Functional significance of the cocoon in two arctic *Gynaephora* moth species

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SUMMARY

Some insects construct cocoons in which pupae complete their metamorphosis to adult form. Despite a variety of proposed benefits, the functional significance of the cocoon remains unclear. Here we experimentally examine the functional significance of the cocoon in two species of moth (*Gynaephora rossii* Curtis and *G. groenlandica* Wöcke, Lepidoptera: Lymantriidae) inhabiting a cold and thermally stressful arctic environment. *G. rossii* larvae spin a single-layered dark cocoon, and *G. groenlandica* spin a double-layered pale cocoon, consisting of an inner dark layer and an outer translucent pale layer. Comparisons of developmental times of pupae in cocoons and naked pupae whose cocoons had been experimentally removed revealed that the pale cocoons of *G. groenlandica* accelerated pupal development substantially. The warming effect of sun was seemingly the principal influence accounting for the enhanced development. In contrast, the presence of a dark cocoon of *G. rossii* did not accelerate development detectably. To generate hypotheses for why these two closely related species might differ in cocoon type and function, we examined ecological correlates associated with each cocoon type. The experimental results for *G. groenlandica* provide clear evidence that cocoons enhance a correlate of fitness in insects.

1. INTRODUCTION

The cocoon is a prominent feature in the life cycle of many holometabolous insects (Borror *et al.* 1976). Its structure varies enormously among taxa, from a simple few threads of silk to an elaborate double-layered silk envelope (Mosher 1969). The allocation of protein to cocoons can be considerable; in the extreme case of the domesticated silkworm (*Bombyx mori*), mature larvae allocate 50% of their total protein to their silk cocoon (Wigglesworth 1972). Presumed functions of the cocoon include crypsis, attachment, protection from parasites, predators, or parasitoids, and enhancing development by improving thermal and/or moisture conditions for the pupa. However, there is surprisingly little empirical support for these functions, and their relative importance is unknown. Solitary tent caterpillars develop more slowly than conspecific social caterpillars in communal silk tents (Knapp & Casey 1986), but it is unclear whether tents are equivalent to cocoons. In general, there is little conclusive experimental evidence for any of the hypothesized functions of cocoons.

In this paper we examine the functional significance of the cocoon in two co-occurring species of arctic moths: *Gynaephora rossii* Curtis and *G. groenlandica* Wöcke (Lepidoptera: Lymantriidae). As arctic and alpine dwelling insects, poikilotherms like *Gynaephora* face harsh and unpredictable developmental conditions (Downes 1964). Reflective of this harsh environment, *Gynaephora* have one of the longest egg to adult developmental periods known for any insect (estimated as 14 years, Kukal & Kevan 1987). The slow growth rate and short growing season may present a developmental challenge, particularly given that larvae must pupate, emerge as adults, and reproduce all within one season (Kukal & Kevan 1987; D. Morewood, personal communication). *Gynaephora* larvae adopt a variety of behavioural mechanisms to increase their body temperature above ambient (Kukal *et al.* 1988), mechanisms that presumably enhance development. One such mechanism is the use of a cocoon, which in this genus is a tightly woven, enveloping silk structure. In one of the species, the cocoon has been shown to act as a 'micro-greenhouse' which increases the temperature of the developing pupa within (Kevan *et al.* 1982). However, the developmental consequences of increased temperature were not examined, nor is it known if the cocoon of the other species also acts in this manner.

The two species of *Gynaephora* construct cocoons that differ strikingly in both structure and colour. *G. rossii* spin a single-layered, dark grey cocoon (hereafter 'dark'), consisting of a single layer of caterpillar pile woven together with silk. *G. groenlandica* spin larger cocoons (hereafter 'pale'), consisting of two distinct layers, separated by a thin layer of air. An inner darker layer is composed of caterpillar pile woven together with silk, and is very similar, if not identical, to the dark cocoons. Enclosing this inner layer is a semi-translucent, whitish-coloured layer composed mainly of silk. Thus, the main difference between the two types of cocoons is that the dark cocoons lack the outer, translucent layer. Intriguingly, both cocoons are found

in similar habitats where they co-occur in the arctic, although one species (*rossii*) is mostly alpine, and the other exclusively arctic (Ferguson 1978; D. Lafontaine, personal communication). Here, we report the results of cocoon removal experiments done to determine whether either cocoon type provides measurable developmental benefits, and if so, what physical factors are responsible.

2. METHODS

(*a*) *Study area and animal*

We studied *G. groenlandica* and *G. rossii* from mid-June to early August, 1984 on Jenny Lind Island, N.W.T. Canada (68°43′ N, 102°47′ W), a flat, low-lying island in Queen Maud Gulf that shares both low and high arctic fauna (Parmelee *et al.* 1967). *Gynaephora* caterpillars and pupae of both types were abundant in areas of marshy tundra and adjacent upland *Dryas*-lichen tundra from late June onward. The two cocoon types have previously been erroneously reported as a polymorphism in both species (Ryan & Hergert 1977), but reference collections matching adults to their specific cocoons in the Canadian National Collection of Insects, Ottawa, indicate that the differences in cocoon morph are differences between species, not polymorphisms within each species (D. Lafontaine, personal communication). Moreover, this conclusion is supported by detailed field studies (W. D. Morewood, personal communication).

Previous work on *Gynaephora* cocoons merits taxonomic comment. Kevan *et al.* (1982) report results of a study of cocoons in *G. rossii*, but their figure 1 clearly illustrates a *groenlandica* individual. Given this, and the fact that their study area is a region where *groenlandica* is expected to be far more common *rossii* (Ferguson 1978) and where all other studies have focused on *groenlandica* (Kukal & Kevan 1987; Kukal *et al.* 1988), we conclude that their study involved *groenlandica*, not *rossii*.

(*b*) *Experiment to assess whether cocoons accelerate pupal development*

To determine if the presence of a cocoon accelerates pupal development, we removed pupae from their cocoons and compared the timing of adult eclosion of naked pupae and of control pupae with cocoons intact. Pupae were collected on the tundra between 18 and 22 June, and set out in two stages, on 21 June and 23 June. Based on patterns of snow melt, we estimate that most larvae could not have pupated earlier than one week before collection. We affixed the cocoons or pupae to a sparsely vegetated region of lichen-dominated tundra using small wire hoops, alternating species and treatments. The entire assemblage was covered with standard poultry mesh to protect the pupae from birds. We set out 20 naked and 20 control pupae for each species, totalling 80 pupae. We then checked the pupae, normally every 1–2 d, to determine when each pupa eclosed. The experiment was terminated on 1 August. Pupation and eclosion invariably occur in the same season (Kevan *et al.* 1982; Kukal *et al.* 1987). However, the sample size of adults that eclosed was less than the number of pupae set out because: (i) some larvae in cocoons were later found to be parasitized; (ii) some pupae died during development; and (iii) some possibly viable pupae failed to eclose as adults by the end of the experiment on 1 August. We included the 'leftover', potentially viable pupae ((iii) above) in the analyses, and assigned them an eclosion date of 2 August. However, when we repeated analyses without these leftover pupae the results were unchanged. We could detect no sexual differences in the timing of eclosion for either species, either in separate analyses for each treatment, or for treatments pooled within species. To increase statistical power, we therefore pooled sexes within each treatment when comparing eclosion times.

(*c*) *Experiment to assess the importance of sun*

To determine whether any effects we observed in the first experiment were caused by exposure to sun per se, as opposed to other ambient factors like wind, humidity, and temperature, we set out a second series of pupae, both naked pupae and pupae in cocoons as above, but covered the entire assemblage under a piece of plywood placed 10 cm above the ground. This cover completely blocked the sun, but the setup was open enough to allow ventilation. We cannot rule out the possibility that the experimental setup reduced exposure to wind to some degree, or altered humidity. Note, however, that reducing wind exposure would have reduced heat loss through convection, a factor that would make our experiment conservative for examining the role of the sun. Sample sizes were initially 20 pupae in cocoons and ten naked pupae for each species but a Lapland longspur (*Calcarius lapponicus*) reduced the sample size to six naked pupae of each species. As above, we checked this second experiment on a regular basis to determine when pupae eclosed. To distinguish between the first experiment where the cocoons and pupae were exposed to the elements, and the second experiment where they were shaded from direct sunlight, we refer to the first experiment as 'exposed' and the second as 'covered'.

(*d*) *Census and statistics*

To assess the rate of avian predation on pupae in cocoons of each species, we walked transects across the tundra and noted the number of intact versus empty cocoons that had been torn open by birds. We conducted censuses on June 18 (*groenlandica* only) and 2 July (both species). On the 2 July census, we also assessed the orientation of intact cocoons on the tundra. Because we could not distinguish posterior from anterior ends, we scored the orientation of the long axis of each cocoon into four directional categories; NW, N, NE, and E. We then used Rayliegh tests (Batschelet 1981) to determine whether the representation of cocoons in these four directional categories deviated from random. We also collected 143 pupae (122 *rossii*, 21 *groenlandica*) to obtain body mass measurements, and weighed pupae to the nearest 0.1 mg after oven-drying them for 24 h at approximately 50 °C. We sexed these pupae, and pupae in the exposed experiment, according to the degree of antennal bulge on the pupal case, with males having larger bulges. This difference is due to sexual differences in antenna size in adult moths: for example, in *rossii* adults, antennae widths were an order of magnitude larger for males (2 ± 0.13 mm, $n = 10$) than females (0.2 ± 0.01 mm, $n = 10$; measurements taken from specimens in the Canadian National Collection of Insects).

Measurements are reported as means and standard errors, or medians and 25th and 75th percentiles. We used one-tailed statistical tests when comparing eclosion dates for naked versus cocooned pupae in the exposed experiment because we specifically tested whether cocoons accelerate development. For the comparison between experiments, one-tailed tests were used for all directional predictions.

3. RESULTS

In the exposed experiment, cocoons enhanced the rate of development in *groenlandica*, but not in *rossii* (figure 1). Adult *groenlandica* eclosed from pupae in cocoons significantly earlier than from naked pupae

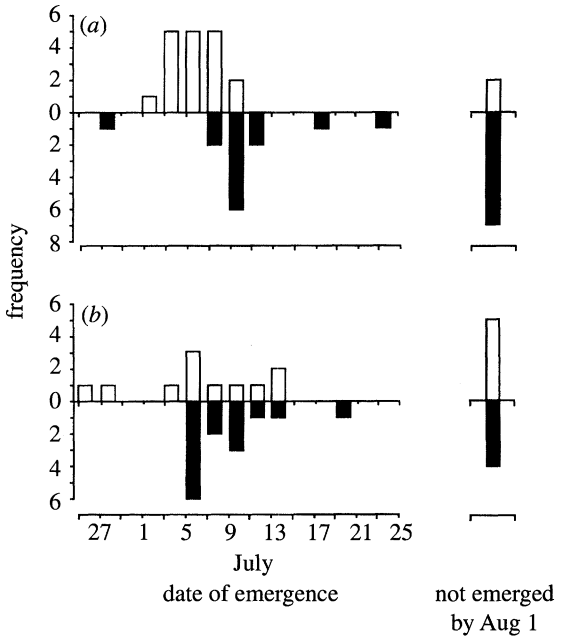

Figure 1. Results from the exposed cocoon removal experiment, contrasting the dates that adults emerged from pupae inside cocoons and from naked pupae lacking cocoons. Dates of emergence are shown separately for: (*a*) pale cocoons (*G. groenlandica*); and (*b*) dark cocoons (*G. rossi*). Pupae remaining at the end of the experiment were classified as 'not emerged by August 1'. Key to figure: shaded = naked pupae; unshaded = in cocoon.

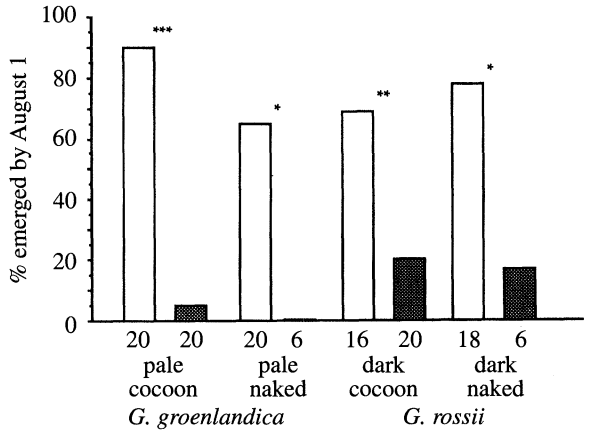

Figure 2. A comparison of the percentage of pupae in the exposed experiment (in sun) and the covered experiment that emerged as adults by 1 August. Separate comparisons are made for each treatment category within experiment. Probabilities for Chi-square tests of independence; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Key to figure: shaded = covered; unshaded = exposed (in sun).

(figure 1*a*; median date of eclosion for cocoons was 6 July (4–8 July), for naked pupae 12 July (10 July–1 August); Mann-Whitney *U* test, $z = 3.65$, one-tailed $p < 0.001$). Based on mean date of eclosion, the presence of a cocoon accelerated development (at least the portion of development spent in the experiment) by 10 d (mean date of eclosion 8 July for cocoon versus 18 July for pupae). Note that this difference is a minimum estimate because most leftover pupae would not have eclosed on the arbitrary date we assigned them (2 August) and more naked pupae were leftover than

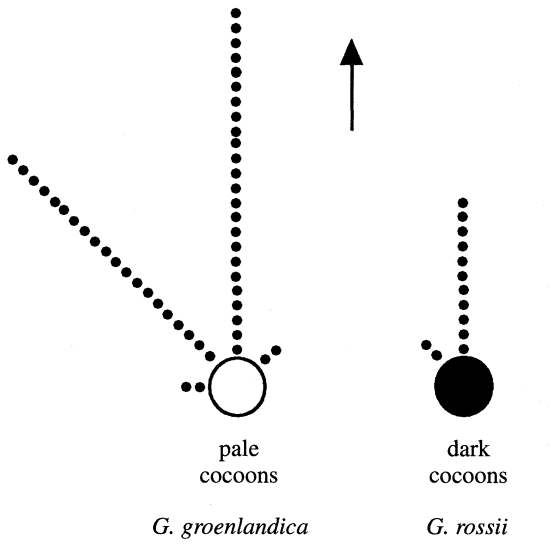

Figure 3. Orientation of the long axis of pale and dark cocoons on the tundra. Orientation classified into four directional categories; west, northwest, north and northeast. Each dot represents one cocoon.

cocoons (figure 1*a*; seven pupae versus two cocoons). In contrast to *groenlandica*, adult *rossii* did not eclose significantly earlier from pupae in cocoons than from their naked counterparts (figure 1*b*; median date of eclosion for cocoons was 11 July (6 July–1 August), for naked pupae 10 July (6–20 July); Mann-Whitney *U* test, $z = 0.18$, one-tailed $p = 0.57$).

Pupae developed more slowly in the covered experiment than in the exposed experiment (figure 2). Only six adults eclosed from pupae in the covered experiment. This small number precludes comparing eclosion dates between the two experiments, so we compared the frequency of eclosed versus non-eclosed pupae in each treatment category. In all four comparisons, a significantly higher proportion of adults eclosed from pupae in the exposed experiment than in the covered experiment (figure 2). These observations suggest that the accelerated development observed for pale cocoons in the exposed experiment was caused, in part, by the effects of exposure to the sun, and that cocoons function as 'micro-greenhouses'.

We examined three ecological factors that might influence the costs and benefits of producing, or developing within, a cocoon, and assessed whether any of these differed between the two species. The orientation of cocoons on the tundra was non-random for both species (figure 3; *groenlandica* $z = 11.3$, $p < 0.01$; *rossii* $z = 27.49$, $p < 0.001$). Most of the cocoons in both species were oriented either in a N or a NW axis (100% of darks, 92% of pales). In addition, there was no difference between species in the proportion of cocoons oriented in a N or NW axis, versus a NE or E axis ($\chi 2 = 0.20$, $p = 0.66$). However, the predation rate on cocoons differed between the species, with the conspicuous pale cocoons of *groenlandica* suffering higher predation (33% of 55 cocoons preyed on) than the more cryptic grey cocoons of *rossii* (2% of 52 cocoons preyed on; $\chi 2 = 15.32$, $p < 0.001$). Finally, the species differed in body size, with *groenlandica* having heavier pupae than *rossii*. In both species, female pupae were

considerably larger than males (two-way ANOVA; species effect $F1,139 = 38.91$, $p < 0.0001$; sex effect $F1,139 = 210.41$, $p < 0.0001$; male *groenlandica* 0.13 ± 0.003 g; female *groenlandica* 0.23 ± 0.017 g; male *rossii* 0.11 ± 0.002 g; female *rossii* 0.18 ± 0.003 g).

4. DISCUSSION

Our experiments provide clear evidence that the pale, bi-layer cocoon of *groenlandica* accelerates development of the pupa within, but that the same result does not obtain for the dark, single-layer cocoon of *rossii*. Comparing the two experiments also suggests that this accelerated development for pale cocoons is caused, at least in part, by the direct effect of solar radiation, rather than the effects of ambient temperature, humidity, and wind alone (see below). These results strengthen previous studies reporting that larval *Gynaephora* are cold-stressed, and adopt several behavioural mechanisms to increase their body temperatures above ambient temperature, presumably to accelerate their rate of development (Kevan *et al.* 1982; Kukal *et al.* 1988). The pale cocoon serves a thermoregulatory function by increasing body temperature of developing pupae (Kevan *et al.* 1982; misidentified as *rossii*). Moreover, the non-random orientation of cocoons on the tundra (Kevan *et al.* 1982 for *groenlandica*; this study for both species) may minimize convective heat loss, although we have no data on prevailing wind direction to test this hypothesis.

It could be argued that the comparison of the two experiments indicates not that sun is important to the function of pale cocoons, but merely that the temperature of the surrounding substrate is important. By blocking sunlight, the covered experiment would not only have blocked radiation contacting the pupae or cocoons directly, but it may have also lowered the substrate temperature relative to that under the exposed experiment. However, reanalysis of data in Kevan *et al.* (1982, their table 5) using partial correlation analysis to hold substrate or air temperatures constant, indicates that air temperature affects the temperature inside cocoons, whereas substrate temperature does not (one-tailed tests; holding substrate temperature constant, partial $r = 0.52$, 8 d.f., $0.05 < p < 0.1$; holding air temperature constant, partial $r = -0.18$, 8 d.f., $p > 0.1$). We assume that the covered treatment was ventilated enough that it should not have appreciably reduced ambient temperature. Air temperature alone, then, is unlikely to account for the striking difference between the covered and exposed experiments.

An alternative explanation is that we inadvertently altered humidity or wind, and that these factors, not sunlight-induced temperatures, explain the differences between the covered and exposed experiments. We cannot rule this possibility out, but results from other studies strongly suggest that temperature is likely to be the most important factor. For example, our results for *groenlandica* pupae provide a logical link between the temperature-enhancing properties of cocoons (unrelated to wind or humidity) demonstrated by Kevan *et al.* (1982) and the well-known relation between ambient temperature and developmental rate (Bursell 1974). By coupling the enhanced temperature and enhanced development effects, our experiments demonstrate for the first time a direct fitness advantage conferred by the presence of cocoon.

Our findings thus support the notion that the translucent pale cocoons function as micro-greenhouses (Kevan *et al.* 1982); sunlight is able to penetrate the outer layer and heat the dark, inner layer, where heat is trapped and protected from the cooling effects of wind. In contrast, the opaque dark cocoon would trap the sun's heat on the cocoon's outer surface, where it could easily be removed by wind. Given this interaction between wind, sun and pupal colour, *rossii* could compensate for wind by pupating in more sheltered locations on the tundra, further within the boundary layer of the ground, where they would be less exposed to wind. Although we have no data to address this question, we did note a tendency for dark cocoons to be found in small depressions on the tundra, rather than near the tops of hummocks. This logic also illustrates a potential weakness in our experiment for assessing the developmental benefits of a dark cocoon, if the species do differ in habitat selection. By placing pupae on the exposed tundra, our experimental setup may have increased exposure to wind above levels typically found at pupation sites chosen by *rossii*. The dark cocoons may actually enhance development when tucked within the boundary layer in the appropriate microhabitat for *rossii*, and enhance it to the extent that it does not pay *rossii* to spin a conspicuous pale cocoon that incurs a high predation risk. The geographic ranges of these species are also consistent with this microhabitat explanation, with *groenlandica* more common in northern (more exposed) habitats, and *rossii* more common in southern alpine (more vegetated) habitats. Moreover, *G. rossii* is smaller-bodied than *groenlandica*, suggesting that the species' difference in cocoon type could also reflect tradeoffs between size-dependent costs or benefits of spinning each cocoon type. Examining differences between the species in habitat choice, and its concomitant developmental consequences, across a broad geographic range, would clearly be worthwhile.

A pale cocoon accelerates development by an average of 10 d. This may be an underestimate because we collected cocoons part way through their development, at which point all pupae had already enjoyed some benefits from the presence of a cocoon. We do not know the amount of development time that had elapsed in these cocoons before collection. However, given that pupal development takes an average of 15 d on Ellesmere Island (W. D. Morewood & P. Lange, personal communication) and that pupae in pale cocoons emerged an average of 17 d after the experiment was started, we probably collected the experimental cocoons within a few of days of pupation. Thus, our estimate of the time saved by developing within a pale cocoon is likely a reasonable one.

Reducing development by 10 d in the short arctic growing season is likely to provide an enormous selective advantage, and we can suggest several possible benefits. Accelerated development would increase the

likelihood that adults will emerge, lay eggs, and that the eggs hatch and first instar larvae successfully molt to second instar (the stage in which the first winter is spent, Kukal & Kevan (1987)). Second, males might benefit from accelerated emergence because it would increase the number of females they would have access to for matings (see, for example, Wiklund & Fagerström 1977). Finally, mortality factors like predation and parasitism favour accelerated growth and development through risky life stages (Sibly *et al.* 1985). Pupae in pale cocoons are vulnerable to avian predation (Kukal & Kevan 1987; 33% in the present study) and a shorter pupation period would reduce the amount of time exposed to this risk (assuming that presence of cocoon does not increase the daily rate of predation compared to its absence, an assumption that is unlikely to hold in the case of pale cocoons).

Our experiments clearly demonstrate the developmental benefits associated with a complex, bi-layered cocoon in a cold environment. Microclimate-enhancing structures like bi-layered cocoons should be of selective advantage whenever the timing of pupation faces severe seasonal constraints such as thermal stress, short season, or stage-dependent predation and/or parasitism. For example, adult females in a bivoltine Megachilid bee produce single-layered cocoons in their first generation, and double-layered cocoons in their second generation (Mello & Garófalo 1986). Only first generation broods are tended by the female, suggesting that the thermal benefits of a bi-layered cocoon may compensate for the lack of parental care. Using an experimental approach, similar to the one we have employed here, to examine the variety of cocoons in other holometabolous insect taxa, seems promising. It would be particularly interesting to combine this experimental approach with a comparative analysis of variation in cocoon types among relatives of *Gynaephora* in the family Lymantriidae, a group that is widespread across a diversity of habitats from the arctic to the tropics.

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