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Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars

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Summary

1. Three hypotheses have been proposed to account for the maintenance of aggregation behaviour in caterpillars. While the benefits of aggregation for defence and for the elevation of growth rates have been investigated in some species, both of these hypotheses have been criticized, and in many cases the alternative hypothesis regarding the benefits of physiological regulation has not been investigated.

2. In this paper the field thermal biology and water relations of solitary caterpillars (instars IV–V) and aggregations of caterpillars (instars I–III) are examined in *Imbrasia belina* (Westwood) (Lepidoptera: Saturniidae), a species where an instar-related change in aggregation behaviour is obligatory (instars I–III always aggregate, instars IV–V are always solitary), and where the benefits of defence and of overwhelming inducible host plant defences are unlikely to be significant.

3. Aggregations of instars II–III maintain body temperatures equivalent to those of larger solitary individuals of instars IV and V, but significantly higher than those of operative models of single caterpillars of instars II–III. In addition, behavioural thermoregulation is not used to elevate body temperatures, although hanging behaviour takes place when upper lethal temperature limits are approached. Nonetheless, solar radiation has a marked influence on the temperature of aggregations because of the accumulation of higher heat loads by the larger masses of large aggregations.

4. Instars IV–V can survive higher temperatures than instars I–III, and this may be because of their accumulation of very high heat loads in the field. Instars I and III can avoid these high heat loads by partial dispersal from the aggregation. In contrast, instars I–III have significantly lower critical thermal minima than the later instars, which may allow larvae to regain aggregations if they are separated from them.

5. Water loss rates of real aggregations, of a given size, within a particular instar are lower than the sum of the rates of the same number of individual caterpillars of the same instar. In addition, survival of water loss tends to be higher in individuals from aggregations than in single individuals.

6. Using this evidence in conjunction with studies that demonstrate significant predation in all instars, aggregation in the early instars irrespective of whether outbreaks take place or not, and the absence of inducible defences in the major host (*Colophospermum mopane*) of *I. belina*, it is concluded that physiological regulation, of temperature and water balance, is the most likely explanation for the maintenance of aggregation behaviour in this species.

Key-words: Body size, gregariousness, thermoregulation, upper lethal limits, water balance

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Introduction

Gregariousness in caterpillars is thought to have two major benefits. First, Hamilton (1971) argued that individual animals are most likely to avoid predation when living in groups. This idea was subsequently elaborated to include caterpillar aggregations (see Sillén-Tullberg 1988, 1990), and a number of authors have provided support for this ‘defence hypothesis’ (e.g. Cornell, Stamp & Bowers 1987; Stamp & Bowers 1988; Lawrence 1990; Sillén-Tullberg 1990). The second advantage is associated with feeding (‘host plant hypothesis’). Various studies have shown that gregariousness enhances the foraging efficiency of caterpillars (Fitzgerald 1993), and that synchronous feeding in groups leads to elevated growth rates (Tsubaki & Shiotsu 1982; Lawrence 1990; Clark & Faeth 1997; Denno & Benrey 1997). The latter is in turn thought to be the consequence either of the induction of a nutrient sink in the plant, the overwhelming of an induced allelochemical response (Denno & Benrey 1997), or of cooperatively overcoming plant structural defences (Dussourd 1993; Fitzgerald 1993; Clark & Faeth 1997).

Although these advantages are not thought to represent the primary causes of aggregation (see Stamp 1980; Courtney 1984 for discussion of the benefits of egg clustering and other behaviours that lead to aggregation), they are thought to be important in maintaining it (Fitzgerald 1993). However, Cappucino, Damman & Dubuc (1995) recently came to the conclusion that there is no clear evidence linking feeding groups either with an ability to circumvent or overcome host defences, or with the avoidance of enemies. Hence, it must either be presumed that the benefits of egg-clustering alone are responsible for both the origin and the maintenance of aggregation, or that other advantages accrue to aggregating species.

The third set of such advantages are those associated with thermoregulation and water balance (the 'regulation hypothesis'). A variety of studies have demonstrated that aggregations of caterpillars can maintain higher, and more stable, body temperatures over longer time periods than can their solitary counterparts (Casey *et al*. 1988; Joos *et al*. 1988; Casey 1993; Fitzgerald 1993). In turn, these higher body temperatures lead to higher growth rates (Scriber & Lederhouse 1983; Reynolds & Nottingham 1985; Knapp & Casey 1986) which hold advantages for caterpillars, including the reduction of parasitism and predation, and the temporal avoidance of poor quality plant material (Casey *et al*. 1988; Stamp & Bowers 1990; Fitzgerald 1993; Benrey & Denno 1997). Furthermore, Willmer (1980) and Clark & Faeth (1997) demonstrated that caterpillar aggregations facilitate water conservation, and this has been shown in a number of other insect species (Yoder, Denlinger & Wolda 1992; Yoder & Grojean 1997; Rasa 1997).

Nonetheless, few studies have examined the regulation hypothesis in a single species, which shows instar, or body size-associated, changes in behaviour, and compared the outcome of such an investigation with the other hypotheses proposed as proximal explanations for aggregation in caterpillars. In this regard, the anomalous Emperor Moth (*Imbrasia belina* (Westwood) (Lepidoptera: Saturniidae)) is of interest. Caterpillars in its first three instars are highly gregarious, and this appears to be obligatory (Oberprieler 1991, 1995), while those in instars IV and V are solitary. *Imbrasia belina* is highly polyphagous, successfully completing its development on 33 host plant species in eight plant families (Oberprieler 1986, 1991, 1995; R. G. Oberprieler, personal communication), of which three species are recent additions to its diet, making the likelihood of specific adaptations to particular hosts (induction of nutrient sinks, overwhelming of allelochemical defences) unlikely. In addition, the larvae of all instars of this species are preyed on by a wide range of vertebrates and invertebrates, both when living in aggregations and as solitary caterpillars, casting some doubt on the utility of aggregation as a means to reduce predation (Gaston, Chown & Styles 1997; J. Bell, M. A. McGeoch & K. J. Gaston, personal communication). Thus if there are any advantages to aggregation in this species (beyond those associated with fecundity and oviposition strategies), they are most likely to be associated with differences in the thermal biology and water relations of solitary caterpillars and aggregations. Hence, in this paper we compare the thermal biology and water relations of both single and gregarious caterpillars of the first three instars with those of solitary caterpillars in the last two instars of the anomalous Emperor Moth.

Materials and methods

STUDY SITE AND ANIMALS

Field work was done at Phalaborwa game ranger camp in the Kruger National Park (23°58'S, 31°10'E) from February to March 1996, and continued in the Hans Merensky Nature Reserve (23°40'S, 30°37'E) from November 1996 to March 1997. Both localities lie in the Mopane Bushveld vegetation type in the Savanna biome of South Africa (Low & Rebelo 1996). They are characterized by relatively low annual rainfall (250–500 mm) and frost-free, high annual temperatures (1·5–42·5 °C with a mean of 22 °C). The vegetation is dominated by Mopane trees and shrubs, *Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léon (Fabaceae), in association with several other tree and herbaceous plant species (see Low & Rebelo 1996).

Imbrasia belina is widely distributed in southern Africa. The moths do not feed and are night flying. Eggs are laid in clusters of 50–200 around twigs or on the leaves of their host plants, and hatch after ≈ 10 days. There are five larval instars. From instars I to III the caterpillars are strictly gregarious and will forage together in aggregations of 20–200 individuals (Oberprieler 1991, 1995), but do not display complex social and cooperative behaviour to the extent found in other caterpillars (see Casey & Knapp 1987; Casey *et al*. 1988; Joos *et al*. 1988; Fitzgerald 1993). Nonetheless, when separated from an aggregation caterpillars cease feeding and search continually for the original or another aggregation (Oberprieler 1991, 1995). After moulting into instar IV caterpillars disperse immediately to become solitary. The larval stage lasts approximately 6 weeks during which the caterpillars undergo a 4000-fold increase in mass, from ≈ 0·0037 to 16 g (Gaston *et al*. 1997; C. V. Styles, personal communication). At the end of the larval stage the fifth instar caterpillars burrow into the soil where they pupate. Across most of its distribution, the species is bivoltine with the first generation in November to December and the second in February to March; only in more arid areas is it univoltine (Oberprieler 1991, 1995).

FIELD THERMAL BIOLOGY

In February/March 1996, the relationship between body temperatures (T_b) of caterpillars and ambient temperatures (T_a) in the field was determined by inserting a 40GG copper–constantan thermocouple, mounted inside a 30GG hypodermic needle and connected to a KaneMay 457XP digital thermometer (KaneMay, UK), into the body core of the caterpillars. At 2-h intervals (from dawn to dusk) temperature measurements were made of 10 individuals of each instar (for instars II to V, small body size excluded instar I) as they became available in the field. Temperature readings were taken within 2 s of insertion of the thermocouple. Directly after this, ambient temperature was measured with a dried thermocouple within 2 cm of the caterpillar. Temperature measurements of instars II and III were taken from caterpillars in aggregations. Caterpillars did not suffer any visible deleterious effects from this invasive technique and individuals of the larger instars continued feeding during the procedure. The behaviour of the caterpillars during the day was also observed to determine whether it varied with changing T_as and the corresponding T_b. Field temperature work could not be conducted at night due to the presence of large vertebrate predators.

From the T_b and T_a measurements, temperature excesses for each caterpillar were calculated ($T_{ex} = T_b - T_a$). Least squares linear regression analyses of T_b on T_a were undertaken to determine whether caterpillars of instars II to V exhibited any form of physiological thermoregulation. The slopes of the lines were tested for differences from each other and for differences from unity (Casey 1988). To determine if caterpillars of instars II to V experienced different T_bs and T_as, measurements of each instar were pooled across all time intervals and compared using single classification analyses of variance (ANOVA).

Over the same period, temperatures of Mopane tree microsites most often frequented by the caterpillars, and wet and dry bulb temperatures inside a miniature Stevenson screen at ground level, were determined using 40GG copper–constantan thermocouples. Two operative thermometers, prepared from dried, chemically preserved caterpillars, with 40GG thermocouples imbedded in their body cores, were placed in natural positions (exposed to both sun and shade at different times of the day) in the tree to determine operative temperatures (T_e) experienced by single caterpillars in the field (Bakken 1992; Walsberg & Wolf 1996). Single preserved specimens for T_e measurements were chosen to represent the instars present in the field at the particular dates. The above measurements were made for 3 days during the presence of instars II and III and for 2 days during the presence of instars IV and V, and for comparisons of T_e and T_b, data from this period were used. However, operative temperatures were also measured during November/December 1996 and February/March 1997. The operative thermometers, of single caterpillars of instars II to V and of a single aggregation each of instars II and III (each in turn comprising 50 caterpillars), prepared from dried, chemically preserved caterpillars, and positioned in natural postures along with a thermocouple measuring T_a, were deployed in the field for 12 days during each generation. The use of models of single caterpillars of the early instars were especially necessary because solitary caterpillars of instars I–III are rarely seen in the field, hence measurements of T_b for solitary instar I–III caterpillars cannot be made. During all three periods wind speed (in m s^{-1}, RM Young wind monitor, RM Young, USA), solar radiation (in W m^{-2}, LiCor silicon pyranometer, LiCor, USA), and saturation deficit (in kPa, non-aspirated psychrometer) were also measured whenever T_es were determined. All sensors were connected to a Campbell Scientific CR10 data logger (Campbell Scientific, USA) programmed to read every second, and to store the averaged values every 10 min.

Single classification ANOVAs were used to compare T_es (single caterpillars) to T_bs measured simultaneously, every 2 h, in live caterpillars during the February/March 1996 generation, and to compare T_es recorded continuously from sunrise to sunset during the same period. The relationship between solar radiation and ambient temperature, and T_{ex} of live caterpillars was determined using multiple regression using data from February/March 1996.

TEMPERATURE TOLERANCE

For determining critical thermal minima (CT$_{min}$) and maxima (CT$_{max}$) the methods of Klok & Chown (1997) were used. For each instar 11 specimens ($n = 15$ for instars IV and V) were placed in a glass beaker. A 40 GG copper–constantan thermocouple was inserted into the body core of one caterpillar to measure body temperature (T_b) during the experiment. It was assumed that the T_b of this operative thermometer adequately represented the T_bs of the 10 experimental caterpillars. The beaker was almost completely submerged in a Grant LTD6 water bath (0·1 °C accuracy) connected to a PZ1 programmable temperature controller (Grant Instruments, UK). For CT$_{min}$, the temperature was decreased at 0·5 °C min^{-1} to 5 °C. The caterpillars were then equilibrated for 15 min after which temperature was increased at the same rate. The temperature at which caterpillars regained coordinated locomotor function was recorded as the CT_{min}. For CT_{max} the caterpillars were equilibrated for 15 min at 35 °C after which the temperature was increased at 0·5 °C min^{-1}. The temperature at which a caterpillar lost coordinated locomotor function was noted as the CT_{max} for that individual.

To determine upper thermal tolerance, 20 caterpillars of instars I to III were placed in each of 13 glass vials on Mopane twigs, and submerged in the Grant LTD6 water bath. Caterpillars were equilibrated for 15 min at 34 °C after which the temperature was increased at 0·1 °C min^{-1} until the first temperature, 35 °C, was reached. After 1 h, one vial was removed from the bath and the specimens were given 24 h to recover at ambient temperature. The temperature was then increased 1 °C at 0·1 °C min^{-1}, and the above procedure repeated (Klok & Chown 1997). This was continued to 48 °C. After 24 h, caterpillars were assessed for survival and only those with full locomotor function (i.e. capable of coordinated movement) were considered survivors. Because of their size, caterpillars of instars IV and V were placed in 375-ml jars in groups of 10. Only two jars could be accommodated within the water bath. Thus each group of 10 was exposed to two temperatures (e.g. 39 and 40 °C) before it was removed after 1 h at the higher temperature. When one jar was removed it was immediately replaced with another containing 10 caterpillars. The procedure was repeated to obtain $n = 20$ for each temperature as with the first three instars. After 24 h, all caterpillars were checked for survival and those with full motor function, i.e. able to climb out of their containers, were considered survivors.

To examine short-term survival of a constant high temperature, vials, each containing 10, instar-matched caterpillars on Mopane twigs, were warmed from 35 °C at a rate of 0·1 °C min^{-1} to 40 °C. This temperature was chosen based on the results of the previous experiment. At 6-h intervals, in a 24-h period, a vial was removed and the caterpillars were given 24 h to recover at ambient temperature. Only caterpillars with full locomotor function were considered survivors (Klok & Chown 1997). This procedure was repeated for each instar.

FIELD WATER RELATIONS AND DESICCATION RESISTANCE

Directly after the field temperature readings (see above) humidity was measured using a Novasina thermohygrometer (Labotec, USA) every 2 h and expressed as saturation deficit. Ten caterpillars of instars II to V were collected at each time interval and weighed on a Sartorius electronic microbalance (sensitive to 0·1 mg). The caterpillars were then dried to constant mass at 60 °C and weighed again. The difference between the fresh and dried mass was taken as the water content of the particular individual. Because of the dramatic differences in body size of caterpillars from instar II to V, the measured body water content data were corrected for body mass as suggested by Packard & Boardman (1988). In this procedure body water content is adjusted either upwards or downwards to a common value (grand mean) for body mass using the average slope derived from an analysis of covariance (similar to the calculation of adjusted means in ANCOVA, see Sokal & Rohlf 1995). A single classification ANOVA was used to determine whether the mass corrected water content (g g^{-1} initial mass) of the 41 groups of caterpillars ($n = 10$) of instars II to V differed at the respective measurement intervals in February/March 1996.

Desiccation resistance was determined for both individual caterpillars of instars I–V and for aggregations of instars I–III using Chown's (1993) methods. Field-fresh caterpillars were kept in a temperature-controlled cabinet at 35 °C with a 14L:10D photoperiod for 24 h with access to water but not to food to allow them to clear their digestive tracts. In instars I–III, caterpillars were kept both individually and in aggregations for both of the 1996/1997 generations.

In the individual-based desiccation trials, 20 individuals of instars I–V were numbered, weighed (see above) and placed separately in desiccation chambers containing silica gel which reduced the relative humidity to < 5% (monitored using a Novasina thermohygrometer). In the aggregation-based desiccation trials, caterpillars of instars I–III were subjected to desiccation in aggregations of 5, 10, 20 and 40 individuals. There were five replicates for each aggregation size. Both the solitary caterpillars and aggregations were transferred to the temperature-controlled cabinet at 35 °C (14L:10D photoperiod), and caterpillars were subsequently weighed every 8 h. The individual-based desiccation trials continued until 100% mortality. Desiccation trials involving aggregations were terminated at a time equivalent to the time of 100% mortality found in the trials for individuals of each particular instar to standardize comparison of water loss rates and amounts.

In the individual-based desiccation trials, maximum tolerable water loss (g), time to maximum water loss (h) and rates of water loss (g h^{-1}) were determined. These parameters were used because water loss via cuticular and respiratory transpiration could not be distinguished. They were calculated from the mass recorded at the time interval directly prior to death in each individual. Because the caterpillars produced no frass, mass loss was assumed to be equivalent to incidental and respiratory water loss (Wharton 1985). The data were also corrected for body mass (see above).

To determine whether aggregation influenced water loss rates of caterpillars in instars I–III, the following method was used. Rates of water loss determined for individuals of each instar were used to calculate water loss rates for increasingly large numbers of solitary individuals of that instar, as a product of the mean individual water loss rate and the number of caterpillars used in the experimental aggregation.

For this calculation it was presumed that the relationship between number of individuals and water loss rate is isometric. Least squares linear regression was then used to determine the relationship between water loss rate (after 65 h, the LT_{100} for the first instar, to standardize for time) and aggregation size for each of the instars in the experiments using real aggregations. For each instar the slope of the line determined for the real aggregations was compared with that calculated for the increasing numbers of solitary individuals using a *t*-test. For the aggregations, percentage survival of caterpillars surviving after 65 h was also noted.

Results

FIELD TEMPERATURE BIOLOGY

Microclimate data collected over all three study periods indicated that ambient temperatures varied between an absolute minimum of 13·8 °C and an absolute maximum of 46·2 °C, with a mean temperature of 24·1 °C, while solar radiation reached a maximum value of 1148 W m^{-2} (mean daily maximum, over all three periods, of 619 W m^{-2}). Summary statistics for T_b and T_a, the mean temperature excesses (T_{ex}) of each of the five instars, and the results of the linear least squares regression analyses of T_b on T_a of instars II–V are provided in Table 1. The slopes of the regression analyses of T_b on T_a for each of the instars did not differ from each other (F = 1·21, df = 622, P = 0·305), but all slopes differed significantly from unity ($t_{\text{s Instar II}}$ = 13·6, $t_{\text{s Instar III}}$ = 15·6, $t_{\text{s Instar IV}}$ = 12·6, $t_{\text{s Instar V}}$ = 12·0, $P_{\text{Instars II–V}}$ < 0·001). In general, instar V had significantly higher T_bs than did instars II–IV (F = 11·88, df = 626, P < 0·0001). At low T_as (20–25 °C) caterpillars had body temperatures similar to ambient, whereas temperature excesses were highest at high ambient temperatures. T_es measured in February/March 1996 were similar to the measured T_bs (Table 1), although the caterpillars in aggregations of instars II and III had significantly higher T_bs than the diurnal T_es measured from single models of these instars (Fig. 1). T_bs of the naturally solitary instars IV and V did not differ from the diurnal T_es of the same instars (Fig. 1). Diurnal T_es measured from the single operative models of instars II and III during the same generation, but continuously from sunrise to sunset, did not differ significantly from each other, nor did the diurnal T_es of instars IV and V. However, solitary instar II and III operative models had significantly lower diurnal T_es than instars IV and V (F = 23·96, df = 729, P < 0·0001). Tukey 95% HSD multiple comparisons indicated that diurnal T_es of operative models of aggregations of instars II and III were significantly higher than the T_es found for the solitary instar IV operative model, and that the operative model of the instar III aggregation had significantly higher T_es than that recorded for the single instar V operative model (F = 6·03, df = 4041, P = 0·0004). Multiple regression analyses indicated that T_a and solar radiation explained a significant portion of the variance in caterpillar T_{ex} (r^2 of 42–43% for instars II–V, and with both variables contributing significantly in all models). Caterpillars did not display any thermoregulatory behaviour early in the morning that could be considered a means to maximize T_b. However, at temperatures above 32 °C caterpillars displayed hanging behaviour (i.e. the caterpillars let go of the branch with the front two-thirds of their body), but did not move towards cooler microsites. Besides hanging, aggregating instar II and III caterpillars also dispersed partially from their aggregations at high temperatures.

CRITICAL THERMAL LIMITS AND UPPER THERMAL TOLERANCE

Tukey 95% HSD intervals for critical thermal maximum and critical thermal minimum for each of the instars indicated that caterpillars of the first three instars had significantly lower CT_{min}s than did the latter two instars, which did not differ (Fig. 2), whereas

Table 1. Mean ± SE, minimum and maximum values (in parentheses), and sample sizes for ambient temperature, body temperature (°C) and temperature excesses (°C) experienced by *Imbrasia belina* caterpillars in the field during February/March 1996. Body temperature measurements of instars II and III were made on caterpillars in aggregations. The results of the linear regression analyses of T_b on T_a are also provided

Variable	Instar II (n = 180)	Instar III (n = 180)	Instar IV (n = 160)	Instar V (n = 110)
Ambient temperature	26·0 ± 0·3	25·6 ± 0·3	25·9 ± 0·3	26·6 ± 0·4
	(18·2–34·1)	(18·3–33·1)	(17·6–33·1)	(18·3–32·4)
Body temperature	28·1 ± 0·4	27·8 ± 0·4	28·7 ± 0·5	31·5 ± 0·6
	(18·4–39·5)	(18·3–39·0)	(18·3–39·0)	(18·3–39·0)
Temperature excess ($T_b - T_a$)	2·0 ± 0·1	2·2 ± 0·1	2·8 ± 0·2	4·9 ± 0·2
	(– 0·3–9·7)	(– 0·1–9·6)	(– 0·2–9·0)	(0·0–8·9)
Regression analyses	r^2	F	P	
T_b instar II = – 5·20 + 1·28T_a	92·99%	2362·8	< 0·000 01	
T_b instar III = – 6·25 + 1·33T_a	92·36%	2152·6	< 0·000 01	
T_b instar IV = – 5·07 + 1·31T_a	90·63%	1529·1	< 0·000 01	
T_b instar V = – 4·81 + 1·37T_a	89·96%	967·9	< 0·000 01	
T_b all instars = – 5·77 + 1·33T_a	89·20%	5185·3	< 0·000 01	

differences in CT_{max} between instars showed no clearly interpretable pattern, although these differences were significant (Fig. 2). Nonetheless, the critical temperature range for the first three instars was markedly larger (34·4, 34·6 and 35·9 °C, respectively) than that of instars IV and V (31·6 and 30·7 °C, respectively).

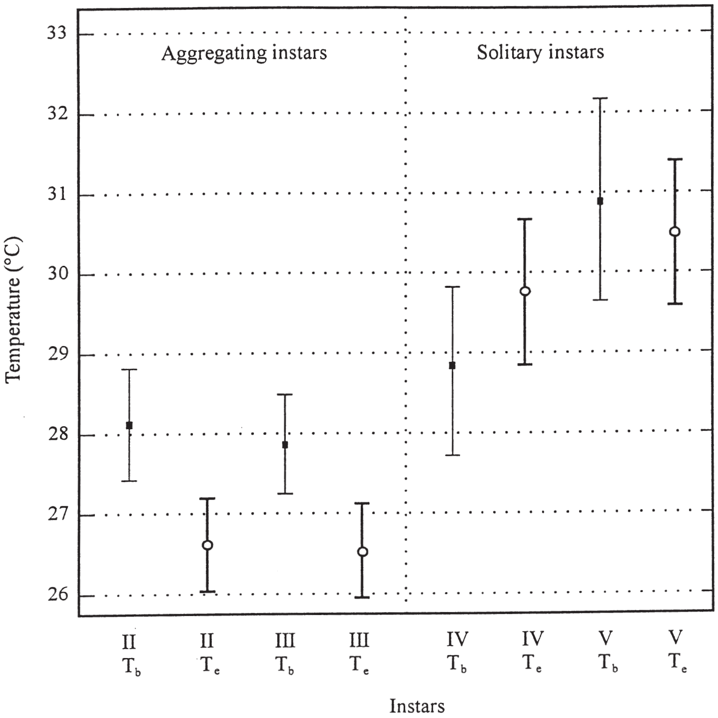

Fig. 1. Tukey 95% HSD intervals showing significant differences in ■ the means of the body temperatures and ○ diurnal operative temperatures of the aggregating instars II and III ($F = 6·06$, df = 796, $P = 0·014$), and the solitary instars IV and V ($F = 1·59$, df = 563, $P = 0·2$). The body temperature of instars II and III were measured from caterpillars in aggregations while the operative temperatures were obtained from single operative models.

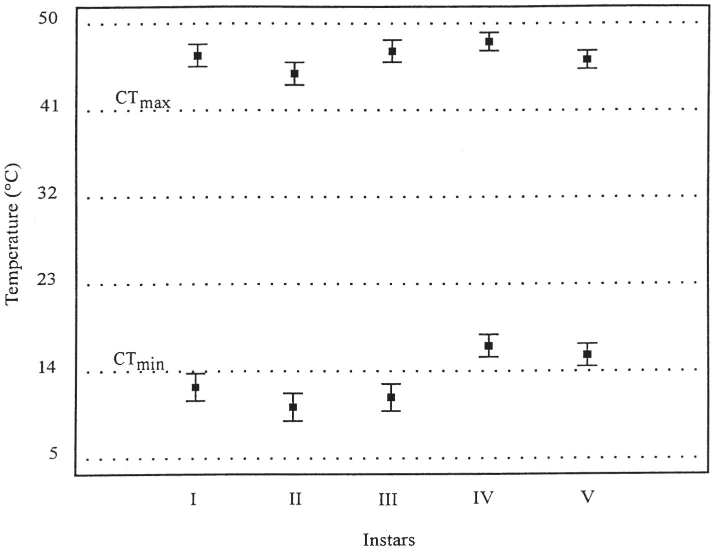

Fig. 2. Tukey 95% HSD intervals showing significant differences in the means of the CT_{min} ($F = 18·678$, $P < 0·0001$, df = 59) and CT_{max} ($F = 5·25$, $P = 0·0012$, df = 59) of the five instars of *Imbrasia belina* caterpillars.

In the upper lethal trials, caterpillars of all five instars showed a drop in survival from 42 °C onwards (Table 2). However, in instars I–III the decline in survival was rapid compared with the steady decline in survival of instars IV and V. By 46 °C, survival had declined to 0% in instars I–III, but remained between 55 and 65% in instars IV and V. Survival of short-term exposures to 40 °C was similar for all five instars, and was reasonably high at 6 h, but declined thereafter (Table 2).

FIELD WATER RELATIONS AND DESICCATION TOLERANCE

Diurnally measured saturation deficits in the trees (0·1–4·1 kPa) were significantly higher ($F = 17·378$, df = 502, $P < 0·0001$) than the saturation deficits measured at ground level by the microclimate station (0·012–2·75 kPa). Because mass-corrected water content differed only between instar IV 30 March 1996 at 12·00, and instar V 1 April 1996 at 10·00 ($F = 2·008$, df = 408, $P = 0·0005$), it is clear that the sometimes high saturation deficit in the tree microsites had little effect on caterpillar water content (which when corrected for body mass by analysis of covariance amounted ≈ 1·75 g, or 86% of fresh mass).

Water content (water content = 0·025 + 0·835 field fresh mass, $r^2 = 99·9\%$, $F = 282\,200$, df = 409, $P < 0·0001$), maximum tolerable water loss (mass loss = – 0·786 + 0·542 fresh mass, $r^2 = 97·3\%$, $F = 3563$, df = 99, $P < 0·0001$), rate of loss (rate = – 0·0014 + 0·0097 fresh mass, $r^2 = 81·0\%$, $F = 419·9$, df = 99, $P < 0·0001$) and survival time (time = 55·1 + 2·72 starting mass, $r^2 = 5·1\%$, $F = 5·25$, df = 99, $P = 0·024$) were significantly related to body size across all five instars. Caterpillars of all instars tolerated between 30 and 50% loss of their body mass over a large range of survival times before they died. As a consequence of their larger size, the later instars were significantly more desiccation resistant than the earlier instars when they were exposed individually to dry conditions (Fig. 3). However, when the data were corrected for body mass, there were no clear differences in rate of water loss and maximum tolerable water loss between the instars. Nonetheless, mean survival time, the ultimate measure of desiccation resistance, was significantly longer in instar IV than in the other instars, which did not differ significantly from each other (Fig. 3).

When rates of water loss for the real aggregations and the increasing numbers of solitary individuals were plotted against aggregation size for each of instars I–III (Fig. 4), the real aggregations appeared to lose water at a lower rate than the 'groups' of individuals in instars II and III only. This was borne out by the comparisons of the slopes of the regressions obtained for the two sets of data. The slopes of these regressions did not differ for instar I ($t_{\text{Instar I}} = 1·667$, $P = 0·1$), and inspection of Fig. 4(a) indicated that the rates did not differ between the aggregations and solitary caterpillars. In contrast, the slopes for the aggregations of instars II and III, and the increasing numbers of solitary caterpillars differed significantly ($t_{\mathrm{Instar\ II}} = 3{\cdot}286$, $P = 0{\cdot}01$ and $t_{\mathrm{Instar\ III}} = 8{\cdot}341$, $P = 0{\cdot}001$, Fig. 4b,c). Likewise, inspection of the figures showed that the rates of water loss in the 'real' caterpillar aggregations clearly decreased as aggregation size increased, in contrast to the additive water loss calculated for increasing numbers of solitary caterpillars. In this context, it is important to note that most of the caterpillars used for the instar I aggregations were newly hatched and did not have the opportunity to build up any reserves by feeding. Nonetheless, they had the highest percentage survival after 65 h (Fig. 4).

Discussion

THERMAL BIOLOGY AND AGGREGATION

Body temperatures of the solitary instar IV and V caterpillars were similar to the T_es of similar-sized operative models, indicating that the models were representative of the core body temperatures of live caterpillars (see Bakken 1992; Walsberg & Wolf 1996). Field measurements of operative and body temperatures indicated that larger-bodied caterpillars, and aggregations of instars II and III, reached higher equilibrium temperatures than did small individual caterpillars. The dependence of T_b on T_a in all instars, and the absence of basking and other regulatory behaviour (see Casey & Knapp 1987; Joos *et al*. 1988; Casey *et al*. 1988), suggested that this is a consequence more of the interaction between solar radiation and caterpillar (or aggregation) mass, than of physiological or behavioural regulation of body temperatures (see also Frears, Chown & Webb 1997; and Stevenson 1985 for general discussion).

Table 2. Percentage survival of *Imbrasia belina* caterpillars at upper temperature limits (specimens were kept for 1 h at each temperature; $n = 20$ per temperature), and 6-h intervals at 40 °C ($n = 20$ per interval)

	Instar I	Instar II	Instar III	Instar IV	Instar V
Upper lethal temperatures (°C)					
35–39	100	100	100	100	100
40	95	100	100	100	100
41	95	100	95	100	90
42	90	80	85	95	90
43	80	70	60	90	80
44	65	10	20	70	65
45	30	0	10	70	45
46	0	0	0	55	65
47	0	0	0	0	15
48	0	0	0	0	0
Time intervals (h)					
6	90	95	90	100	90
12	55	65	65	75	50
18	15	30	40	60	20
24	5	5	0	20	0

Higher heat loads associated with larger body masses should also result in greater thermal tolerances of the larger instars. Although this was not reflected in the $\mathrm{CT}_{\mathrm{max}}$ values, exposure of caterpillars for 1-h intervals at steadily increasing temperatures, indicated that the decline in survival from 42 °C onwards was more pronounced in the first three instars than in the latter ones, although this difference disappeared during longer-term exposures. Thus it appears that over the short term, instars IV and V are more resistant to increased temperatures than are the first three instars. A notable exception occurred when ambient conditions occasionally lead to T_es of operative models of instar II and III aggregations approaching or exceeding $\mathrm{CT}_{\mathrm{max}}$ levels (for up to 3 h). However, live instar I–III caterpillars usually disperse from the cylindrical aggregations to more sheet-like ones at high T_as (> 32 °C), thus dramatically changing the physical dimensions of the aggregation, effectively reducing its collective equilibrium temperature, and thus preventing T_bs from approaching $\mathrm{CT}_{\mathrm{max}}$ levels (see also Stevenson 1985). In contrast, the large solitary cylindrical instar IV and V caterpillars are unable to change their physical dimensions. Their only recourse is to hanging behaviour (which is also shown by the aggregating instars, Frears *et al*. 1997) and a greater resistance to high temperatures.

In contrast, the lower $\mathrm{CT}_{\mathrm{min}}$ found in the earlier instars would be an advantage to them if they were separated from their aggregations at fairly low temperatures. Oberprieler (1995) noted that if individuals from instars I–III are separated from an aggregation they cease feeding and immediately search for another (see also Cornell *et al*. 1987 and Joos 1992).

AGGREGATION AND WATER LOSS

The water relations of *I. belina* caterpillars are also influenced by their aggregation behaviour. During the larval stage *I. belina* caterpillars consume vast quantities of leaf material containing nutrients and water. This probably accounts for the stable and similar (when discounted for body mass) body water contents during the course of the day in all instars, despite fluctuations in T_a and saturation deficit. Therefore desiccation under normal circumstances in the field, when leaf material is available, probably does not pose a significant threat to the survival of the caterpillars. However, when leaf material is not available, for instance very early in the season when instar I caterpillars hatch before leaf emergence is completed (C. J. Klok & S. L. Chown, personal observation), or in later instars when many trees are defoliated (Oberprieler 1991, 1995), desiccation may negatively influence survival considerably. Under these conditions, small, solitary individuals (instars I–III) would be at considerable risk because of their unfavourable surface

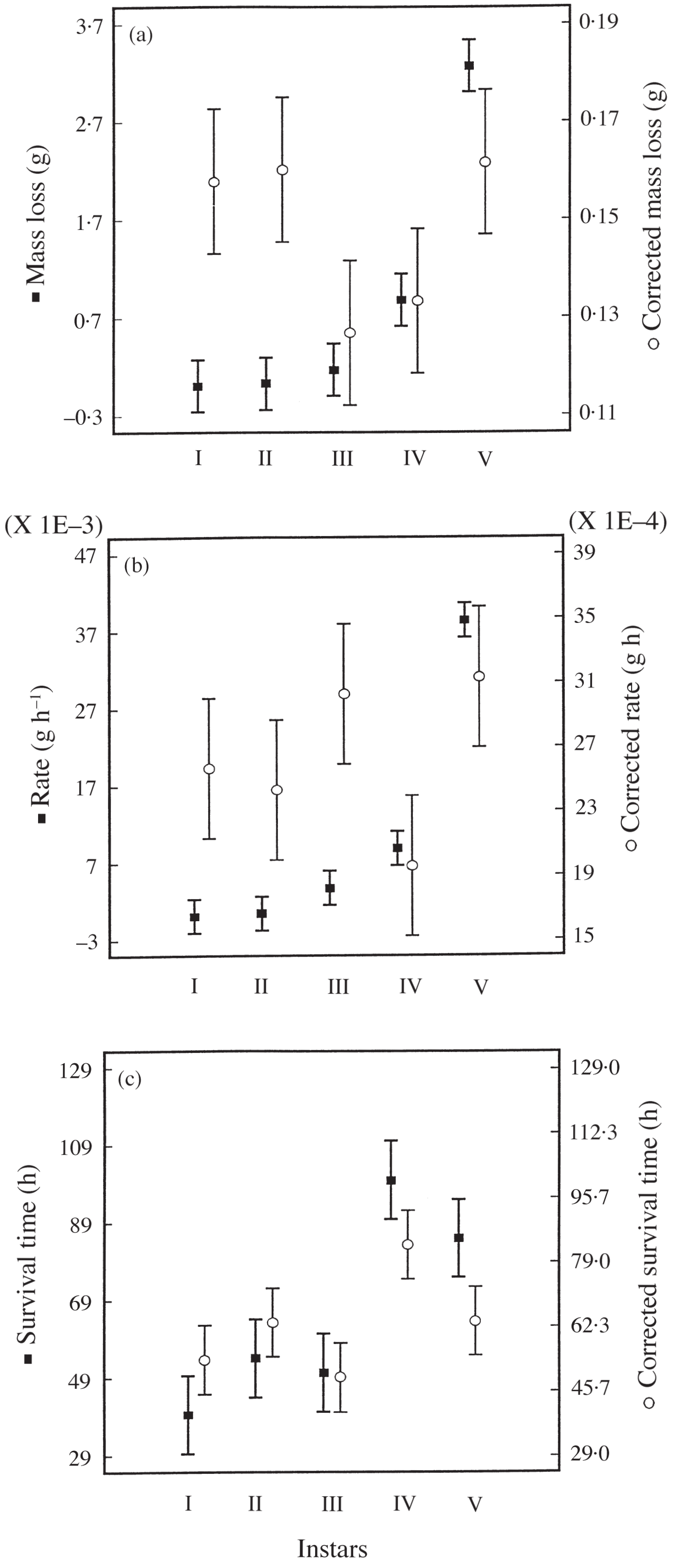

Fig. 3. Tukey 95% HSD intervals showing significant differences, associated with increasing body size, in the means of ■ untransformed and ○ mass-corrected: (a) maximum tolerable mass loss (untransformed $F = 105{\cdot}83$, $P < 0{\cdot}0001$, df = 99, corrected $F = 4{\cdot}86$, $P = 0{\cdot}0013$, df = 99); (b) rate of mass loss (untransformed $F = 206{\cdot}86$, $P < 0{\cdot}0001$, df = 99, corrected $F = 4{\cdot}61$, $P = 0{\cdot}0019$, df = 99); and (c) survival time (untransformed $F = 25{\cdot}02$, $P < 0{\cdot}0001$, df = 99, corrected $F = 8{\cdot}51$, $P < 0{\cdot}0001$, df = 99) of the five instars of *Imbrasia belina*.

area:volume ratios. However, in the field, individuals of these three instars form aggregations, of which some may exceed 200 individuals, and the data presented here show that both survival times (instar I) and rates of water loss (instars II–III) in aggregations are improved compared with solitary individuals (see also Willmer 1980; Yoder *et al.* 1992; Clark & Faeth 1997; Rasa 1997; Yoder & Grojean 1997), to the advantage of the individuals in these aggregations, especially during dry conditions and food shortages.

Instar IV and V caterpillars may also be challenged with desiccation, but more as a consequence of their defoliation of their hosts, especially during outbreak periods. However, the very large body size (and concomitantly high body water contents) of these instars confers considerable desiccation resistance on them. In this context the significantly higher body water content of instar IV caterpillars is of note. Because this is the first solitary instar, which has to contend with water balance as a solitary individual for the first time, there may be some premium for considerable desiccation resistance (see also Willmer 1980).

BENEFITS OF AGGREGATION

Our data therefore suggest that aggregations have a considerable advantage to the early instars of *I. belina* because they allow individuals in these instars to attain stable and high body temperatures, and to reduce rates of water loss, as is the case in many other caterpillars (Willmer 1980; Casey *et al.* 1988; Joos *et al.* 1988; Fitzgerald 1993). The maintenance of stable and favourable temperatures and body water levels throughout the period of larval development is likely to lead to increased growth rates (Scriber & Lederhouse 1983; Reynolds & Nottingham 1985). In turn, high growth rates shorten development time, and are thus likely to reduce the caterpillars' exposure to predators and parasitoids, the likelihood of adverse abiotic conditions, and declining food quality (Casey *et al.* 1988; Stamp & Bowers 1990; Fitzgerald 1993). *Imbrasia belina* has numerous predator and parasitoid species, occurs in areas where rainfall can be relatively unpredictable, and regularly defoliates its hosts in outbreak periods.

Even though we have found support for the regulation hypothesis for the maintenance of aggregation behaviour in *I. belina*, it should be noted that benefits of elevated growth rates (or even their existence in small *vs* large aggregations) have not been investigated. In addition, the alternative hypotheses (defence and host plant) have not been rigorously examined. However, there is strong reason to suspect that in this case these latter hypotheses are not suitable explanations for aggregation.

First, *I. belina* caterpillars form the prey of at least 30 insect, 4 reptile, 34 bird and 10 mammal

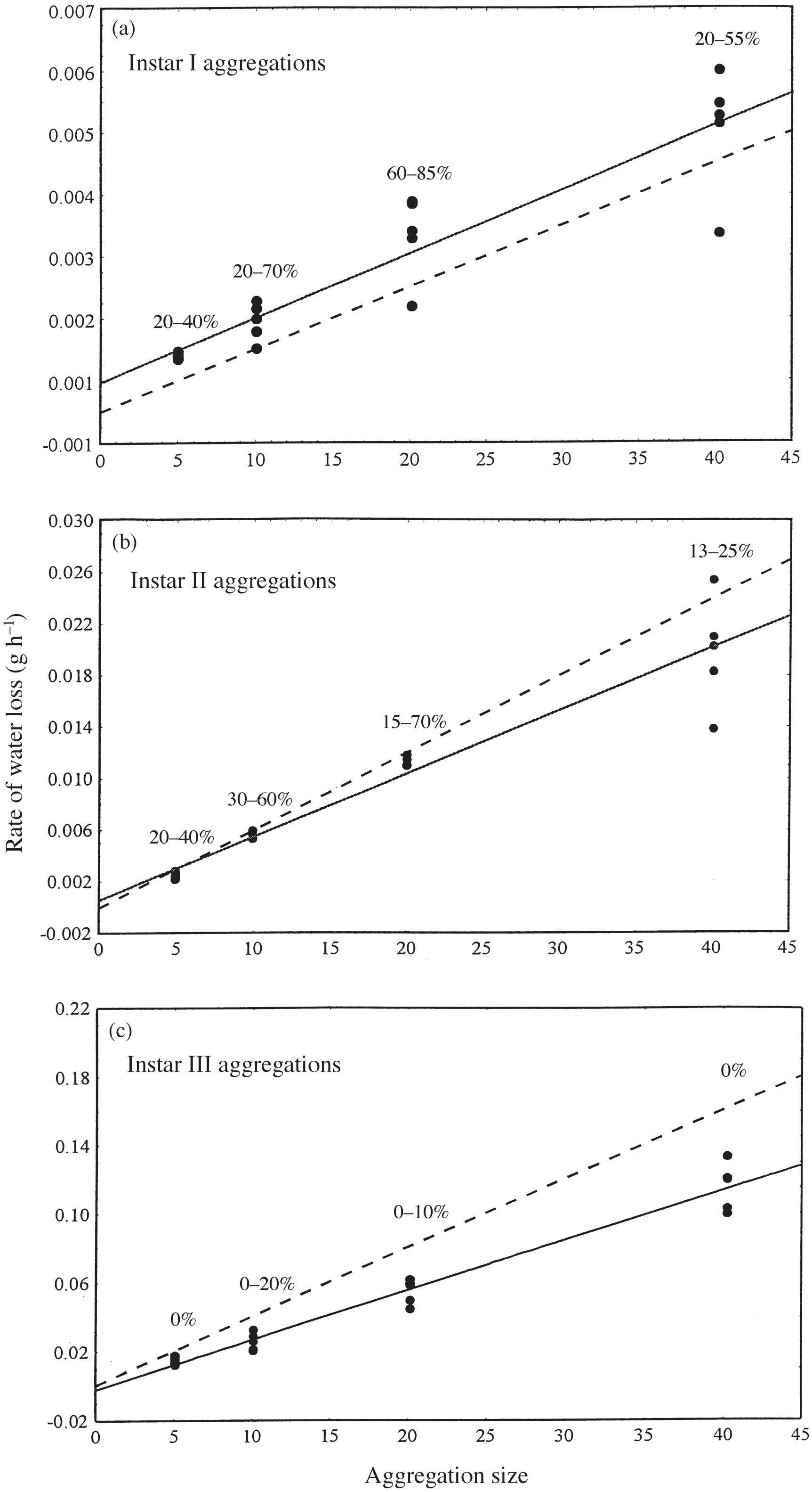

Fig. 4. Bivariate plots indicating the effect of group size on the hypothetical rate of water loss for groups of individual caterpillars, and on water loss rates measured in real aggregations of (a) instar I, (b) instar II and (c) instar III. Hypothetical linear regression lines (dashed lines), for the isometric increases of the rate of water loss with increases in aggregation size, calculated from data obtained in the desiccation experiments involving single caterpillars, are given by $Y = 0{\cdot}000085 + 0{\cdot}00012X$ (instar I), $Y = 0{\cdot}000001 + 0{\cdot}0006X$ (instar II) and $Y = 0{\cdot}000001 + 0{\cdot}004X$ (instar III). Linear regression lines of rate of water loss on aggregation size (solid lines) are also shown. The equations for these lines are: instar I, $Y = 0{\cdot}000085 + 0{\cdot}00012X$, $r^2 = 85{\cdot}36\%$, $F = 104{\cdot}92$, $P < 0{\cdot}0001$; instar II, $Y = 0{\cdot}000587 + 0{\cdot}000485X$, $r^2 = 91{\cdot}21\%$, $F = 196{\cdot}1$, $P < 0{\cdot}0001$; instar III, $Y = 0{\cdot}001085 + 0{\cdot}002899X$, $r^2 = 96{\cdot}36\%$, $F = 480{\cdot}26$, $P < 0{\cdot}0001$). The percentage caterpillars surviving in each aggregation after 65 h is also shown.

species (Gaston *et al*. 1997; J. Bell, K. J. Gaston & M. A. McGeoch, personal communication), and Gaston *et al*. (1997) have noted that the seeming aposematism of the later instars has no effect on predators (i.e they are pseudo-aposematic, Stamp 1980). Grant & Moran (1986) also showed that tree-feeding lepidopteran caterpillars in the southern African savannah are highly susceptible to predation by ants. In addition, irrespective of whether these caterpillars occur in areas where outbreak densities are likely (Mopane Bushveld) or unlikely (most other areas) (Oberprieler 1995; R. G. Oberprieler, unpublished data), aggregation in the first three instars is obligatory. Therefore, although the defence hypothesis has not been explicitly tested, it seems unlikely that aggregation behaviour is effective in reducing larval mortality in this species.

Second, the ‘host plant’ hypothesis seems equally inappropriate as an explanation for the maintenance of aggregation behaviour in *I. belina*. Not only do the caterpillars of this species feed on an extremely wide range of host plants, but their major host plants (e.g. *Burkea africana* and *Colophospermum mopane*, Oberprieler 1986, 1995) are slow growing (Coates-Palgrave 1995; Bryant *et al*. 1991). Such slow growing savannah species do not generally show long-term induced defences to defoliation, and may indeed be more susceptible to insect herbivory following initial defoliation (Bryant *et al*. 1991). In addition, in the eastern parts of its range (i.e. the mesic savannah areas, Low & Rebelo 1996), *I. belina* has two generations per season which feed on leaf material that is very different in terms of its age and quality. The first generation’s caterpillars hatch just after leaf flush of the host, providing the young caterpillars with young leaf material, while in the second generation there is no new leaf flush, and the caterpillars have to contend with mature leaves from the first instar onwards. Thus aggregations in the two generations experience rather large differences in host-plant quality. Wiggins (1997) also found that *I. belina* shows no clear preference for leaves that may have lowered defence capabilities. Caterpillars also disperse immediately after moulting into instar IV, suggesting that cooperative overwhelming of plant defences is of little importance.

In sum, the regulation hypothesis seems to be the most plausible of the three hypotheses proposed for the maintenance of aggregation, at least in the case of *I. belina*. This species has overcome the considerable developmental variation in heat and water fluxes, caused by a 4000-fold change in body size, by large modifications in its behaviour and only small physiological adjustments. In this way it appears to have retained the developmental flexibility so characteristic of Lepidoptera larvae which also alter their feeding habits (Gaston, Reavey & Valladares 1991) and defensive behaviour (Cornell *et al*. 1987) in response to a rapidly changing body size.

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References

Bakken, G.S. (1992) Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist* **32,** 194–216.

Benrey, B. & Denno, R.F. (1997) The slow-growth–high-mortality hypothesis: a test using the cabbage butterfly. *Ecology* **78,** 987–999.

Bryant, J.P., Heitkonig, I., Kuropat, P. & Owen-Smith, N. (1991) Effects of severe defoliation on the long-term resistance to insect attack and on leaf chemistry in six woody species of the southern African savanna. *American Naturalist* **137,** 50–63.

Cappucino, N., Damman, H. & Dubuc, J.F. (1995) Spatial behavior and temporal dynamics of outbreak and nonoutbreak species. *Population Dynamics: New Approaches and Synthesis* (eds N. Cappucino & P. W. Price), pp. 65–83. Academic Press, San Diego.

Casey, T.M. (1988) Thermoregulation and heat exchange. *Advances in Insect Physiology* **20,** 119–146.

Casey, T.M. (1993) Effects of temperature on foraging of caterpillars. *Caterpillars: Ecological and Evolutionary Constraints on Foraging* (eds N. E. Stamp & T. M. Casey), pp. 5–28. Chapman & Hall, London.

Casey, T.M. & Knapp, R. (1987) Caterpillar thermal adaptation: Behavioral differences reflect metabolic thermal sensitivities. *Comparative Biochemistry and Physiology* **86A,** 679–682.

Casey, T.M., Joos, B., Fitzgerald, T.D., Yurlina, M.E. & Young, P.A. (1988) Synchronized group foraging, thermoregulation and growth of eastern tent caterpillars. *Physiological Zoology* **63,** 372–377.

Chown, S.L. (1993) Desiccation resistance in six sub-Antarctic weevils (Coleoptera: Curculionidae): humidity as an abiotic factor influencing assemblage structure. *Functional Ecology* **7,** 318–325.

Clark, B.R. & Faeth, S.H. (1997) The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology* **22,** 408–415.

Coates-Palgrave, K. (1995) *Trees of Southern Africa*. Struik, Cape Town, South Africa.

Cornell, J.C., Stamp, N.E. & Bowers, M.D. (1987) Developmental change in aggregation, defense and escape behavior of buckmoth caterpillars, *Hemileuca lucina*. *Behavioral Ecology and Sociobiology* **20,** 383–388.

Courtney, S.P. (1984) The evolution of egg clustering by butterflies and other insects. *American Naturalist* **123,** 276–281.

Denno, R.F. & Benrey, B. (1997) Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology* **22,** 133–141.

Dussourd, D.E. (1993) Foraging with finesse: caterpillar adaptations for circumventing plant defenses. *Caterpillars: Ecological and Evolutionary Constraints on Foraging* (eds N. E. Stamp & T. M. Casey), pp. 92–131. Chapman & Hall, London.

Fitzgerald, T.D. (1993) Sociality in caterpillars. *Caterpillars: Ecological and Evolutionary Constraints on Foraging* (eds N. E. Stamp & T. M. Casey), pp. 372–404. Chapman & Hall, London.

Frears, S.L., Chown, S.L. & Webb, P.I. (1997) Behavioural thermoregulation in the mopane worm (Lepidoptera). *Journal of Thermal Biology* **22,** 325–330.

Gaston, K.J., Reavey, D. & Valladares, G.R. (1991) Changes in feeding habit as caterpillars grow. *Ecological Entomology* **16,** 339–344.

Gaston, K.J., Chown, S.L. & Styles, C.V. (1997) Changing size and changing enemies: the case of the mopane worm. *Acta Oecologica* **18,** 21–26.

Grant, S. & Moran, V.C. (1986) The effects of foraging ants on arboreal insect herbivores in an undisturbed woodland savanna. *Ecological Entomology* **11,** 83–93.

Hamilton, W.D. (1971) Geometry for the selfish herd. *Journal of Theoretical Biology* **31,** 295–311.

Joos, B. (1992) Adaptations for locomotion at low body temperatures in eastern tent caterpillars, *Malacosoma americanum*. *Physiological Zoology* **65,** 1148–1161.

Joos, B., Casey, T.M., Fitzgerald, T.D. & Buttemer, W.A. (1988) Roles of the tent in behavioral thermoregulation of eastern tent caterpillars. *Ecology* **69,** 2004–2011.

Klok, C.J. & Chown, S.L. (1997) Critical thermal limits, temperature tolerance and water balance of a Sub-Antarctic caterpillar. *Journal of Insect Physiology* **43,** 685–694.

Knapp, R. & Casey, T.M. (1986) Thermal ecology, behaviour, and growth of gypsy moth and eastern tent caterpillars. *Ecology* **67,** 598–608.

Lawrence, W.S. (1990) The effects of group size and host species on development and survivorship of a gregarious caterpillar *Halisidota caryae* (Lepidoptera: Arctiidae). *Ecological Entomology* **15,** 53–62.

Low, A.B. & Rebelo, A.G. (1996) *Vegetation of South Africa, Lesotho and Swaziland*. Department of Environmental Affairs and Tourism, Pretoria, South Africa.

Oberprieler, R.G. (1986) *Pinus radiata* D. Don: a new foodplant record for *Imbrasia (Gonimbrasia) belina* (Westwood) (Lepidoptera: Saturniidae). *Journal of the Entomological Society of Southern Africa* **49,** 168–169.

Oberprieler, R.G. (1991) Die Nachtpfauenaugen (Saturniidae) Südwestafrikas. 6. Das Braune oder Mopane-Nachtpafauenauge *Imbrasia belina* (Westwood). *Mitteilungen der SWA Wissenschaftlichen Gesellschaft* **31** (11/12)**,** 133–140.

Oberprieler, R.G. (1995) *The Emperor Moths of Namibia*. Ecoguild, Hartbeespoort, South Africa.

Packard, G.C. & Boardman, T.J. (1988) The misuse of ratios, indices and percentages in ecophysiological research. *Physiological Zoology* **61,** 1–9.

Rasa, O.A. (1997) Aggregation in a desert tenebrionid beetle: a cost/benefit analysis. *Ethology* **103,** 466–487.

Reynolds, S.E. & Nottingham, F. (1985) Effects of temperature on growth and efficiency of food utilization in fifth-instar caterpillars of the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* **31,** 129–134.

Scriber, J.M. & Lederhouse, R.C. (1983) Temperature as a factor in the development & feeding ecology of tiger swallowtail caterpillars, *Papilio glaucus* (Lepidoptera). *Oikos* **40,** 95–102.

Sillén-Tullberg, B. (1988) Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. *Evolution* **42,** 293–305.

Sillén-Tullberg, B. (1990) Do predators avoid groups of aposematic prey? An experimental test. *Animal Behaviour* **40,** 856–860.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry. The Principles and Practice of Statistics in Biological Research*, 3rd edn. W. H. Freeman, San Francisco.

Stamp, N.E. (1980) Egg deposition patterns in butterflies: Why do some species cluster their eggs rather than deposit them singly? *American Naturalist* **115,** 367–380.

Stamp, N.E. & Bowers, M.D. (1988) Direct and indirect effects of predatory wasps (*Polistes* sp. Vespidae) on gregarious caterpillars (*Hemileuca lucina*: Saturniidae). *Oecologia* **75,** 619–624.

Stamp, N.E. & Bowers, M.D. (1990) Variation in food quality and temperature constrain foraging of gregarious caterpillars. *Ecology* **71,** 1031–1039.

Stevenson, R.D. (1985) Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist* **125,** 102–117.

Tsubaki, Y. & Shiotsu, Y. (1982) Group feeding as a strategy for exploiting food resources in the burnet moth *Pryeria sinica*. *Oecologia* **55,** 12–20.

Walsberg, G.E. & Wolf, B.O. (1996) A test of the accuracy of the operative temperature thermometers for studies of small ectotherms. *Journal of Thermal Biology* **21,** 275–281.

Wharton, G.W. (1985) Water balance of insects. *Comparative Insect Biochemistry, Physiology and Pharmacology*, Vol. 4 (eds G. A. Kerkut & L. I. Gilbert), pp. 565–601. Pergamon Press, Oxford.

Wiggins, D.A. (1997) Fluctuating asymmetry in *Colophospermum mopane* leaves and oviposition preference in an African silk moth *Imbrasia belina*. *Oikos* **79,** 484–488.

Willmer, P.G. (1980) The effects of a fluctuating environment on the water relations of larval Lepidoptera. *Ecological Entomology* **5,** 271–292.

Yoder, J.A. & Grojean, N.C. (1997) Group influence on water conservation in the giant Madagascar hissing-cockroach, *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). *Physiological Entomology* **22,** 79–82.

Yoder, J.A., Denlinger, D.L. & Wolda, L. (1992) Aggregation promotes water conservation during diapause in the tropical fungus beetle, *Stenotarsus rotundus*. *Entomologia Experimentalis et Applicata* **63,** 203–205.

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