Defensive responses by a social caterpillar are tailored to different predators and change with larval instar and group size

MELANIE McCLURE[1] AND EMMA DESPLAND[1,*]

[1]*Biology Department, Concordia University, Montreal, Quebec, Canada*

**Corresponding author. E-mail:* despland@alcor.concordia.ca; *Tel.:* 1 514 848-2424 ext 3426

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Abstract:

Gregariousness in animals is widely accepted as a behavioral adaptation for protection from predation. However, predation risk and the effectiveness of a prey's defense can be a function of several other factors, including predator species, and prey size or age. The objective of this study was to determine if the gregarious habit of *Malacosoma disstria* caterpillars is advantageous against invertebrate natural enemies, and whether it is through dilution or cooperative defenses. We also examined the effects of larval growth and group size on the rate and success of attacks.

Caterpillars of *M. disstria* responded with predator-specific behaviors, which led to increased survival. Evasive behaviors were used against stinkbugs, while thrashing by fourth instar caterpillars and holding on to the silk mat by second instar caterpillars was most efficient against spider attacks. Collective head flicking and biting by groups of both second and fourth instar caterpillars were observed when attacked by parasitoids.

Increased larval size decreased the average number of attacks by spiders but increased the number of attacks by both stinkbugs and parasitoids. However, increased body size decreased the success rate of attacks by all three natural enemies, and increased handling time for both predators.

Larger group sizes did not influence the number of attacks from predators but increased the number of attacks and the number of successful attacks from parasitoids. In all cases, individual risk was lower in larger groups. Caterpillars showed collective defenses against parasitoids but not against the walking predators.

These results show that caterpillars use different tactics against different natural enemies. Overall, these tactics are both more diverse and more effective in fourth instar than in second instar caterpillars, confirming that growth reduces predation risk. We also show that grouping benefits caterpillars through dilution of risk, and, in the case of parasitoids, through group defenses. The decreased tendency to aggregate in the last larval instar may therefore be linked to decreasing predation risk.

Introduction:

Many animals live in groups, and gregariousness has been shown to provide protection from predation in a variety of taxa such as anuran larvae (DeVito 2003; Smith and Awan 2009), fish (Krause and Godin 1995), invertebrates (Clark and Faeth 1997; Uetz *et al.* 2002; Lemos *et al.* 2005), small mammals (Hass and Valenzuela 2002; Rogovin *et al.* 2004), ungulates (Mooring and Hart 1992) and many others. Predation risk and the effectiveness of a prey's defense can be a function of several variables, including prey group size, and individual prey size as a function of age (Botham *et al.* 2006; Smith and Awan 2009). Although larger groups of prey may be more easily discovered and may suffer more frequent attacks due to increased conspicuousness, hunting success of predators and per capita predation risk of prey have also been shown to decrease in larger groups (Lawrence 1990; Clark and Faeth 1997; Hunter 2000; Botham *et al.* 2005). Group members may suffer a lower risk of capture because of cooperative defense, enhanced advertisement of unprofitability in aposematic species, shared and more effective vigilance and a reduced probability of predation by virtue of a dilution effect when a predator can take only a limited number of individuals from the group (e.g Seyfarth *et al.* 1980; Peterson *et al.* 1987; Vulinec 1990; Mooring and Hart 1992; Uetz *et al.* 2002; DeVito 2003). In addition, animals in the centre of a group can decrease their risk of predation by surrounding themselves with others (Tostowaryk 1971; Mooring and Hart 1992; Krause *et al.* 1998), which Hamilton (1971) termed the selfish herd effect.

As prey individuals grow, their vulnerability to predators can also change. Smaller predator species may not be physically capable of handling large prey, or the costs of subduing them may be too great (Peters 1983; Warren and Lawton 1987; Cohen *et al.*

1993), whereas larger predator species may avoid small prey because they are too costly to handle for the energy gains. Gaston *et al.* (1997) found that the body masses of the bird species feeding on successive instars of the mopane worm were strongly correlated with the larvae's mass. The ability of pentatomid predators to subdue caterpillars also depends on the larvae's size and behavior (Iwao and Wellington 1970).

Most prey are subject to predation from multiple predators, and different defenses are thought to have evolved in response to selective pressures from different types of predators. As such, different predators may elicit different responses, or a prey species may adopt a general response which provides protection from many different types of predator (Botham *et al.* 2006). Generalized rather than species-specific responses may benefit prey in species that co-occur with multiple similar predators (Webb *et al.* 2010), hence the importance of testing the effectiveness of a prey's defensive mechanisms against different predators. Yet many studies investigating behavioral responses in predator-prey interactions have focused on single predators, and experimental evidence that prey benefit in terms of survival by adopting different responses to different predators appears to be lacking (Botham *et al.* 2006; Castellanos and Barbosa 2006).

We examined the responses of *Malacosoma disstria* caterpillars against three natural enemies and tested the effects of larval growth and gregarious behavior on the rate and success of attacks. Caterpillars of *M. disstria* are gregarious until the final larval stadium, and decreased predation risk is often listed among the benefits of group-living for this (Parry *et al.* 1998) and other gregarious caterpillar species (Reader and Hochuli 2003). *Malacosoma disstria* caterpillars are collective nomadic foragers and use pheromone trails to travel as a cohesive group between feeding sites. These caterpillars hatch in early spring when food quality is high and they develop rapidly to escape predation (Parry *et al.* 1998), as predation risk is thought to decrease with increasing larval size (Costa 1993; Reavey 1993). The importance of predation in shaping the gregarious and fast-developing life history traits is not known, nor is the identity of the predators exerting the selection pressure. Caterpillars of *M. disstria* are unpalatable to most vertebrates (Heinrich 1983; Heinrich 1993a), but little is known of the defensive mechanisms against invertebrate predators (see Fitzgerald 1995). Synchronous flicking of the body has been described for many social caterpillars (see Fitzgerald and Costa 1999 and references therein), and some, such as the closely-related *Malacosoma americanum*, also combine these displays with defensive regurgitation of enteric fluid containing host-derived benzaldehyde when attacked by predatory ants (Peterson *et al.* 1987).

The objective of this study was to determine if the gregarious habit of *M. disstria* is advantageous against invertebrate predation, and whether it is through dilution or cooperative defenses. We also hypothesized that the rate and success of attacks would decrease with increasing group size and caterpillar size (as a function of larval instar), but that these could vary between the three natural enemies tested, depending on the behavioral response exhibited in each case.

Methodology:

Unhatched egg masses of *M. disstria* were collected from Southern Ontario, Canada (44°33.5N, 76°24.1W) in March 2009 and stored at 4ºC with 80% R.H. until use. To minimize mortality from pathogens, egg bands were sterilized by soaking in 5% sodium hypochlorite as described by Grisdale (1985). Caterpillar colonies arising from a single egg mass were kept in plastic rearing containers and kept in a rearing chamber under a controlled light and temperature regime of 21°C, 70% RH and 16L: 8D. Caterpillars were fed *ad libitum* on a nutritionally balanced, standard wheat germ-based meridic artificial diet (Addy 1969). Although *M. disstria* caterpillars have never been observed to regurgitate, gut content may affect predation and so caterpillars were given fresh leaves of their primary host, trembling aspen (*Populus tremuloides*), 24 hrs before being used in experiments with the walking predators. Leaves were collected from multiple trees in Montreal, Quebec and were sterilized using 1% hypochlorite solution and rinsed with tap water against the possible presence of pathogens. All experiments were conducted at temperatures ranging between 20-23°C and 50-60% RH.

Fifteen species of hemipteran stinkbugs are known to prey on tent caterpillars, but *Podisus maculiventris* Say is one of the most common and it is distributed over most of the United States and southern part of Canada. Stinkbugs overwinter as adults and are active in early spring, searching for prey and responding within a short distance or after physical contact (Evans 1982). When a prey is detected, stinkbugs stretch out their proboscis before eventually attacking by inserting their stylets. Stinkbugs appear limited to attacking caterpillars of 20 mm or less (Evans 1982). Beetles in the genus *Calosoma* are also well-known predators of tent caterpillars, which are grasped and cut in half with sharp mandibles (Fitzgerald and Costa 1999 and references therein). Spiders are also important generalist predators, especially of earlier instars (McClure and Despland 2010; Ronnas *et al.* 2010). Although many species of parasitoids attack the eggs or pupae of *Malacosoma*, a few families also attack the larval stage (see Fitzgerald 1995 and references therein; Williams *et al.* 1996). *Malacosoma* caterpillars are known for flicking the anterior portion of their body when attacked by parasitoids, and this behavior quickly propagates through the group into a synchronized behavior. Prop (1960) found that such group displays in gregarious sawflies deterred oviposition by an ichneumonid parasitoid.

Three invertebrate predators, which co-occur with *M. disstria*, were therefore initially selected: stinkbugs (*Podisus maculiventris*) were obtained from The Bug Factory (Canada) and carabid beetles (*Calosoma sp.*) and spiders (*Thanatus vulagaris*) were collected in Montreal (Quebec, Canada). However, in preliminary trials (N=6) carabid beetles were found to be too mobile, with beetles escaping the set-up often without contacting the group of caterpillars (N=4), and were subsequently not used. A generalist parasitoid wasp (*Hyposoter fugitivus*) was also selected and was obtained from Dr. Stoltz's rearing colony (Dalhousie Univeristy in Halifax, Canada). All walking predators were starved 24h before use and a predator used in a test was not used again until it had fed and again been deprived of food. The predators were fed larvae of the greater wax moth, *Galleria mellonella*, and were supplied with moisture via a soaked paper towel. The parasitoids were fed with honey droplets. All walking predators were maintained in rearing chambers under a controlled light and temperature regime of 21°C, 70% RH and 16L: 8D, and parasitoids were stored at 10°C until use.

Tested group sizes were of 2, 10 and 30 second or fourth instar caterpillars. Only second and fourth instar caterpillars were studied during our experiments, as they reflect distinct differences in both body size and group behaviour (older caterpillars exhibit more independent locomotion). The experimental set-up consisted of a plastic arena (43 cm long x 3 cm) covered in brown paper. The arena was balanced on rubber stoppers covered in acetate, placed in a tray containing 2 cm of water in order to prevent caterpillars from leaving. Caterpillars were placed at one end of the arena 20 minutes before the introduction of a predator or 2 parasitoids to allow them to acclimatize and caterpillars were only used once. When using parasitoids, the experimental setup was placed in a mesh cage.

All group size, instar and natural enemy combinations were repeated 20 times. Experiments were terminated after 20 min for predators and 40 min for parasitoids. This was considered enough time to observe an attack, as on average predators attacked in less than 1 minute (mean±SE of 49.22±11.49 secs), and parasitoids did so in less than 8 minutes (mean±SE of 7.96±1.10 min). A video camera was mounted above the arena and all experiments were recorded for further analysis. The likelihood of attack in each treatment was analysed using chi-square. A multivariate analysis of variance (MANOVA) was used for each natural enemy to determine if the number of caterpillars attacked and the number of those attacks that were successful was significantly affected by group size and/or larval instar. The MANOVA for both walking predators also included the latency to attack (i.e. the time from the moment the predator is introduced into the arena to the first attack observed) and the handling time (i.e. the time required for a predator to subdue its prey) as dependent variables. In addition, the MANOVA for the stinkbugs also included the time needed to perceive the caterpillars (determined as when the proboscis was raised). The MANOVA for the parasitoids included the time caterpillar groups spent head flicking after an attack as a dependent variable. Behavioral descriptions of predator or parasitoid attacks and escape responses of caterpillars were also noted for every predator-prey combination. Parasitizing success was determined by rearing some of the groups (a minimum of 7 replicates per group size-instar combination was used for a total of N=45) until parasitoid emergence. Mortality risk per caterpillar from each natural enemy was also calculated by dividing the number of individuals within a group by the number of successful attacks and averaging them for all larval instar and group sizes.

Results:

Although these caterpillars are covered in setae, especially in the later instars, observations during this study gave no indication that they played any role in defense against the predators and parasitoids that were used. Because spiders and stinkbugs are only capable of predating one individual at a time and require time to consume it, there could not be more than one successful attack per given trial. However, when unsuccessful, multiple attacks by these predators could be made within a single trial. *Malacosoma disstria* caterpillars were never observed to regurgitate. Group activity (defined as either active or resting) was never a significant predictor of either attacks or the success of these attacks for any of the natural enemies.

Carabid beetles

Preliminary trials with carabid beetles (N=6) were done with groups of 30 fourth instar caterpillars, but proved to be difficult as the carabid beetles were large and too mobile for the chosen experimental set-up. In 4 of the trials, the beetle repeatedly escaped the set-up without making contact with the caterpillars. In 2 trials, the beetle attacked 1 caterpillar within the group and quickly devoured it. Predated individuals thrashed vigorously, but were never successful at escaping. The group's response consisted of all caterpillars walking away and relocating at the opposite end of the bridge set-up while the predator was occupied with its prey. Although carabid beetles have been described as being aggressive predators which often attack multiple caterpillars within a group, only one individual was observed to be attacked (N=2). Both beetles subsequently escaped the set-up shortly after the predation events.

Spiders

Spiders attacked by pouncing on the caterpillars and rapidly piercing them with their chelicerae. Responses of caterpillars attacked by spiders were different for second and fourth instars (Table 1). Although 42% of second instar caterpillars thrashed when grasped and a small number bit the spiders (5%), this was never successful. Surprisingly, 52% of the attacked caterpillars responded by gripping onto the silk mat: when spiders were unable to dislodge the caterpillar from its silk mat, they abandoned it. This tactic was successful in evading a predation event 80% of the time and bitten caterpillars that were abandoned always survived. Although this is not a group response per se, a group is needed to build a silk mat and this response was therefore not possible for individuals in groups of two. The larger fourth instar caterpillars were more aggressive in their responses. All individuals that were attacked thrashed vigorously. When not combined with any other behavior, this was successful in only 37% of attacks. Survival was similar when thrashing was combined with biting, but increased if caterpillars dropped off the bridge, which was always an effective evasive tactic. This would also be advantageous in the field as larger caterpillars can survive in the absence of conspecifics (Fitzgerald and Costa 1999).

For spiders, the time to attack (i.e. the latency for the spider to attack from the moment it is introduced) was not significantly influenced by group or larval size, but handling time was much longer for fourth instar caterpillars than for second instar caterpillars (162.00±33.87s *vs.* 2.58±0.33s; Table 2). The probability of at least one attack occurring during the trial decreased with larval instar (χ^2=4.805; df=1; p=0.028) but was not affected by group size (χ^2=1.669; df=2; p=0.434). The number of attacks per trial increased with group size for second but decreased for fourth instar caterpillars (Fig. 1). Attacks on fourth instar caterpillars were less likely to be successful than on second instar caterpillars. Attack success rate was not affected by group size (Table 2), and therefore the per capita mortality risk decreased in larger groups (Fig. 4). Position within the group was also found to be important, as the centre of the group sustained fewer attacks.

<u>Stinkbugs</u>

Caterpillars responded differently to stinkbugs, which after detecting the caterpillars raised their proboscis and approached them slowly, than they did to spiders. When second instar caterpillars detected the stinkbug before an attack (which occurred in 10% of cases), they took evasive responses by jerking away (Table 1). This was always successful as stinkbugs retreated. Once the predator had inserted its stylets into the caterpillar, none succeeded in escaping despite 60% of caterpillars thrashing in response to the attack. Fourth instar caterpillars showed a larger range of behavioral responses to stinkbugs, which occurred either singly or in various combinations. Caterpillars took evasive measures in 37% of cases, either by walking quickly out of the predator's path or by jerking away, and this was always successful in evading an attack. Predators were sometimes seen pursuing an escaping caterpillar with extended proboscis, but they never succeeded in catching them and quickly gave up the chase. Caterpillars responded to the stylets being inserted into their body by thrashing 56% of the time, but this was only effective in 17% of cases, even when combined with biting. Although only very few attacked caterpillars were able to both thrash and fall off the bridge (2%), this was always a successful tactic and these caterpillars always survived the piercing of their cuticle.

Whether stinkbugs attacked at least once was not significantly affected by either instar (χ^2=1.634; df=1; p=0.201) or group size (χ^2=2.467; df=2; p=0.291). Stinkbugs launched more attacks per trial against fourth instar caterpillars, but were more successful in capturing second instar caterpillars (Fig. 2). Again, because the number of successful attacks was not affected by group size, the mean mortality risk decreased with group size (Fig. 4). The time needed for stinkbugs to perceive the caterpillars (i.e. the time between introducing the stinkbug to the arena and the first moment they raised their proboscis) was not affected by either larval instar or group size (Table 2), but both the amount of time required to attack (35.68±10.43s *vs.* 200.57±43.29s; Table 2) and to subdue the prey (i.e. for the attacked caterpillar to stop moving) (25.58±3.64s *vs.* 168.95±32.15s; Table 1) was significantly longer for fourth instar caterpillars. Position within the group was again found to be important, as the centre of the group did not sustain any attack for either the second or fourth instar caterpillars.

Parasitoid wasps

Caterpillars reacted to parasitoid attacks, which stung the caterpillars with their ovipositor, both individually and as a group by flicking their heads, and in some cases, head flicking was accompanied by biting, which made it a far more effective tactic (Table 1). Groups of two second instar caterpillars almost never reacted to being attacked. However, for both second and fourth instar caterpillars, groups of 30 were more likely to use biting along with flicking than groups of 10 caterpillars.

The probability of at least one attack by a parasitoid per trial significantly increased with group size (χ^2=9.872; df=2; p=0.007) but was not affected by larval instar (χ^2=0.573; df=1; p=0.449). The number of attacks increased with caterpillar instar (Fig. 3), but the number of successful attacks (i.e. determined by the subsequent emergence of a parasitoid from the caterpillar) decreased with instar (Table 2). The number of attacks and of successful attacks increased with group size (Fig. 3), but the individual mortality risk still decreased with group size (Fig. 4). The time before an attack (i.e. time elapsed between the start of the experiment and the first attack observed) was not influenced by either caterpillar instar or group size, but the amount of time caterpillars spent head flicking after an attack significantly increased with group size (Table 2). Position within the group was again found to be important, as the centre of the group sustained fewer attacks.

Discussion

Caterpillars of *M. disstria* responded to attacks with predator-specific behaviors, which in many cases were successful in warding off attacks. When stinkbugs were used as predators, evasive behaviors were the most efficient in increasing survival, as has also been observed for *Nezara viridula* (De Clercq *et al.* 2002) and *Bombyx mori* (Lemos *et al.* 2005) caterpillars. These behaviors were never observed against spiders. Many fourth instar caterpillars thrashed when attacked by either spiders or stinkbugs, but this behavior was most successful when used against spiders. Second instar caterpillars that were attacked by spiders sometimes responded by holding onto the silk mat. This behavior was never observed with stinkbugs, and it would not likely have been successful, as shriveled caterpillar carcasses are often found still attached to naturally occurring tents and silk mats of *Malacosoma* colonies attacked by *Podisus* stinkbugs. Head flicking and biting were observed in both second and fourth instar caterpillars when attacked by parasitoids, but not when attacked by walking predators. Predator specific responses of *M. disstria* groups were also observed during preliminary trials using *Calosoma* beetles: attacked individuals thrashed vigorously, but unsuccessfully as even fourth instar caterpillars are much smaller than the beetles. But while the beetle was busy with one prey, the rest of the caterpillar group moved away together and relocated to a new bivouac elsewhere, which is important because a single beetle can eradicate an entire colony (Fitzgerald and Costa 1999). Other studies (e.g. Clark and Faeth 1997) have shown that, if predators are not satiated by a single prey item, or if they show a strong and very rapid numerical response, they can annihilate entire groups and group relocation may beneficial. Indeed, groups of *M. disstria* caterpillars have also been shown to relocate their bivouac in response to attacks by *Polistes* wasps (McClure and Despland 2010). However, relocation of the entire group before a food patch is depleted is likely costly, and it makes sense that this response would only be observed when caterpillar groups are attacked by predators capable of successfully predating most, if not all, of the group.

Although different responses to different predators is believed to be adaptive and has previously been suggested, little experimental work has been done to empirically demonstrate the behavioral ecology of defenses (Botham *et al.* 2006).

The escape responses of *M. disstria* caterpillars to predator attacks also varied with larval instar. Smaller caterpillars had fewer defensive behaviors and never dropped off the bridge, probably because the cost of being separated from the group is much higher for younger caterpillars (Despland and Le Huu 2007). Although second instar caterpillars were at times aggressive against parasitoids, biting their legs and antennae, they never successfully bit either the spiders or the stinkbug predators, both of which are larger than the parasitoids. The larger fourth instar caterpillars, however, were more likely to defend themselves with aggressive retaliation such as biting against all predators, as the value of this defense increases with the size of the prey relative to its predator.

As such, the number of caterpillars successfully predated or parasitized decreased with increasing body size, and the time required to subdue the prey increased for both spiders and stinkbugs. However, stinkbugs and parasitoids did attack fourth instar caterpillars more often. Because stinkbugs are cautious predators that slowly approach their prey, failed attempts often occurred before any physical contact was made. As such, attempting to attack a larger caterpillar was possibly less costly for stinkbugs than for spiders, and in fact, stinkbugs were more likely to try again. However, this is likely to change with continued growth of the caterpillars and more aggressive defensive behaviours (Morris 1963), and in fact Evans (1983) observed that stinkbugs experienced increasing difficulty in capturing *Malacosoma* caterpillars as the season advanced. As the caterpillars grew, they rapidly gained the ability to defend themselves from attacking adult stinkbugs by thrashing vigorously and forcing the timid stinkbugs to retreat and abandon the attack.

Although parasitoids can develop in second instar caterpillars, they face a higher risk of the host dying before the parasitoid larvae can complete its development (pers. obs.). Therefore fourth instar caterpillars are better hosts and this is most likely why parasitoids preferentially attacked more of the fourth instar caterpillars. Yet the proportion of caterpillars successfully parasitized decreased with increasing larval size, which suggests a trade-off for parasitoids. This may be due to both an increasing difficulty in successfully parasitizing the caterpillars due to defensive behaviours such as biting, and a stronger immune system in older caterpillars. As such, successful parasitism is likely to continue decreasing with increasing growth of the caterpillars. Thus overall increased body size lowers likelihood of successful attack for all three natural enemies but, at least for parastioids, larval body size appears to increase attractiveness of prey.

Grouping appeared to lower individual risk from all three natural enemies via dilution and the selfish herd effect. In all three cases, individual risk decreased with increasing group size and individuals in the center of the group were at a lesser risk of sustaining attacks than individuals situated at the periphery.

For spiders and stinkbugs, group size had no effect on the number of attacks or the number of successfully captured caterpillars. Because the number of prey successfully attacked was never more than one per trial, mortality risk always decreased with group size. There were no group responses for either second or fourth instar caterpillars attacked by either of the walking predators and therefore, against these predators, dilution of risk appears to be the only group benefit. Presumably, larger aggregations would be beneficial in the field if they do not attract more predators. For the gregarious caterpillar *Halisidota caryae* (Lawrence 1990), larger aggregations did not attract more invertebrate predators than did smaller ones, and so the likelihood of being taken was lower in a larger group. For *Malacosoma* species, Evans (1983) found that the density of caterpillars in a group was always high enough that the functional response of a pentatomid predator was independent of larval density.

By contrast, collective defense was observed against parasitoids. The parasitoids attacked more than one caterpillar once a group was located. However, despite multiple attacks and a higher attack success rate, mean mortality still decreased for individual caterpillars living in larger groups. The number of individuals successfully parasitized did not increase as rapidly as the number of individuals within a group. This may in part be because the optimal foraging time spent at a patch for parasitoids is limited by a diminishing return (Wajnberg 2006), but may also be due to the increasing difficulty in attacking defensive groups.

Indeed, although there was no evidence for group vigilance in trials done with spiders or stinkbugs, caterpillars appeared to benefit from the warning of a parasitoid's presence, possibly through the wing vibrations of parasitoids, vibrations in the silk mat generated by flicking caterpillars, and/or through the direct physical contact with flicking caterpillars, although they don't appear to respond to vibrations caused by approaching predators or by thrashing conspecifics. Caterpillars attacked by the parasitoids usually aggregated as tight flicking groups and displayed co-operative defenses such as simultaneous biting of the wasps' legs and antennae. Individuals who started flicking before having sustained an attack themselves therefore appear to be benefiting from the signaling of other individuals, but those who have already been attacked also benefit as they may be attacked more than once (pers. obs.). Although groups of two caterpillars occasionally displayed these behaviors, they occurred less often, at a lower intensity and for a shorter time. The time spent flicking by groups after the first attack also increased with group size, which suggests that the effectiveness of this behaviour increases for larger groups.

In conclusion, we show that *M. disstria* exhibit different behaviours in response to different predators and at different larval stadia. Like guppies (Botham *et al.* 2006) and monkeys (Seyfarth *et al.* 1980), these caterpillars are able to discriminate between different predators, likely as a result of very different modes of attack, and respond appropriately. Indeed, this study shows experimentally that prey benefit in terms of survival by adopting different responses, although how these caterpillars are able to identify the predator and decide which response to makes has yet to be determined.

In general, fourth instar caterpillars showed more varied defensive responses, including falling off the bridge and biting the aggressor, and were more successful against all three natural enemies. Our results confirm that larval vulnerability is greatest in the early larval instars, supporting the idea that rapid growth constitutes a defensive benefit. An extended development time in herbivorous insects increases larval exposure to natural enemies, termed the slow-growth-high-mortality hypothesis, and has been shown in many species (Schultz 1983; Benrey and Denno 1997). For example, Parry *et al.* (1998) found that survivorship of later hatching *Malacosoma* caterpillars was drastically reduced by invertebrate predation and Evans (1982) observed that during unfavorable weather in the spring, the activity of predatory stinkbugs was temporarily suppressed and enabled the tent caterpillars to escape predation by growing to sizes too large to be subdued by the predators. We show that increased size is advantageous for caterpillars against three very different modes of attack, due not only to the predator's difficulty in handling larger prey, but also to the caterpillar's broader range of defensive behaviors. Our results also show a lower per capita predation risk in larger groups. In the case of spiders and stinkbugs, the benefits of grouping could only be attributed to dilution of risk, but against parasitoids, caterpillars also exhibited group defenses. Improved anti-predator defense has been suggested as a benefit to group living in a wide range of taxa (e.g. Hass and Valenzuela 2002; Uetz *et al.* 2002; e.g. DeVito 2003; Rogovin *et al.* 2004; Lemos *et al.* 2005; Smith and Awan 2009), including many caterpillars (see Vulinec 1990). We confirm that grouping does indeed protect *M. disstria* caterpillars against predation and that they use group defenses in some contexts. Aggregations of early instars of *M. disstria* have also been shown to benefit from group thermoregulation (McClure *et al.* 2010) which enhances larval growth rates (Levesque *et al.* 2002), and thus the aggregated larval lifestyle may also indirectly reduce predation by decreasing exposure to predators. Grouping thus appears to protect *M disstria* against predation via several simultaneously acting mechanisms: predator dilution, group defenses, faster development and possibly aposematism (Heinrich 1993b). Hunter (2000), who compared the shapes of published survivorship curves of gregarious and solitary Lepidoptera and Symphyta, concluded that there was something in addition of the possession of defenses that explains the higher larval survival of gregarious species. This study further supports their suggestion that dilution of risk, possibly in concert with increased group defense behaviors, and reduced duration of exposure to enemies because of rapid development time may explain the survival advantage of gregariousness.

Finally, the decreased tendency to aggregate of later instars of *Malacosoma* species has been tied to an increase in food competition (Despland and Le Huu 2007) and a reduced need for thermoregulation (McClure *et al.* 2010); our results suggest that it may be further enabled by caterpillars' increased ability to defend themselves against invertebrate predators.

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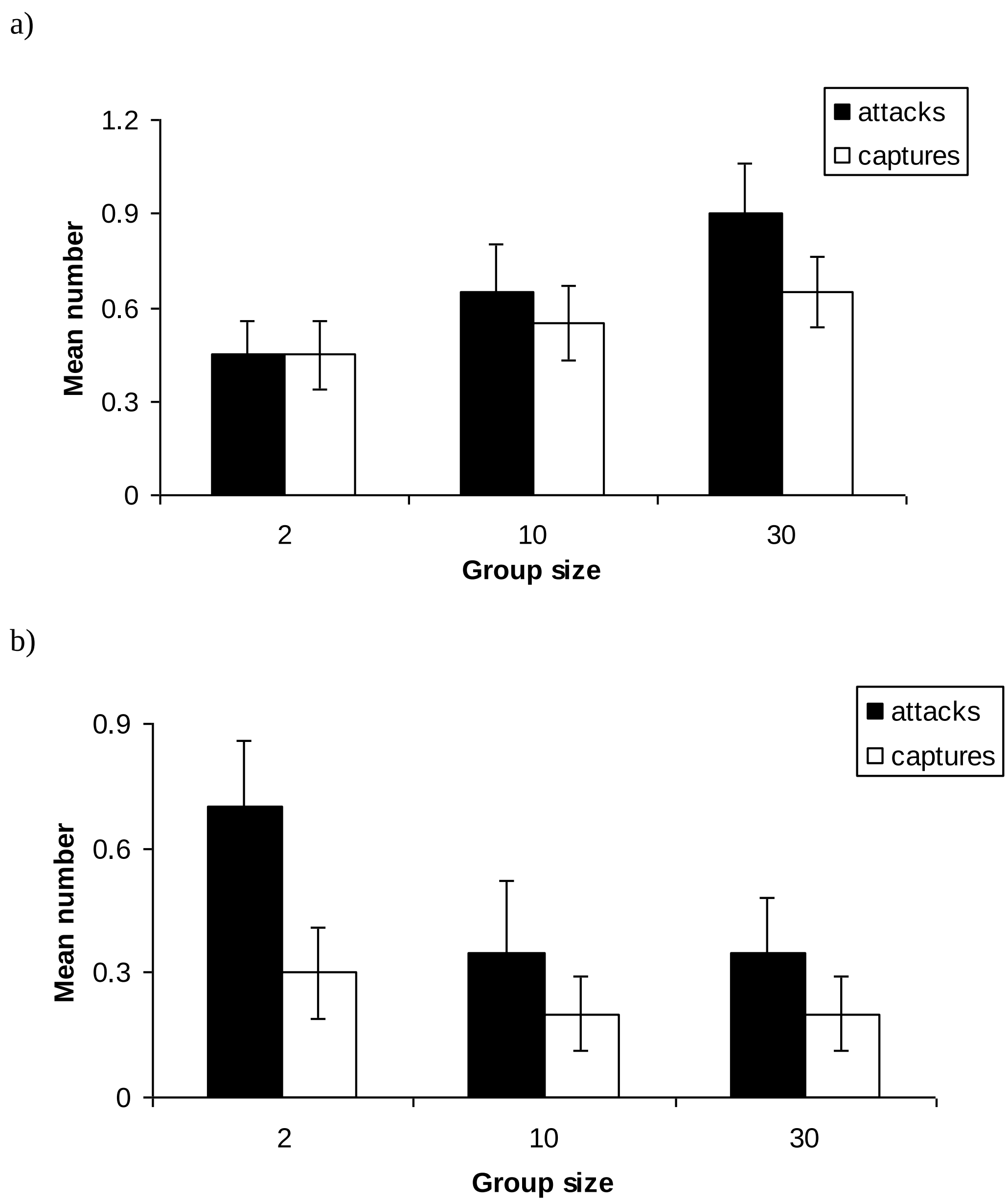
List of figures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Mean number
2
10
30
Group size
b)
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captures
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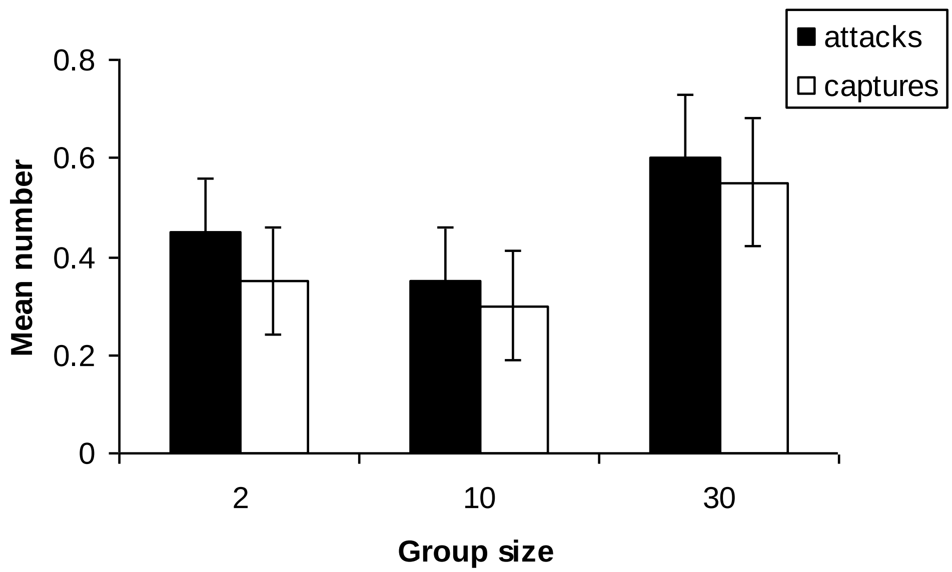
Mean number
2
10
30
Group size

Fig. 1

a)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Group size

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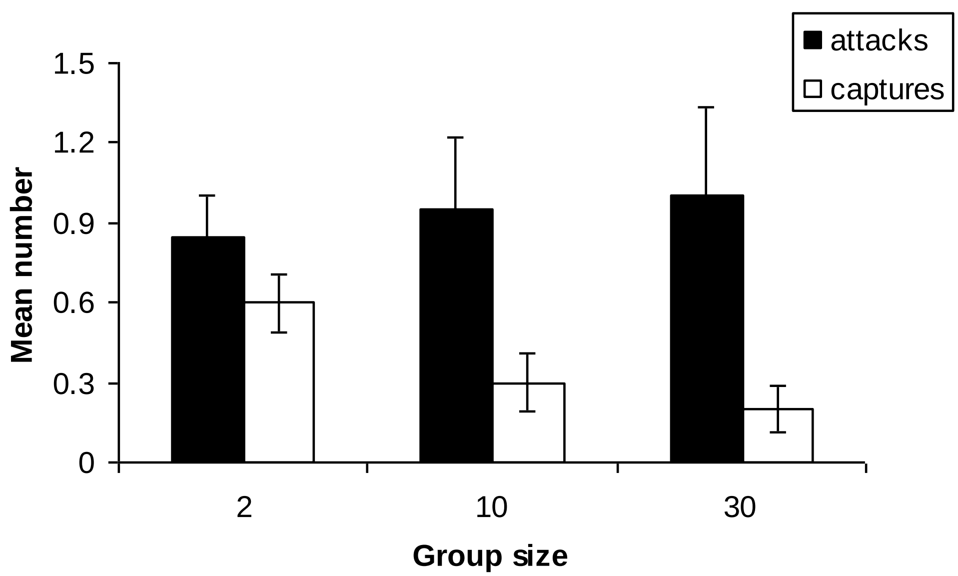
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Mean number
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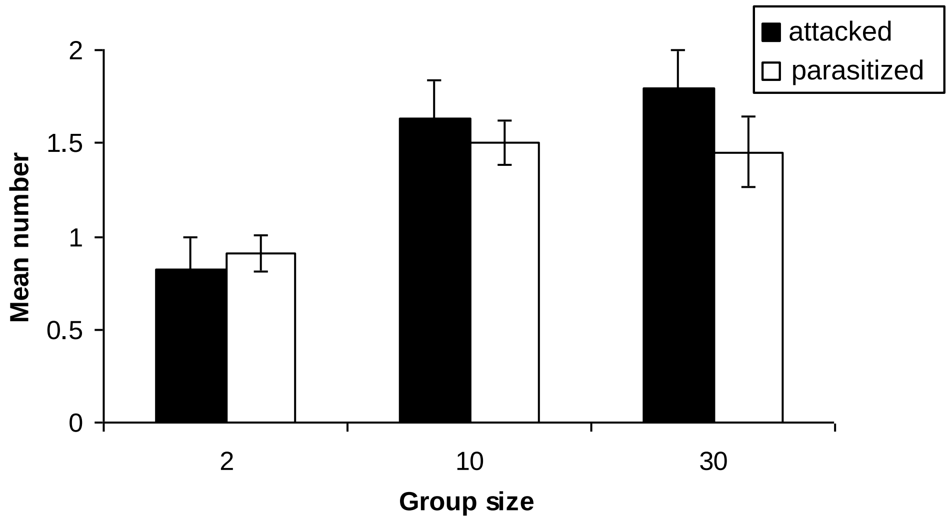
Group size

Fig. 2

a)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parasitized
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1
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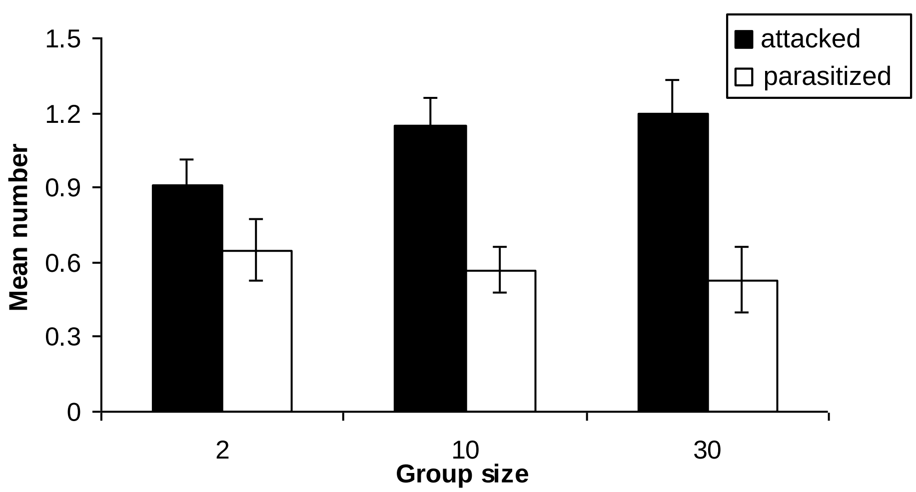
2
10
30
Group size

b)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parasitized
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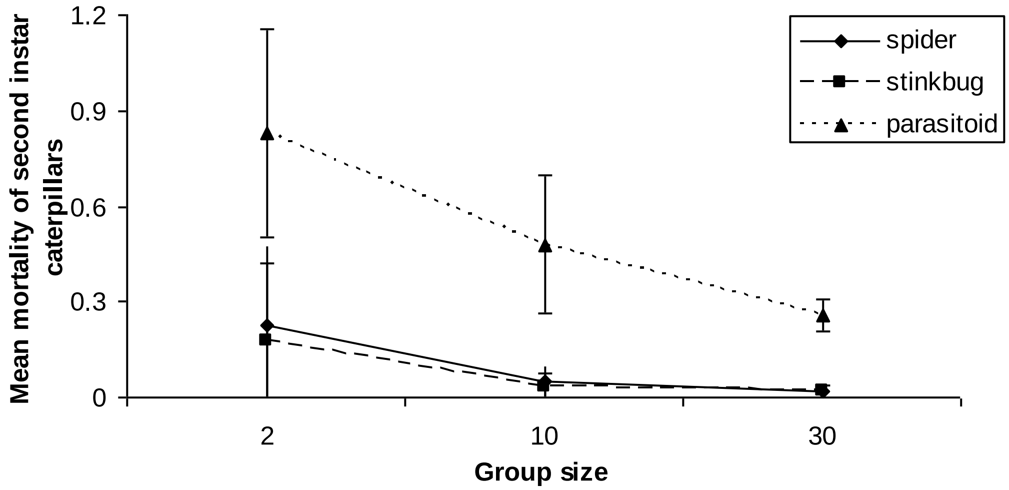
0.6
0.9
1.2
1.5
2
10
30
Group size

Fig. 3

a)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0.9
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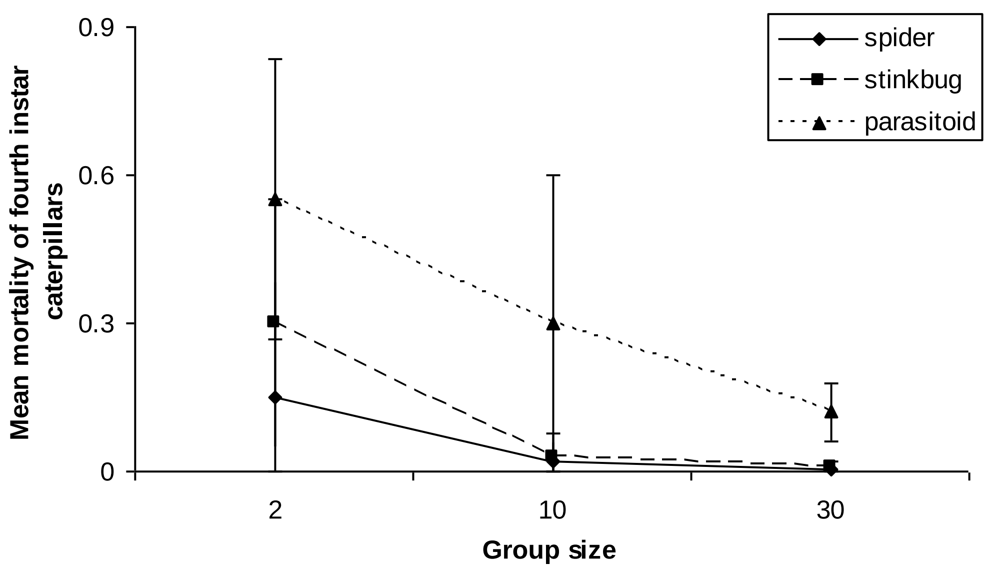
Mean mortality of second instar caterpillars
2
10
30
Group size
spider
stinkbug
parasitoid

b)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0.6
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Mean mortality of fourth instar caterpillars
2
10
30
Group size
spider
stinkbug
parasitoid

Fig. 4

Table 1: The behavioural response (when one was observed) elicited by an attack by an invertebrate predator or parasitoid, the proportion of *Malacosoma disstria* caterpillars responding and the proportion of those that were successful in escaping predation or parasitisation.

Type of predator	Instar	Behavioral response	% responding	% responding successfully
Spider	2	Thrashing	42	0
		Biting	5	0
		Holding the silk mat	53	80
	4	thrashing	37	38
		thrashing & biting	30	38
		thrashing & falling	33	100
Stinkbug	2	jerking back	11	100
		thrashing	61	0
	4	walking away	12	100
		jerking back	26	100
		thrashing	56	17
		thrashing & biting	5	0
		thrashing & falling	2	100
Parasitoid	2	head flicking	70	9
		head flicking & biting	30	30
	4	head flicking	66	32
		head flicking & biting	34	66

Table 2: Statistical results for 3 separate MANOVAs done for each natural enemy as a function of group size and larval instars of *Malacosoma disstria* caterpillars (N=120 groups per analysis).

Type of predator	Measured variable	Experimental factor	F value	df	p value
Spider	Number of attacks	Caterpillar instar	2.7	1, 114	0.103
		Group size	0.36	2, 114	0.701
		Interaction	3.77	2, 114	**0.026***
	Number of captures	Caterpillar instar	12.26	1, 114	**0.001***
		Group size	0.27	2, 114	0.768
		Interaction	1.02	2, 114	0.36
	Time to attack (secs)	Caterpillar instar	0.15	1, 59	0.698
		Group size	0.08	2, 59	0.923
		Interaction	0.93	2, 59	0.402
	Handling time (secs)	Caterpillar instar	86.38	1, 40	**>0.001***
		Group size	0.81	2, 40	0.453
		Interaction	0.85	2, 40	0.434
Stinkbug	Number of attacks	Caterpillar instar	7.94	1, 114	**0.006***
		Group size	0.37	2, 114	0.695
		Interaction	0.16	2, 114	0.851
	Number of captures	Caterpillar instar	0.31	1, 114	0.58
		Group size	1.27	2, 114	0.286
		Interaction	4.42	2, 114	**0.014***
	Time to perceive (secs)	Caterpillar instar	1.6	1, 78	0.214
		Group size	0.3	2, 78	0.741
		Interaction	0.23	2, 78	0.798
	Time to attack (secs)	Caterpillar instar	15.96	1, 78	**>0.001***
		Group size	2.63	2, 78	0.087
		Interaction	1.8	2, 78	0.181
	Handling time (secs)	Caterpillar instar	14.28	1, 53	**>0.001***
		Group size	0.01	2, 53	0.994
		Interaction	0.02	2, 53	0.984
Parasitoid	Number of attacks	Caterpillar instar	4.16	1, 114	**0.042***
		Group size	4.75	2, 114	**0.009***
		Interaction	1.47	2, 114	0.232
	Successfully parasitized	Caterpillar instar	16.69	2, 39	**>0.001***
		Group size	20.02	1, 39	**>0.001***
		Interaction	3.22	2, 39	0.051
	Time to attack (secs)	Caterpillar instar	6.29	1, 72	0.594
		Group size	0.002	2, 72	0.998
		Interaction	0.33	2, 72	0.719
	Time spent flicking (secs)	Caterpillar instar	0.6	1, 72	0.441
		Group size	26.03	2, 72	**>0.001***
		Interaction	1.32	2, 72	0.276