nymphs emigrated (*i.e.* nymphs from both treatments that never returned to their host) had significantly fewer healthy leaves than plants from sessile nymphs (*i.e.* nymphs from both treatments that never left their host) (Figure 5-3). In contrast, there was no difference between leaf numbers of plants from remigrating and sessile nymphs.

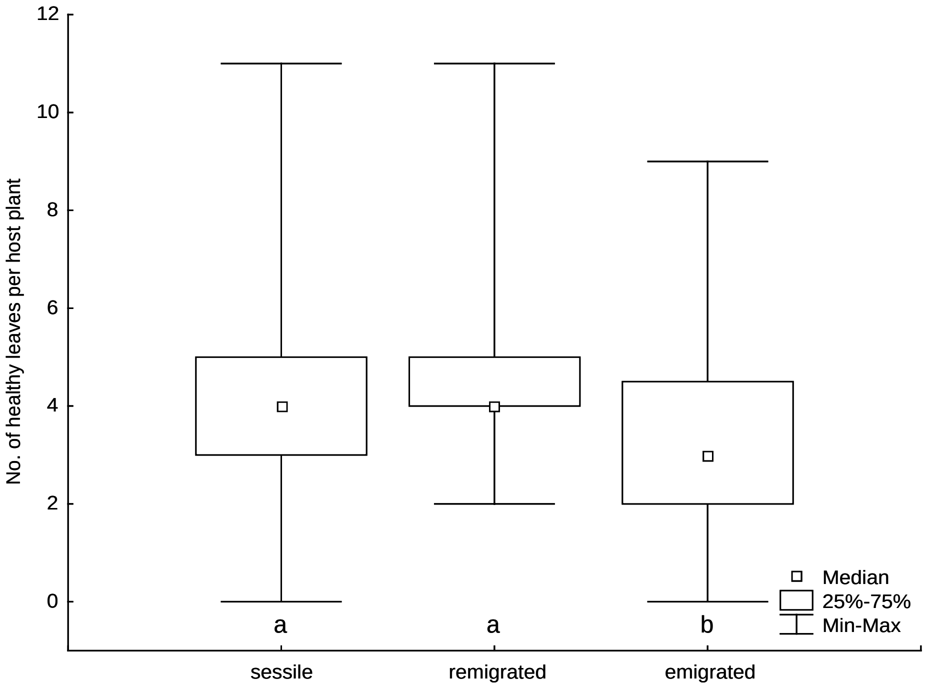

Figure 5-3: Plant size (in terms of healthy leaves per plant) for nymphs that left their hosts (emigrated, $N = 24$) was significantly lower than for nymphs that continuously stayed on their host during the experiment (sessile, $N = 96$; Mann-Whitney U = 712, $P < 0.01$) as well as for nymphs that left and returned to their host (remigrated, $N = 38$; U = 292, $P < 0.05$). Different letters indicate significant differences.

5.4 Discussion

5.4.1 The top-down view of *M. diocles* population regulation

The presented study is the first to quantify predation-related mortality of early life stages of phasmids. First instar nymphs of *M. diocles* suffered 73 % mortality in a two-week period, and the significant reduction in mortality in exclosures indicates that natural enemies were an important source of mortality. These results correspond with earlier studies showing predation pressure as a severe factor influencing survival of insect herbivores (*e.g.* Chang 1991, Floyd 1996, Moran & Hurd 1998) and in particular immature stages of holometabolous external feeding insects (Cornell & Hawkins 1995, Cornell *et al.* 1998). Similar patterns have been described for temperate hemimetabolous grasshoppers. For example, for the whole 48 d nymphal period of two grasshoppers Belovsky *et al.* (1990) estimated the loss to predators to be *ca* 40 %. Oedekoven & Joern (1998) accounting 19 % of grasshopper nymph mortality (3rd instar) while total stage-specific mortality exceeded 90 %. Here, I provided evidence that nymphs of a hemimetabolous tropical phasmid suffered approximately 54 % predation-related mortality in a 14-day period. As phasmids in general have much longer nymphal periods than grasshoppers, total predation impact on immature stages may not be comparable between the two herbivore groups. It is reasonable to assume that the extended larval development in phasmids (*ca* 100 d in *M. diocles*, *cf* Chapter 3) may increase the impact of predation as compared to grasshoppers. High enemy-induced mortality in late developmental stages of holometabolous insects originates as high parasitation of the pupal stage (Hawkins *et al.* 1997). Hemimetabolous insects lack this immobile phase of reorganisation. In the case of phasmids their extended larval development could compensate for the lack of parasitoid-mediated control.

My findings support the top-down view of population regulation in *M. diocles*. Such high predator induced mortality as presented here leads to a drastic reduction of the rate of population increase in only the first two weeks of the developmental period of this phasmid. Individuals that had so far survived still need another 12 to 13 weeks before they enter the reproductive stage. However, my results do not allow for an extrapolation of predation impact over the whole life cycle of *M. diocles*, because different life-stages of an insect may experience different susceptibilities to predation, often in a size-selective manner (Oedekoven & Joern 1998).

Predation pressure in the present study may be underestimated for two reasons: First, a proportion of the host plant specific predator community (Dyer *et al.* 1999) could have been missed by the use of a gap associated *Piper* species. In addition, I could not measure parasitoid attack. Parasitoids may live in their host a long time only becoming lethal in later developmental stages (Begon *et al.* 1996). However, based on observations from specimens collected in the field, parasitoids probably play a minor role in early mortality of *M. diocles* nymphs.

My results need to be discussed with regard to two major problems involved in conducting field experiments. My design of exclosures could have caused microclimatic changes leading to differential mortality. I think matching proportions of intrinsic mortality in both the field and the migration experiment in greenhouses do enforce my results and suggest that influence of microclimatic differences in the exclosures was negligible. Further, in my study disappearance of nymphs in control plots (54 %) could just reflect emigration, and emigration then was higher at night as phasmids are nocturnal. I presented evidence that emigration of nymphs most likely did not affect the presented results, because my searching area was well above the average distance of 47.53 cm nymphs moved between checking the plots. Similar movement ranges in phasmids have been reported previously (0.55 m d^{-1}, Willig *et al.* 1986), and phasmid nymphs were shown to behave philopatric while adults represent the dispersal stage (Willig *et al.* 1986, 1993). In addition, similar proportions of migrating nymphs in both treatments indicate that migratory behavior was observed adequately.

5.4.2 Is off-plant migration mediated by natural enemies or by resources?

The initially stated hypothesis that migration behavior of *M. diocles* nymphs would reflect predator avoidance could not be supported by observations in the field experiment. Preys are expected to invest in predator avoidance tactics when predation risk is sufficiently high (Charnov *et al.* 1976, Lima & Bednekoff 1999, Luttbeg & Schmitz 2000). Experimental studies show that prey display migratory predator avoidance based on the risk imposed by predators (Snyder & Wise 2000, Venzon *et al.* 2000). This may not apply for the presented study system. I showed that nymphs of *M. diocles* suffered significant pressure from the third trophic level, but I did not find the expected increase in migratory activity of nymphs in controls.

Increased migratory activity of nymphs in the field as compared to nymphs in greenhouses may reflect odor-released migration. For example, herbivorous mites migrate in-between their host plant as a specific reaction to odors of their predators (Magalhães *et al.* 2002). The general observation that part of *M. diocles* nymphs moved in both the field and the greenhouse experiment may be intrinsically triggered as a consequence of enemy free space. This has been shown for herbivores moving in non-feeding periods to patches of reduced predation risk (for a review see Beredegue *et al.* 1996). However, migration may be of minor importance for predation avoidance in phasmids, because they show a variety of behavioral and morphological adaptations to escape from predators, such as chemical defense (*e.g.* Eisner 1965; Carlberg 1985; Eisner *et al.* 1997), acoustic and color display (Robinson 1968a, 1968b, 1969), as well as camouflage and mimicry (Bedford 1978).

As an alternative to top-down related explanations of migration, data indicated that emigration may be attributed to ‘plant size’ (in terms of leaf number). Mean number of healthy leaves per host plant was significantly lower for emigrating nymphs than for sessile or remigrating nymphs. Plant size may reflect both food availability and quality. As first instar nymphs consume minimal quantities of leaf tissue per day (pers. obs.) food in general was available and hence the described emigration pattern seems not to be related to resource availability. I suggest that nymphs left their host because of significant bottom-up pressure in terms of reduced suitability of the host plant. Leaf traits such as nutrient or toxin content can be plant size related. Plants with lower leaf area suffer relatively higher damage by larval feeding and consequently are at higher risk of reduced fitness (Marquis 1992). The greater impact of herbivory on small plants should have selected for constitutive defenses that decline during development (Coley *et al.* 1985; but see Kearsley & Whitham, 1989). Plant size was shown to positively affect larval performance of insect herbivores (Price 1991; Teeder & Tammaru 2002) and according to the plant vigor hypothesis, bigger plants or plant modules are more vigorous and preferred by herbivores (Price 1991). Both increased constitutive defenses and reduced plant vigor in smaller plants result in diminished food quality. Poor quality food is known to decrease survivorship (*e.g.*, Lill & Marquis 2001) and development (Damman 1987) of herbivores and as a consequence larva may move on to a new host (van Dam *et al.* 2001). The presented relation of emigration and plant size supports this view but further research is needed to evaluate whether smaller individuals in *Piper marginatum* are of minor quality as food a source for *M. diocles* nymphs.

If reduced suitability was the motivation to leave a host plant, there are still costs involved in searching for a new host. First the herbivore may not encounter a suitable new host in time and it is deprived of food while searching. Very young caterpillars, for example, often have trouble finding neighboring food plants (van Dam *et al.* 2000) and temporal deprivation of food can retard development more strongly than feeding on an induced low quality food source (van Dam *et al.* 2001). Second, while searching, the herbivore runs risk of being attacked by enemies (Price *et al.* 1980). In contrast, staying can result in higher enemy-caused mortality as development is decelerated (slow growth/ high mortality) (Feeny 1976; Clancy & Price 1987). Hence bottom-up factors can affect mortality directly through starvation and indirectly through a feedback loop to predation.

5.4.3 Who are the predators?

In my study, disappearance of nymphs was significantly higher at night. I showed that disappearance to a high extent may have reflected predation. As a consequence, I concluded that birds, a prominent group of predators on herbivorous insects (*e.g.*, Marquis & Whelan 1994; Floyd 1996; Murakami 1999) seem to be of no importance as mortality source of *M. diocles* nymphs. In accordance with Belovsky *et al.* (1990) I suggest that arthropods are the principal predators on immature individuals. During the day, nymphs of *M. diocles* mainly hide on the underside of leaves. They huddle against the leaf midrib with their morphology perfectly blending in with the substrate (pers. obs.). Due to this camouflage, they may prevent predation during the day from visually searching natural enemies. At night, when moving from their hiding location to a feeding site, they are most vulnerable to attack. Nocturnal activity is a common behavioral adaptation to decrease predation risk. Nonetheless, many predators search for prey at night. I found spiders, bugs (*Reduviidae*) and ants of the genus *Ectatomma* preying upon nymphs. The relevance of *Ectatomma* ants as predators of *M. diocles* is supported by high densities of these ants in the BCI forest (Levings & Franks 1982), and by the fact that *E. ruidum* workers preyed upon nymphs in lab experiments (data not shown). *E. ruidum* is mainly foraging in the litter and on the soil whereas *E. tuberculatum* searches different plant strata (Levings & Franks 1982). *Ectatomma* spp., like many other ant species show no expressed pattern in diurnal activity (Kaspari & Weiser 2000). In contrast, *Pheidole radowskowskii* FOREL (*Formicidae*) was shown to preferentially forage at night (Kaspari & Weis 2000) and to forage in high frequencies on leaves of *Piper marginatum* (Dyer *et al.* 1999).

5.4.4 Conclusions

M. diocles and many other phasmids occur in particularly low densities in humid tropical forests (*cf.* Chapter 2; Novotny & Basset 2000). Yet, the factors influencing population densities in phasmids have never been addressed in an empirical study. My findings support the top-down view of population regulation in *M. diocles*. While my data cannot uncover any impacts on population dynamics, for example because predation rates alone fall short to including density-dependence of predator-prey interactions (Sih *et al.* 1985), the high rates of predator-induced mortality I found may directly translate into a reduction of the intrinsic rate of population increase and hence explain the low abundances of this stick insect.

Contrary to my predictions, emigration of nymphs was not related to top-down pressure. Hence, the function of observed behavior in one prey may not be assignable to another. In accordance with Witz (1990), I emphasize experimental confirmation before assuming a behavior as predator avoidance. My results suggest host plant size as an alternative factor influencing the movement patterns of herbivores. Host plant size may be directly related to suitability as food source. Herbivore survivorship and fecundity can then be affected directly by plant size related leaf traits reducing quality and indirectly by increasing migratory behavior thereby increasing exposure to predators.

6 Concluding remarks

6.1 Is *M. diocles* a herbivore specialist?

This study could not provide clear evidence whether the restrictions in *M. diocles* host range reflect specialization. The distant relationship of its host plant families, the Araceae and Piperaceae, disagrees with the predictions of the concept of biochemical coevolution (Ehrlich & Raven 1964; Strong *et al.* 1984). Alternatively, restricted host range of *M. diocles* may reflect 'clouded' biochemical coevolution, *i.e.* the taxonomic relationship among host plants is disconnected by other factors such as predation. This concept of diffuse coevolution (Janzen 1979; 1980; Fox 1981) bases on multitrophic interactions simultaneously exerting selective pressures on one another and being affected by changes in other component members. For example, strong differential predation pressure on host plant species as demonstrated by Sandoval (1994), may have led to the exclusion of missing links between Araceae and Piperaceae in the host plant range of *M. diocles*. Such a scenario must remain purely speculative because so far there is no incidence that these families are in a closer taxonomoic relation. In contrast, the Araceae belong to the monocots and the Piperaceae remain on a somehow uncertain taxonomic position among the paleoherbs between mono- and dicots (Judd *et al.* 2002). At least future field-experiments assessing predation pressure on *M. diocles* on different host plants would give a principle idea, whether diffuse coevolution may be involved.

However, Jermy (1976) suggested an alternative 'null' model of coevolution. Sequential evolution may best apply to herbivores like *M. diocles* that feed on distantly related plant taxa. Jermy (1976) argued that, because most insect herbivores have low population densities, they could hardly be important selection factors for plants. Insect-host relationships need not be antagonistic, they may as well interact in a mutually advantageous way by insects keeping their hosts at an ideal density. Consequently secondary compounds may not reflect defense, as resistance is not necessary. According to sequential evolution, biochemical plant diversity origins from abiotic selection factors and from plant-plant interactions. Herbivores just adapt to plant changes but don't influence them markedly. Generally, this model applies when evidence for coevolution is not forthcoming, and parallel evolutionary lines of plants and insects are few (Hartley & Jones 1995). As a consequence of sequential evolution, plants and their interactions with the abiotic and biotic environment replace herbivores. Thus the initially stated supposition that herbivores are central factors in maintaining forest dynamics and ecosystem stability of tropical forests (Lowman 1984; Brokaw 1985; Collins *et al.* 1985; Schowalter 1985; Brown *et al.* 1987) cannot hold if herbivore specialization is based on sequential coevolution.

Concluding, this study showed that *M. diocles* as a tropical herbivore is clearly restricted in its host range, particularly facing the fact that this species seems to feed only on some plants from two families out of more than 1200 plant species representing 120 families occurring on BCI (Croat 1978). Host plant use of *M. diocles* may even be more restricted as shown here, because differential interspecific effects among host plants on nymph performance were great.

6.2 Bottom-up or top-down or both

This study provided evidence that herbivores suffer significant impact from both bottom-up and top-down factors. In the debate about the relative roles of higher and lower trophic levels in regulating tropical herbivore populations several authors recently reasoned that natural enemies (Pace *et al.* 1999; Persson 1999; Polis 1999; Dyer & Coley 2001) and in particular their marked effect on early lifestages (Cornell & Hawkins 1995; Cornell *et al.* 1998) exceeded the effects from plants.

My results give further support to this view. Predation seems be the stronger factor explaining low abundances of *M. diocles* in the BCI forest because of its direct effects and because of indirect effects mediated by host plants.

In two weeks nymphs suffered 54 % mortality from predators in their natural environment and predation pressure seemed to be spread equally in the forest understory (*cf.* Chapter 5). On the other hand I provided evidence that nymphs, when confronted with an inadequate host may suffer up to 100 % mortality in a comparable time span (*cf.* Chapter 4). I also showed that nymphs may leave a host of minor suitability (although the evidence was weak; *cf.* Chapter 5). Sedentary behaviour on such a host involves two risks. The nymph may die because of toxic or minor nutritive host quality (particularly if this host was *Piper grande* or *Dieffenbacchia longispatha*, *cf.* Chapter 4 or *e.g.*, Lill & Marquis 2001)) or it will suffer decreased growth thereby remaining longer in the more vulnerable immature stage (Damman 1987). Leaving the host involves the risks of starvation, of not finding any or a more suitable host (van Dam *et al.* 2001), and of predation (Price *et al.* 1980).

At least in the understory of the BCI forest *M. diocles* nymphs may find an acceptable host in the abundant *Philodendron inaequilaterum* (Croat 1978). This species proofed to be of high suitability for nymphs as they suffered least mortality. Albeit, growth on *Phil. Inaequilaterum* was decreased as compared to some other species. Despite the fact that a plant species may be of minor suitability considering growth, phasmids were shown to modulate feeding in their natural setting rather to availability than to acceptability (Willig *et al.* 1993).

Consequently, low suitability of a host plant may either kill a nymph(demonstrated in Chapter 5), it may translate into higher predation pressure via decelerated growth (slow-growth high mortality; Feeny 1976; Clancy & Price 1987), or it may increase the exposure to predators if the nymph searched a new host. In other words: effects of host suitability feed back into predation and thereby add on to direct top-down effects.

Closing, I would like to come back to Lawton & McNeill (1979): *the Devils can only be that evil because the Sea is so Deep and Blue*.

7 Abstract

Herbivory is discussed as a key agent in maintaining dynamics and stability of tropical forested ecosystems. Accordingly increasing attention has been paid to the factors that structure tropical herbivore communities.

The aim of this study was (1) to describe diversity, density, distribution and host range of the phasmid community (Phasmatodea) of a moist neotropical forest in Panamá, and (2) to experimentally assess bottom-up and top-down factors that may regulate populations of the phasmid *Metriophasma diocles*.

The phasmid community of Barro Colorado Island was poor in species and low in density. Phasmids mainly occurred along forest edges and restricted host ranges of phasmid species reflected the successional status of their host plants. Only *M. diocles* that fed on early and late successional plants occurred regularly in the forest understory.

A long generation time with a comparably low fecundity converted into a low biotic potential of *M. diocles*. However, modeled potential population density increased exponentially and exceeded the realized densities of this species already after one generation indicating that control factors continuously affect *M. diocles* natural populations. Egg hatching failure decreased potential population growth by 10 % but was of no marked effect at larger temporal scale.

Interspecific differences in defensive physical and chemical leaf traits of *M. diocles* host plants, amongst them leaf toughness the supposedly most effective anti-herbivore defense, seemed not to affect adult female preference and nymph performance. Alternatively to these defenses, I suggest that the pattern of differential preference and performance may be based on interspecific differences in qualitative toxic compounds or in nutritive quality of leaves. The significant rejection of leaf tissue with a low artificial increase of natural phenol contents by nymphs indicated a qualitative defensive pathway in *Piper* evolution. In *M. diocles*, oviposition may not be linked to nymph performance, because the evolutionary prediction of a relation between female adult preference and nymph performance was missing. Consequently, the recruitment of nymphs into the reproductive adult phase may be crucially affected by differential performance of nymphs.

Neonate *M. diocles* nymphs suffered strong predation pressure when exposed to natural levels of predation. Concluding from significantly increased predation-related mortality at night, I argue that arthropods may be the main predators of this nocturnal herbivore. Migratory behavior of nymphs seemed not to reflect predation avoidance. Instead, I provided first evidence that host plant quality may trigger off-plant migration.

In conclusion, I suggest that predation pressure with its direct effects on nymph survival may be a stronger factor regulating *M. diocles* populations, compared to direct and indirect effects of host plant quality, particularly because slow growth and off-host migration both may feed back into an increase of predation related mortality.

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9 Appendix

Please continue to next page.

A newly hatched nymph of *Metriophasma diocles* (© Christian Ziegler)

Appendix 1: Phasmid species previously reported from Panama and BCI. The table lists current names of species and synonyms.

No	Genus	Species	Type-Author	Reported locations	BCI	Synonyms.	Reference
1	*Acanthometriotes*	*crassus*	Hebard	Panama, Ecuador			Robinson 1969, Bedford 1978
2	*Agrostia*	*amoena*	Redtenbacher	Panama			Hebard 1923
3	*Agrostia*	*viridipes*	(Rehn)	Panama (Cabima) / Costa Rica (Cache)		*Olcyphides viridipes* Rehn 1905	Hebard 1923
4	*Bacteria*	*nodulosa*	Redtenbacher	Panama, Surinam			
5	*Bacteria*	*ploiaria*	(Westwood)	Panama (Gatun, Canal Zone, coll. by Zetek, Chiriqui)	Berger	*Phibalosoma ploiaria* Westwood 1859 *Bacteria subvolans* Redtenbacher 1908	Hebard 1923, Robinson 1969
6	*Bostra*	*dorsuaria*	Stal	Panama (Chiriqui)			Hebard 1923
7	*Bostra*	*lobata*	Stal			*Phantasis lobata* Redtenbacher	Hebard 1923
8	*Bostra*	*martini*	Griffini				
9	*Bostra*	*nodulosa*	Redtenbacher				Hebard 1923
10	*Bostra*	*podagrica*	Stal				Hebard 1923
11	*Brizoides*	*annulicornis*	Hebard	Panama (Portobello)			Hebard 1923
12	*Brizoides*	*graminea*	Redtenbacher	Panama (Chiriqui-Bugaba)			Hebard 1923
13	*Brizoides*	*lacteipennis*	Redtenbacher				Hebard 1923
14	*Brizoides*	*nigricornis*	Redtenbacher				Hebard 1923
15	*Calynda*	*bicuspis*	Stal	Panama (Chiriqui)			Hebard 1923
16	*Chlorophasma*	*hyalina*	Redtenbacher				Hebard 1923
17	*Damasippus*	*fuscipes*	Redtenbacher				Hebard 1923
18	*Damasippus*	*striatus*	Redtenbacher				Hebard 1923
19	*Damasippus*	*westwoodii*	Stal				Hebard 1923
20	*Dyme*	*chiriquensis*	Brunner			*Bacteria*	Hebard 1923
21	*Dyme*	*discors*	Brunner			*Bacteria*	Hebard 1923
22	*Dyme*	*magnifica*	Hebard	Panama (BdT)		*Bacteria*	Hebard, 1933
23	*Dyme*	*modesta*	Brunner			*Bacteria*	Hebard 1923
24	*Isagoras*	*dentipes*	Redtenbacher	Panama (Chiriqui-Bugaba)	Berger		Hebard 1923, Robinson 1969

Appendix 1: Phasmid species previously reported from Panama and BCI. The table lists current names of species and synonyms.

No	Genus	Species	Type-Author	Reported locations	BCI	Synonyms.	Reference
25	*Isagoras*	*vigneri*	(Redtenbacher)	Panama (Darien-Paya, Portobello)		*Xerosoma vignieri* Redtenbacher	HEBARD 1923
26	*Libethra*	*panamae*	Hebard	Panama (Paraiso, Panama vieja)			HEBARD 1923
27	*Metriophasma*	*diocles*	Westwood	Panama (BCI)	Berger	*Metriotes diocles*	HEBARD 1923, 1933, ROBINSON 1969
28	*Metriophasma*	*iphicles*	Redtenbacher	Panama (Chiriqui-Bugaba, Rio Trinidad) / Honduras (San Pedro-Sula)	Berger		HEBARD 1923
29	*Olcyphides*	*grisescens*	(Redtenbacher)			*Perliodes grisescens* Redtenbacher	HEBARD 1923
30	*Olcyphides*	*nigro-granulosus*	(Redtenbacher)			*Perliodes nigrogranulosus* Redtenbacher	HEBARD 1923
31	*Oncotophasma*	*armata*	(Brunner)	Panama (Chiriqui)		*Paradiapheromera armata* Wattenwyl, sp. n.	HEBARD 1923
32	*Oncotophasma*	*martini*	(Griffini)	Panama (Darien-Lake Pita, Chiriqui & Darien, Portobello, Alhajuelo, Gatun, BCI)	Berger	*Bostra martini* Griffini 1896 *Paradiapheromera strumosa* Brunner 1907 (Darien) Wattenwyl, sp. n.	HEBARD 1933
33	*Paranisomorpha*	*insignis*	Redtenbacher	Panama (Boquete) / Costa Rica			REDTENBACHER 1908
34	*Prisopus*	*ariadne*	Hebard	Panama (Alhajuela, Paraiso) / Costa Rica (Carillo)			HEBARD 1923
35	*Prisopus*	*berosus*	Westwood	Panama (coll. by Zetek)/ Costa Rica (in coll.)	Berger		HEBARD 1923
36	*Pseudoceroys*	*harroweri*	Hebard	Panama (Portobello)			
37	*Pseudophasma*	*menius*	(Westwood)	Panama (Portobello, Gatun, Culebra, BCI) / Colombia	Berger	*Phasma menius* Weswood	HEBARD 1923, ROBINSON 1969
38	*Pterinoxylus*	*spinulosus*	Redtenbacher	Panama (Chiriqui, BCI)	Berger		BEDFORD, 1978 REDTENBACHER, 1908
39	*Stratocles*	*cinctipes*	Stal				HEBARD 1923
40	*Stratocles*	*multilineatus*	Rehn	Panama (Bugaba-Chiriqui, Gatun, Guamina-Rio Chagres) / Costa Rica (San Carlos)	Berger	*Stratocles forcipatus* Redtenbacher	HEBARD 1923, 1929, ROBINSON 1969, BEDFORD 1978
41	*Tenerella*	*tenerrima*	Redtenbacher	Ancon			HEBARD 1923, 1933

Appendix 1: Phasmid species previously reported from Panama and BCI. The table lists current names of species and synonyms.

No	Genus	Species	Type-Author	Reported locations	BCI	Synonyms.	Reference
42	*Trychopeplus*	*laciniatus*	(Westwood)	Panama (Chiriqui, Chiriqui-Bugaba, Rio Trinidad) / Costa Rica (Rio Sucio) / Nicaragua	Aiello	*Ceroys laciniatus* Westwood *Pericentrus multilobatus* Redtenbacher *Pericentrus laciniatus* Shelford *Trychopeplus multilobatus* Shelford	HEBARD 1923

Tabellarischer Lebenslauf

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Citable publications:

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Berger J., Wirth R., 2001. Physical antiherbivore defense does not determine preference of a specialist stick insect. In: G. Zotz, Ch. Körner (eds.) The functional importance of biodiversity. Verhandlungen der Gesellschaft für Ökologie 31: 311.

Berger J., Wirth R., 2001. Diversity and diet breadth of stick insects (Phasmatodea) in a Panamanian semi-deciduous rainforest). In: G. Zotz, Ch. Körner (eds.) The functional importance of biodiversity. Verhandlungen der Gesellschaft für Ökologie 31: 338.

Contributions to meetings:

Berger J., Wirth R. 2004. Resource and predator-mediated control of a rare specialist herbivore. In: Biodiversity and dynamics in tropical ecosystems. 17th annual meeting of the Society for Tropical Ecology. Poster

Berger J., Wirth R., 2003. Top-down and bottom-up effects on survival and migration of a neotropical herbivore. Field experiments with nymphs of the stick insect *Metriophasma diocles*. Tagungsband der 16. Jahrestagung der Gesellschaft für Tropenökologie. Poster

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Glöggler J., Berger J., Schaefer M., 1998. Untersuchungen zu gebäudebesiedelnden Stechimmen (Hymenoptera) in einem Freilichtmuseum. In: Osten, T. (Hrsg.) Beiträge der Hymenopterologen-Tagung in Stuttgart. Oral Presentation

Berger J., Schaefer, M., 1997. Wildbienen als Blütenbesucher in Bauerngärten. Tagungsband der Jahrestagung der Gesellschaft für Ökologie. Poster

Popular scientific articles:

Berger J., In press. The Masters of Camouflage. BBC Wildlife Magazine. (appears in September 2004)

Erklärung:

Hiermit versichere ich, dass ich die vorliegende Dissertation in allen Teilen selbständig angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Darüber hinaus erkläre ich, dass die vorliegende Dissertationsschrift weder vollständig noch teilweise einer anderen Fakultät mit dem Ziel vorgelegt worden ist, einen akademischen Grad zu erwerben.

Kaiserslautern, den 27. Mai 2004