Predation-mediated Mortality of Early Life Stages: A Field Experiment with Nymphs of an Herbivorous Stick Insect (*Metriophasma diocles*)

ABSTRACT

We quantified predation pressure on first instar nymphs of a stick insect in predator-exclusion experiments in the forest of Barro Colorado Island, Panamá. After considering intrinsic mortality (19%) and potential emigration (negligible), we estimated that 54 percent of the nymphs died due to predation in a two-week period. Predation on nymphs was highest at night and may explain the low abundance of *Metriophasma diocles* in the understory.

Key words: early life stages; hemimetabolous insects; nocturnal predation; Panamá; Phasmatodea; top-down; tropical rain forest.

HIGH SPECIES DIVERSITY AND LOW POPULATION DENSITIES OF HERBIVOROUS INSECTS are widely acknowledged features of tropical forest ecosystems (Basset 1999, Novotny & Basset 2000). For example, many species of stick insects (Phasmatodea) are known to occur in low abundances in humid tropical forests (Novotny & Basset 2000). Besides bottom-up forces and competition, several levels of top-down control are considered important determinants of the abundance and distribution of prey organisms, and numerous studies have documented that predators and parasitoids can have major impacts on insect herbivore populations (for review see Sih et al. 1985, Walker & Jones 2001). This is especially true for the tropics, where predation pressure is thought to be greater than in temperate systems (Hawkins et al. 1997 and references therein). Generally, predator-mediated mortality is not equally distributed among feeding guilds or developmental stages of insects. In particular, exophytic and immature herbivores have been shown to experience the greatest top-down pressure (reviewed in Hawkins et al. 1997); however, support for these patterns has been derived primarily from studies on holometabolous herbivores, the immobile pupal stage of which is particularly susceptible to natural enemies (Cornell et al. 1998). Relatively little attention has been paid to predation pressure on exophytic feeding hemimetabolous herbivores, and published studies have focused largely on orthopterans in temperate grassland habitats (Joern & Gaines 1990, Oedekoven & Joern 1998).

In this study, we quantified the predation-related mortality of nymphs of *Metriophasma diocles* (Westwood), a hemimetabolous exophytic herbivore that occurs in low abundances in the understory of Neotropical rain forests (6.7 individuals/ha; Berger 2004). The assessment of predation impact on survivorship of *M. diocles* nymphs was achieved 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). A general shortcoming of such field studies of survival is that they cannot distinguish between death versus emigration as the cause of disappearance. To account for the potential emigration of *M. diocles* nymphs out of control plots, we recorded the migratory activity of individual nymphs. For the description of natural enemies and the temporal pattern of their impact, the study was complemented by observations on (3) the identity of potential predators and (4) the diurnal patterns of nymph survival.

The study took place in a semi-deciduous tropical moist forest on Barro Colorado Island (BCI, Republic of Panamá) from September to December 2001. At each of three sites in the forest understory, we installed ten experimental plots. Each plot included both an enclosure and a control plant (1 m apart from each other). We chose the experimental sites on the basis of prior field records (Berger 2004) indicating that *M. diocles* occurs at forest edges and in moist areas of the understory where several species of host plant families (Araceae, Piperaceae) were abundant. The three sites (20–50 m apart from each other) were located along a slope in old-growth forest that was heterogeneous in respect to density of the understory vegetation and microclimatic setting. The experimental plots were at least 5 m apart. In each enclosure and each control plot, we planted one greenhouse-grown sapling of *Piper marginatum* JACQ. (Piperaceae). This *Piper* species proved particularly suitable for the experiment because palatability

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to *M. diocles* was high (Berger 2004) and bottom-up effects were shown to be minimal. The enclosures (50 cm diam, 60 cm height, 0.1 cm mesh diam) were sufficiently large to prevent leaves of the enclosed plant from touching the mesh. The experiment was repeated four times (runs 1–4), each lasting 14 days and all with a new set of plants. Plants were monitored daily in each experimental run. Before starting an experimental run, we checked that enclosures and the plants therein were free from herbivores and predators. Likewise, we checked the surrounding vegetation of each control plant to ensure that no individuals of *M. diocles* were present (no *M. diocles* specimens were found). At day 1 (before 1000h), one first instar nymph (average weight: 14.82 ± 7.10 mg; length: 12–15 mm; *N* = 219) reared from eggs collected in a laboratory colony, was placed on each plant within a plot (exclosure and control). Each individual plant was used only once. In each run, we recorded (1) survival and migratory activity of the nymphs and (2) the presence of potential predators on control plants, daily in the morning and in the evening. Hereafter, we will use the term "survival" to refer to all nymphs present in control plots (on- or off-host) and in enclosures at a given time. Nymphs in the enclosures 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 enclosures. Nymphs that disappeared from control plants, or any subsequently marked position in the surrounding vegetation, were systematically searched in a column of 1 m radius and 2 m height. If found alive off the host, a nymph was recorded as "migrating"; its location was then marked and the traveled distance measured (only two dead nymphs were found off-host). 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, we obtained data for 238 nymphs in both enclosures and controls.

To control for effects of the enclosure on mortality and migratory activity of nymphs and to better allow for an estimation of predation-mediated disappearance, we designed an additional 14-day experiment in the greenhouse (mesh-protected against intruders) with seven experimental plots. An individual sapling of *P. marginatum* was planted in the center of a 1 m² bed (15 cm high) and eight individuals at the outer borders. To prevent nymphs from leaving a plot, we constructed an adhesive barrier. A single nymph was then set on the central plant and checked twice daily for survival, migration, and remigration. A small number of predation events may have occurred as workers of *Ectatomma ruidum* Roger sporadically crossed the adhesive barrier. In total, we followed 53 nymphs in this experiment. Survival times were calculated using the Kaplan–Meier estimate (also known as the product–limit estimate). Differences in survival times were tested by Mantel–Cox log-rank analysis.

Survival times of *M. diocles* nymphs differed significantly between enclosures and controls (Mantel–Cox test; *χ²* = 74.08, df = 1, *P* < 0.0001; Fig. 1). In total, nymph survival was three times higher on caged plants than on exposed plants (81 vs. 27%; Table 1). In the enclosures, survival times varied neither spatially among the three forest sites nor temporally throughout the study period. In contrast, spatiotemporal variation increased in the controls, where nymphs were exposed to natural levels of predation. Most notably, residence times differed significantly among runs in the controls (Mantel–Cox test; *χ²* = 23.62, df = 3, *P* < 0.0001).

In controls, significantly more nymphs disappeared at night compared to the daytime (Mantel–Cox test; *χ²* = 31.13, df = 1, *P* < 0.0001; Fig. 2). In contrast, there was no diurnal variation in mortality in the enclosures, where predation was absent (Mantel–Cox test; *χ²* = 1.96, df = 1, *P* < 0.16). Various invertebrate predators were recorded (Table 2). Spiders (2 cases) and bugs (*Reduviidae*; 1 case) were seen preying upon nymphs. *Ectatomma* ants were repeatedly found on control plants prior to the disappearance of 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 (J. R. Berger 2004).

The fate of any lost nymph in the control plots was 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 enclosures and controls and was therefore approximated as the proportion of nymphs having died on caged plants (19%; i.e., 23 of 119 nymphs; Table 1). This estimate proved legitimate as mortality levels in the greenhouse experiment did not differ significantly (32%; i.e., 17 of 53 nymphs; *χ²* = 2.66, df = 1, *P* = 0.10). The latter correspondence also suggests that mortality levels were not considerably influenced by the enclosures. We 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 (distances per nymph were averaged as some nymphs moved multiple times). Similar movement ranges in phasms have been reported previously (0.55 m/d; Willig et al. 1986). Second, overall migratory activity in controls appeared to be adequately estimated, as the proportion of moving nymphs corresponded well with exclosures (Table 1). This was further supported by the fact that the proportion of migrating nymphs in the predation-free greenhouse experiment (8 of 53 nymphs) was not different from the exclosures (30 of 119 nymphs; \( \chi^2 = 1.49, df = 1, P = 0.22 \)). 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 percent.

The present study on phasms is the first to quantify mortality of early life stages for an exophytic hemimetabolous insect in the tropics. Nymphs of *M. diocles* suffered 73 percent mortality in their first two weeks after eclosion and the significant reduction in mortality in exclosures indicates that natural enemies were the most prominent source of mortality. These results correspond with earlier studies showing predation particularly influencing survival of immature stages of holometabolous insects (Cornell & Hawkins 1995, Hawkins et al. 1997, Cornell et al. 1998). Similar patterns have been described for temperate hemimetabolous grasshoppers. For example, for the whole 48-day nymphal period of two grasshoppers, Belovsky et al. (1990) estimated the loss to predators to be ca 40 percent. Here, we provided evidence that nymphs of a hemimetabolous tropical phasmid suffered ca 54 percent predation-related mortality in a 14-day period. Furthermore, extended larval development in phasms (ca 100 d in *M. diocles*; Berger 2004) may have increased the impact of predation compared to grasshoppers. Predation pressure in the present study may have been underestimated because the duration of the experiment did

| TABLE 1. Disappearance or death and migratory activity of *Metriophasma diocles* nymphs in a field experiment with predation exclusion. |
|---------------------------------|---------|---------|------------------|
| No. of *M. diocles* nymphs      | Exclosure | Control | chi-square test |
| Total                           | 119      | 119      | \( \chi^2 = 67.09, P < 0.01 \) |
| Disappeared or dead after 14 days| 23       | 87       | \( \chi^2 = 0.75, P = 0.39 \) |
| Migrated                        | 30       | 37       |                   |
TABLE 2. *Potential predators of Metriophasma diocles nymphs observed on 119 control plants. Each plant was surveyed 29 times in runs 1–3 and 15 times in run 4 (including one night check per run).*

<table>
<thead>
<tr>
<th>Predator group</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ants (Formicidae): <em>Ectatomma</em> spp.</td>
<td>38</td>
</tr>
<tr>
<td>Army ants (<em>Eciton</em> spp.)</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
</tr>
<tr>
<td>Spiders (Araneae)</td>
<td>44</td>
</tr>
<tr>
<td>Reduvid bugs (Hemiptera)</td>
<td>3</td>
</tr>
<tr>
<td>Frogs (Anura)</td>
<td>2</td>
</tr>
<tr>
<td>Katydids⁴ (Orthoptera); adults</td>
<td>35</td>
</tr>
</tbody>
</table>

Katydids are often omnivorous.

not allow for the detection of parasitoid attack (although we found no evidence of parasitoids in field collections). In addition, 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 our study, predation on nymphs was significantly higher at night. Phasmids are known to possess behavioral and morphological adaptations to avoid predators (Bedford 1978, Sandoval 1994). During the day, nymphs of *M. diocles* mainly hide on the underside of leaves (J. R. Berger 2004) and may thereby avoid predation from visually searching natural enemies such as birds, a prominent group of predators on herbivorous insects (Van Bael et al. 2003). In accordance with observations on phasmid nymphs (Bedford 1978) and studies on other insect herbivores, our records of observed and suspected predators indicated that immature individuals were primarily susceptible to invertebrate predators (see Belovsky et al. 1990 for grasshoppers).

*Metriophasma diocles* and many other phasmids occur in particularly low densities in humid tropical forests (Berger 2004; Novotny & Basset 2000). Yet, the factors influencing population densities in phasmids have never been addressed in an empirical study. Our findings support the top-down view of population regulation in *M. diocles*. While our 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 we found may directly translate into a reduction in the intrinsic rate of population increase and hence explain the low abundances of this stick insect.

The correspondence of migration rates in both the experimental treatments and under the predator-free greenhouse conditions may indicate that moving off the host plant is not a matter of predation avoidance (Venzon et al. 2000) and may rather reflect reduced suitability of host plants (van Dam et al. 2001). Preliminary evidence suggested that the mean number of healthy leaves per host plant was significantly lower for emigrating than for sessile nymphs (Berger 2004). Whether movements of nymphs are intrinsically triggered due to enemy-free space (Beredegue et al. 1996), odor released migration (Magalhães et al. 2002), or stimulated by poor food quality, may become apparent in future studies.

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**References**


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