

MYRMECOPHILY AND PARASITOID INFESTATION OF SOUTH-EAST ASIAN LYCAENID BUTTERFLY LARVAE

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INTRODUCTION

Parasitoids are major selective factors in the life-history evolution of many insects (Weseloh 1993, Godfray 1994). Among the Lepidoptera, early stages such as larvae are often heavily attacked by parasitoids. Larval life-history traits like gregariousness (Fitzgerald 1993) or host plant selection (Gauld & Gaston 1992) have been shown to reduce the risk of parasitoid infestation. Larvae of many species in the butterfly family Lycaenidae possess an additional means of protection against enemies: they are attended by ants, at least during the later part of their development. These butterfly-ant interactions, termed "myrmecophily" (Pierce 1987, Fiedler 1991), are thought to be mutualistic in most cases. Relationships between myrmecophilous caterpillars and ants range from loose, unspecific, facultative interactions to obligatory, species-specific associations (e.g., Fiedler *et al.* 1996). The larvae of some lycaenid species, however, never associate with ants ("myrmecoxenes"). Attendant ants have been shown in a few cases to protect lycaenid immatures from attack by certain enemies, including parasitoids. So far, the scanty available data suggest that the protective services of ants are very important among obligatory and specific myrmecophiles (Pierce *et al.* 1987, Seufert & Fiedler 1996a), while among facultatively myrme-

cophilous species this protective effect was less pronounced (Pierce & Eastal 1986) or not detectable at all (Peterson 1993, Wagner & Kurina 1997). However, most previous studies have concentrated on single species and have been conducted in temperate or subtropical regions with rather depauperate faunas, such as North America or Australia. More information for a broader range of taxa and including species-rich tropical faunas is required before reliable generalizations about the relative importance of parasitoids and predators in the evolutionary ecology of lycaenid-ant interactions can be made. We here present the results of a multi-species comparison as a first rough approach to assessing the relationship between enemy pressure and myrmecophily in a species-rich tropical rainforest fauna. Specifically, we investigate whether an overall correlation exists between levels of parasitism and intimacy of the ant-association among South-East Asian members of the butterfly family Lycaenidae, using our data on natural parasitization rates collated over some years.

MATERIAL AND METHODS

During extended field studies on the life cycles and ecology of Malaysian lycaenids, as many lycaenid larvae as possible were sampled and reared in the laboratory until emergence of either adult butterflies or parasitoids. This was carried out under ambient light and temperature in closed transparent plastic con-

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rainers. Freshly-cut foliage or inflorescences of natural host plants were provided every second day, or as needed. Collections took place in 1988 and from 1991 to 1995 in the Gombak valley (Peninsular Malaysia, located about 20 km north of Kuala Lumpur, 3°21'N, 101°42'E, elevation 200–400 m), and in 1994 and 1997 in Mt Kinabalu National Park (vicinity of Poring, 6°05'N, 160°33'E, 500–800 m) on the island of Borneo. All collections were made in dipterocarp forests (ranging from almost pristine to advanced secondary forest) and were restricted to the vegetation layer that could be reached from the ground (i.e., up to 3 m in height).

We excluded from the analyses caterpillars that had been sampled as first instars, because first-instar larvae turned out never to be infested by parasitoids. Likewise, available field observations and experiments indicate that parasitoid females oviposit mainly on or into second or third instars of lycaenids, but not in younger and rarely in older larvae (Seufert 1997, Baumgarten & Fiedler 1998). Furthermore, all larvae were excluded from analysis (1) that died from infections or unknown reasons during rearing, (2) that were killed and preserved for morphological studies prior to completion of their development, or (3) if the species concerned was represented in the sample with less than six individuals. Data were pooled for the genus *Curetis*, because species of this taxon cannot be reliably identified using larval morphology only (Fiedler *et al.* 1995a). However, larvae of *C. bulis*, *C. santana*, *C. tagalica*, and *C. regula* co-occurred on the same host plants and were indistinguishable with regard to their feeding habits and relationships towards ants, so that specific differences with regard to parasitoid infestation are most unlikely to occur. Altogether we were thus able to assemble 576 larvae representing 26 species (Table 1) over a cumulative fieldwork period covering 93 weeks.

From this data set the rate of parasitoid infestation for each species was estimated as the percentage of parasitized individuals of the respective species total. This simple measure does not account for spatial or temporal variation in parasitoid pressure. Given the low abundance of most rainforest butterflies most of the time, however, more precise estimates are impossible for all but a small minority of the species. Furthermore, since we are interested here in differences across a wide range of species in their overall parasitoid load and possible trade-offs with myrmecophily, rather than in the population dynamics of particular host-parasite systems, this rough esti-

mate is considered as sufficient for the present comparative analysis.

Information on intensity of interactions with ants and parasitoid infestation among the 26 lycaenid species under investigation is presented in Table 1, together with data on some other characteristic larval life-history traits. This information was derived from our own field observations and experiments, supplemented by host plant records from the literature. Intensity of lycaenid-ant interactions was scored in four classes: (1) not ant-attended (= myrmecoxenous), (2) weakly ant-attended (< 50% of larvae associated with ants), (3) regularly ant-attended (> 50% of larvae associated with ants), (4) obligately attended by specific ants. The distinction between categories (2) and (3) was more pronounced than suggested by our numerical division, because in weakly ant-attended species less than 25% of the larvae were found in association with ants, whereas in the regularly ant-attended class usually more than two-thirds were actually tended (e.g., Seufert 1997). All statistical analyses were performed using STATISTICA 5.1 (StatSoft 1995).

RESULTS AND DISCUSSION

Lycaenid larvae in our sample were parasitized by species of Tachinidae (81 cases), Braconidae (58), Ichneumonidae (16), and Chalcidoidea (1). Proportions of parasitoid infestation ranged from 0 to 90% across species (Table 1). Parasitism increased significantly with the intensity and intimacy of interactions with ants (gamma rank correlation for multiple ties: $\gamma = 0.3832$, $P = 0.027$, see Fig. 1), generating the counter-intuitive pattern that obligately myrmecophilous lycaenid larvae often suffer from very high rates of parasitoid infestation. The strongest evidence for protection of caterpillars from predation or parasitoid attacks through ant guards has so far been found among obligate myrmecophiles (Pierce *et al.* 1987, Seufert & Fiedler 1996a). Such data obtained from ant-exclusion experiments and intraspecific comparisons might suggest that specialization and optimization of larval communication signals (Fiedler *et al.* 1996) could enhance the protective efficiency of attendant ants, for example by attracting a greater number of ant workers per larva and stabilizing the mutualistic association.

Our data derived from an interspecific comparison, however, challenge this view. Two arguments can be proposed to explain why more strongly ant-

TABLE 1. Larval life-history traits and incidence of parasitoid infestation of 26 South-East Asian Lycaenidae butterfly species. Degree of myrmecophily: 1 = not ant-attended; 2 = < 50 % of larvae ant-attended; 3 = > 50 % of larvae ant-attended; 4 = obligately attended by specific ants.

Lycaenid species	Degree of myrmecophily	Number of host plant families	Social status	Feeding position	Number of larvae parasitized by				Total number parasitized	Percentage parasitized	Total n
					Tachinidae	Braconidae	Ichneumonidae	Chalcidoidea			
<i>Poritia sumatrae</i>	1	1	aggregated	exposed	0	0	0	0	0	0.00	18
<i>Curetis</i> spp.	1	1	solitary	exposed	2	4	0	1	7	50.00	14
<i>Arhopala amphimuta</i>	4	1	solitary	exposed	12	0	0	0	12	32.43	37
<i>Arhopala dajagaka</i>	4	1	solitary	exposed	1	8	0	0	9	90.00	10
<i>Arhopala major</i>	3	1	solitary	exposed	0	2	0	0	2	16.67	12
<i>Arhopala metamuta</i>	3	1	solitary	concealed	1	0	0	0	1	7.14	14
<i>Arhopala muta</i>	3	1	solitary	concealed	0	0	0	0	0	0.00	6
<i>Surendra florimel</i>	3	1	solitary	exposed	0	8	0	0	8	38.10	21
<i>Cheritra freja</i>	1	8	solitary	exposed	0	2	0	0	2	7.41	27
<i>Drupadia ravindra</i>	3	7	solitary	exposed	0	1	5	0	6	21.43	28
<i>Drupadia theda</i>	4	6	aggregated	exposed	9	11	0	0	20	37.04	54
<i>Eooxylides tharis</i>	3	1	aggregated	exposed	0	0	0	0	0	0.00	13
<i>Hypolycaena othona</i>	2	1	solitary	concealed	0	1	0	0	1	3.13	32
<i>Rapala dienece</i>	4	12	solitary	exposed	11	0	0	0	11	55.00	20
<i>Rapala pheretima</i>	4	11	solitary	exposed	0	0	0	0	0	0.00	13
<i>Anthene emolus</i>	4	10	aggregated	exposed	0	17	0	0	17	36.69	46
<i>Jamides pura</i>	3	2	solitary	exposed	7	0	6	0	13	28.26	46
<i>Jamides caeruleus</i>	2	2	aggregated	concealed	13	0	0	0	13	34.21	38
<i>Jamides virgulatus</i>	3	2	aggregated	concealed	0	0	2	0	2	10.53	19
<i>Jamides alecto</i>	3	1	solitary	concealed	0	0	0	0	0	0.00	18
<i>Jamides malaccanus</i>	3	1	aggregated	exposed	18	3	0	0	21	77.78	27
<i>Prosotas dubiosa</i>	2	4	solitary	exposed	0	0	0	0	0	0.00	23
<i>Caleta manovus</i>	1	1	solitary	exposed	0	1	0	0	1	14.29	7
<i>Caleta roxus</i>	1	1	solitary	exposed	0	0	0	0	0	0.00	9
<i>Actyolepis pusa</i>	3	14	solitary	exposed	7	0	0	0	7	53.58	13
<i>Plautella cossaea</i>	3	1	solitary	exposed	0	0	3	0	3	27.27	11
Sum					81	58	16	1	156		576
(% of total)					(14.1)	(10.1)	(2.8)	(0.1)	(27.1)		

attended lycaenids experience higher rates of parasitism. First, evidence already exists that certain parasitoids can use ants as cues to locate their hosts (Pierce *et al.* 1987, Nash 1989). Specialized ichneumonids even endure attacks from ants when stinging their hosts (Thomas & Elmes 1993). Second, obligate myrmecophiles may provide particularly rewarding

targets for parasitoids, because the close association of such caterpillars with ecologically dominant ant species usually results in clumped and rather predictable distributions of host larvae (Seufert & Fiedler 1996b). The use of myrmecophilous hosts might further provide enemy-free space for primary parasitoids through protection from hyperparasitoids

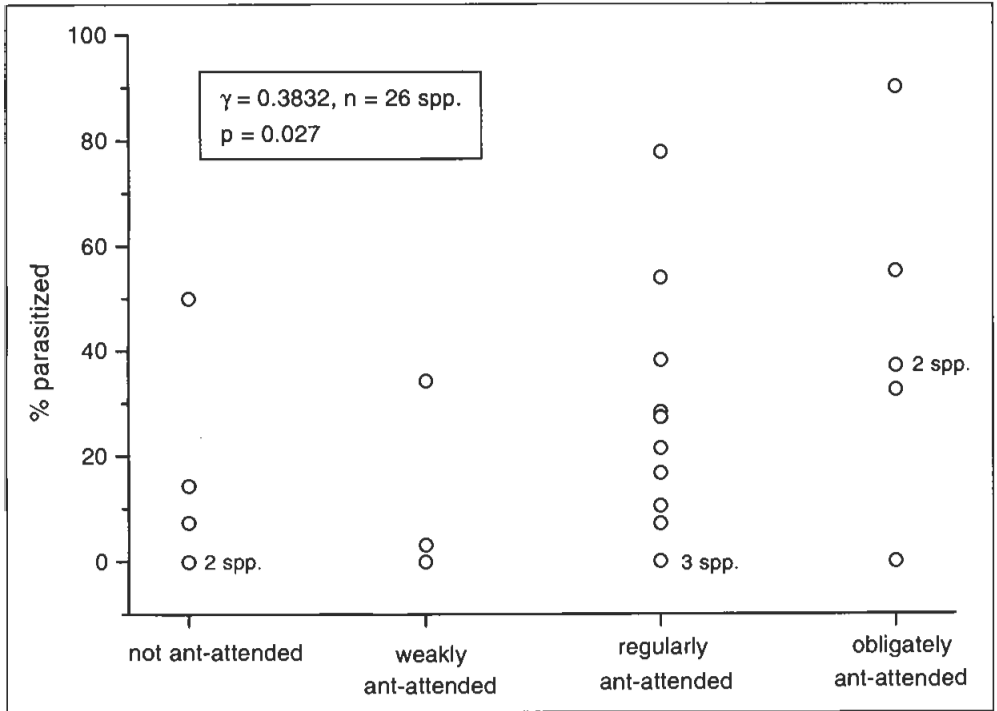


FIG. 1. Relationship between intimacy of ant-association and the incidence of larval parasitism in 26 South-East Asian Lycaenidae species. The gamma rank correlation γ indicates that parasitism increases significantly with intensity of myrmecophily.

(Völkl 1997, see also Fiedler *et al.* 1995b). Therefore, ant-adapted parasitoids can substantially dilute the benefits which otherwise accrue to obligately myrmecophilous lycaenids from their specialization on aggressive and dominant ant partners. Accordingly, unspecific and less intense mutualism with ants is not *per se* an inferior strategy of trophobionts, which is in accordance with the worldwide preponderance of facultative mutualism among lycaenids (Fiedler 1991), homopterans (Bristow 1990), and plants (Fiala & Linsenmair 1995).

One might assume that parasitoids of obligate myrmecophiles are more strongly host-specific than those of facultative myrmecophiles, where the ant guard is less well developed. Available taxonomic evidence, however, does not support this idea. Most of the braconids recorded, which all belonged to the *Apanteles* group (subfamily Microgasterinae), were so far found in one particular lycaenid species only (J. Papp, pers. comm.), irrespective of the intensity

of their hosts' myrmecophily. By contrast, all tachinids reported in Table 1 belonged to a single species, *Aplomya flavisquama* Wulp (H. Shima, pers. comm.), which is evidently neither specialized with regard to its lycaenid hosts nor to the ants which might attend them.

We observed that exposed-feeding larvae were more heavily attacked by parasitoids than their concealed-feeding relatives (mean parasitization rate per species, ± 1 SE; exposed feeders: $29.32 \pm 5.88\%$, $n = 20$; concealed feeders: $9.17 \pm 5.28\%$, $n = 6$). Also on the basis of caterpillar individuals, the advantage of concealed feeding habits was pronounced (exposed feeders: 30.96% parasitized, total $n = 449$; concealed feeders: 13.38% parasitized, total $n = 127$, $\chi^2_{1df} = 8.2$, $P < 0.005$). This might indicate that it is more difficult for parasitoid females to locate their potential hosts when hidden inside plant tissue or shelters (Hawkins *et al.* 1990, Mills 1993), although studies on species-rich leaf-miner parasitoid communities

demonstrate that concealed feeding does not create a strictly "enemy-free space" (Mommott *et al.* 1994).

If compared across species, larval aggregation behavior had no apparent influence on parasitoid infestation (mean parasitization rate per species, ± 1 SE; solitary species: 23.42 ± 7.72 %, $n = 19$; aggregated species: 28.07 ± 10.41 %, $n = 7$). Larval aggregations are common among strongly myrmecophilous lycaenids and may help to reduce the costs associated with maintaining the mutualism (Axén & Pierce 1998). Therefore, our observation that group-living does not inflict another severe cost in terms of parasitism is important for the cost-benefit balance. If analyzed on the individual level, solitary larvae seem to suffer less heavily from parasitism than gregarious ones (aggregated larvae: 33.95 % parasitized, total $n = 215$; solitary larvae: 23.0 % parasitized, total $n = 361$; $\chi^2_{df=1} = 8.2$, $P < 0.005$). However, an analysis of larval groups, rather than individuals, as independent data points would be required to address this question more adequately, and our database does not allow this type of comparison. Hence, the potential role of aggregation as a cost factor in relation to parasitism cannot yet be conclusively addressed.

Life-history traits such as gregariousness, intimacy of ant-association, diet breadth, and feeding habits may often be intercorrelated (e.g., Fiedler 1995, Axén & Pierce 1998), rendering statistical analyses of their effects on rates of parasitism more complicated. A factor analysis, however, indicated that in our data set the four aforementioned variables were reasonably independent of each other.

A more serious general critique, which might be put forward against an analysis such as that presented above, is that we have treated species as independent data points, without accounting for phylogenetic relatedness (Harvey & Pagel 1991). Clearly, life-history traits often show phylogenetic idiosyncrasies (Miller & Wenzel 1995), and this is generally true for the relationships of lycaenid butterflies to ants (Fiedler 1991) and host plants (Fiedler 1995). However, in the sample of 26 South-East Asian species assembled for the present study, life-history variation within genera (*Arhopala*, *Jamides*) or groups of related genera (*Drupadia*, *Cheritra*: Seufert & Fiedler 1996a) is not smaller than between distantly related taxa, and the same applies to variation in rates of parasitism. Furthermore, when we collapsed all pairs of closely related species, for which the life-history characters mentioned in Table 1 were identical, into operational taxonomic units (i.e., *Arhopala amphimuta* + *dajagaka*;

A. metamuta + *muta*; *Caleta roxus* + *manovus*), all patterns concerning parasitism in relation to myrmecophily or feeding habits persisted. Hence, the patterns emerging from our comparative analysis are robust with regard to phylogenetic effects. Nevertheless, as more precise phylogenetic information becomes available, it will be interesting to re-examine the interrelationships between the incidence of parasitism and other life-history traits more rigorously.

As it stands, the straightforward idea that more intimate associations with ants generally provide stronger protection from parasitoids does not receive support for a relatively large set of lycaenid butterfly species from one of the most diverse biotas in the world. Furthermore, our data on a broad range of lycaenids suggest that the role of parasitoids in the evolutionary ecology of butterfly-ant interactions may have been overemphasized. As pointed out by DeVries (1991) for the facultatively myrmecophilous Neotropical rioidid *Thisbe irenea*, generalist predators such as polistine wasps may account for a significant fraction of caterpillar mortality in tropical rainforests. In the South-East Asian obligate myrmecophile *Drupadia theda*, we likewise observed that flying predators such as polistine wasps can play a major role (Seufert & Fiedler 1996a, Seufert 1997). Hence, a better understanding of the selective forces acting on lycaenid-ant interactions should involve both a more complete assessment of mortality costs due to predators and parasites, and a broader coverage of the diversity of lycaenid butterflies.

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REFERENCES

- Axén, A.H., & N.E. Pierce. 1998. Aggregation as a cost-reducing strategy for lycaenid larvae. *Behav. Ecol.* 8: 109–115.
- Baumgarten, H.-T., & K. Fiedler. 1998. Parasitoids of lycaenid butterfly caterpillars: different patterns in resource use and their impact on the hosts' symbiosis with ants. *Zool. Anz.* 236: 167–180.
- Bristow, C.M. 1990. Why are so few aphids ant-tended? Pp. 104–119 in Huxley, C.R., & D.F. Cutler (eds.). *Ant-plant interactions*. Oxford.
- DeVries, P.J. 1991. Mutualism between *Thise irenea* butterflies and ants, and the role of ant ecology in the evolution of larval-ant associations. *Biol. J. Linn. Soc.* 43: 179–195.
- Fiala, B., & K.E. Linsenmair. 1995. Distribution and abundance of plants with extrafloral nectaries in the woody flora of a lowland primary forest in Malaysia. *Biodiv. Conserv.* 4: 165–182.
- Fiedler, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). *Bonner Zool. Monogr.* 31: 1–210.
- Fiedler, K. 1995. Lycaenid butterflies and plants: is myrmecophily associated with particular hostplant preferences? *Ethol. Ecol. Evol.* 7: 107–132.
- Fiedler, K., Seufert, P., Maschwitz, U., & I. Azarac. 1995a. Notes on larval biology and pupal morphology of Malaysian *Curetis* butterflies (Lepidoptera: Lycaenidae). *Tyô to Ga* 45: 287–299.
- Fiedler, K., Seufert, P., Pierce, N.E., Pearson, J.G., & H.-T. Baumgarten. 1995b. Exploitation of lycaenid-ant mutualisms by braconid parasitoids. *J. Res. Lepid.* 31: 153–168.
- Fiedler, K., Hölldobler, B., & P. Seufert. 1996. Butterflies and ants: the communicative domain. *Experientia* 52: 14–24.
- Fitzgerald, F.O. 1993. Sociality in caterpillars. Pp. 372–403 in Stamp, N.F., & T.M. Casey (eds.). *Caterpillars: ecological and evolutionary constraints on foraging*. New York.
- Gauld, I.D., & K.J. Gaston. 1992. Plant allelochemicals, tritrophic interactions and the anomalous diversity of tropical parasitoids: the "nasty" host hypothesis. *Oikos* 65: 353–357.
- Godfray, H.C.J. 1994. *Parasitoids: behavioural and evolutionary ecology*. Princeton.
- Hawkins, B.A., Askew, R.R., & M.R. Shaw. 1990. Influences of host feeding-niche and foodplant type on generalist and specialist parasitoids. *Ecol. Entomol.* 15: 275–280.
- Harvey, P.H., & M.D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford.
- Memmott, J., Godfray, H.C.J., & I.D. Gauld. 1994. The structure of a tropical host-parasitoid community. *J. Anim. Ecol.* 63: 521–540.
- Miller, J.S., & J.W. Wenzel. 1995. Ecological characters and phylogeny. *Annu. Rev. Entomol.* 40: 389–415.
- Mills, N.J. 1993. Species richness and structure in the parasitoid complexes of tortricoid hosts. *J. Anim. Ecol.* 62: 45–58.
- Nash, D.R. 1989. Cost-benefit analysis of a mutualism between lycaenid butterflies and ants. PhD thesis, Oxford University.
- Peterson, M.A. 1993. The nature of ant attendance and the survival of *Icaricia acmon* (Lycaenidae). *J. Lepid. Soc.* 47: 8–16.
- Pierce, N.E. 1987. The evolution and biogeography of associations between lycaenid butterflies and ants. *Oxford Surv. Evol. Biol.* 4: 89–116.
- Pierce, N.E., & S. Eastal. 1986. The selective advantage of attendant ants for the larvae of a lycaenid butterfly, *Glanopsyche hygdamus*. *J. Anim. Ecol.* 55: 451–462.
- Pierce, N.E., Kitching, R.L., Buckley, R.C., Taylor, M.F.J., & K.F. Benbow. 1987. The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evigorns*, and its attendant ants. *Behav. Ecol. Sociobiol.* 21: 237–248.
- Seufert, P. 1997. Bläulinge auf *Saraca thaipingensis* – eine Fallstudie zu diversitätserhaltenden Faktoren im Tropischen Regenwald. PhD thesis, University of Würzburg.
- Seufert, P., & K. Fiedler. 1996a. Life-history diversity and local co-existence of three closely related lycaenid butterflies (Lepidoptera: Lycaenidae). *Zool. Anz.* 234: 229–239.
- Seufert, P., & K. Fiedler. 1996b. The influence of ants on patterns of colonization and establishment within a set of coexisting lycaenid butterflies in a South-East Asian tropical rain forest. *Oecologia* 106: 127–136.
- StatSoft 1995. STATISTICA 5.1. Vol. I. Tulsa, Oklahoma.
- Thomas, J.A., & G.W. Elmes. 1993. Specialized searching and the hostile use of allomonas by a parasitoid whose host, the butterfly *Maculinea rebeli*, inhabits ant nests. *Anim. Behav.* 45: 593–602.
- Völkl, W. 1997. Interactions between ants and aphid parasitoids: patterns and consequences for resource utilization. Pp. 225–240 in Dettner, K., G. Bauer & W. Völkl (eds.). *Vertical food web interactions: evolutionary patterns and driving forces*. *Ecol. Stud.* 130.
- Wagner, D., & L. Kurina. 1997. The influence of ants and water availability on oviposition behaviour and survivorship of a facultatively ant-tended herbivore. *Ecol. Entomol.* 22: 352–360.
- Weseloh, R.M. 1993. Potential effects of parasitoids on the evolution of caterpillar foraging behavior. Pp. 203–223 in Stamp, N.E., & T.M. Casey (eds.). *Caterpillars: ecological and evolutionary constraints on foraging*. New York.

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