

REPORT

Good housekeeping: why do shelter-dwelling caterpillars fling their frass?

Martha R. Weiss
Biology Department,
Georgetown University,
Washington DC 20057-1229,
USA

Abstract

Although both feeding and elimination of waste are imperatives for all animals, ecologists and evolutionary biologists have devoted considerable attention to foraging, while largely ignoring defecation. Many organisms, however, exhibit defecation behaviours that appear to have been shaped by natural selection in a range of ecological contexts. Accumulation of waste may pose particular challenges for animals that exhibit high site fidelity or live within enclosed spaces. In a taxonomically widespread but largely unexamined behaviour, many caterpillars that construct and inhabit leaf shelters ballistically eject their individual faecal pellets (frass) great distances at great speeds. Here, I show that elimination of chemical cues for natural enemies is likely to have been a driving force behind the evolution of frass ejection behaviour in skipper caterpillars; hygiene and crowding are less important in this system.

Keywords

Anal comb, defecation, *Epargyreus clarus*, faecal pellets, Hesperiiidae, leaf-roller, *Polistes*, shelter-builder.

Ecology Letters (2003) 6: 361–370

INTRODUCTION

All animals must ingest food and eliminate the waste products. Both of these essential biological processes take place in an ecological context, with potentially important consequences for the animal itself, as well as for its relationships with other organisms. While studies of foraging have been a cornerstone of ecological research and a source of major theoretical insights (see Schoener 1971), analogous issues related to defecation have received much less attention (Wotton & Malmqvist 2001).

Scattered throughout the literature are reports of defecation behaviours that appear to have been shaped by natural selection in a range of ecological contexts, including predator–prey interactions (Boonstra *et al.* 1996; Brown *et al.* 1996; Eisner & Eisner 2000), exposure to pathogens or toxins (Hölldobler & Wilson 1990; Hart & Ratnieks 2001), nutrition (Kenagy & Hoyt 1980; Troyer 1982), and social signalling (Ralls 1971; Stewart *et al.* 2001). Animals that exhibit high site fidelity or live within enclosed spaces face particular challenges, in that proximity to accumulated waste may (i) increase exposure to pathogens or toxins; (ii) crowd the animal physically; or (iii) provide signals to natural enemies.

Larvae in a number of diverse lepidopteran families have evolved an unusual behaviour, whereby they ballistically eject individual faecal pellets (frass) great distances (Frohawk 1892, 1913; Scoble 1995; Caveney *et al.* 1998). Within the family Hesperiiidae, for example, larvae have been reported to shoot frass pellets a meter or more away (Frohawk 1913; Caveney *et al.* 1998); I have observed a 4-cm long *Epargyreus clarus* (silver-spotted skipper) larva launch a pellet a remarkable 153 cm, or 38 times its body length.

Frass ejection is commonly found among larvae that construct simple structures externally on a host plant by tying, folding or rolling plant structures with silk (Fig. 1). Larvae can move freely inside or outside these structures when feeding, and tend to remain within a narrowly circumscribed area for days to weeks. *Epargyreus clarus* larvae, in addition to ejecting frass, also consistently remove any pellets that are inserted into their shelters, either by butting them out with their heads or picking them up in their mandibles (MRW, pers. obs.). In the field, frass is almost never found in or around *E. clarus* shelters or even on host plant leaf surfaces, as ejection distances are generally sufficient to propel a pellet beyond the margin of the shelter-bearing leaflet, and any pellets that land on another leaflet roll off as the leaves move.

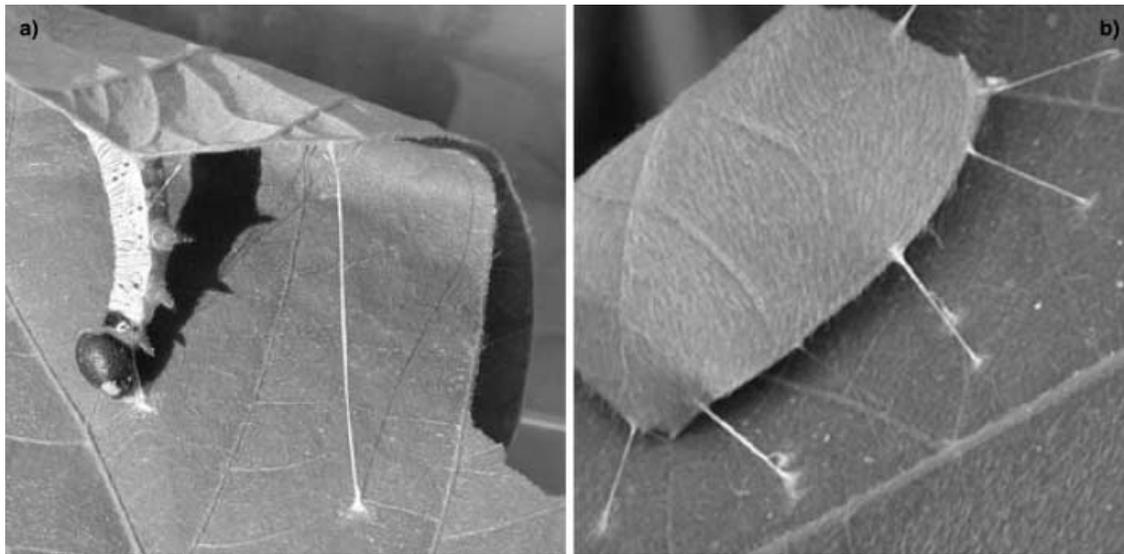


Figure 1 (a) Fourth instar *Epargyreus clarus* larva laying down a silk 'guy-wire'. (b) Shelter built by a third instar larva.

Caterpillars that eject frass generally bear a sclerotized comb or fork on the lower surface of their anal plates, above the anus (Frohawk 1913; Stehr 1987; Scoble 1995). This structure, originally thought to function as a catapult that flicked the pellet away (Frohawk 1913, Scoble 1995), is now known to serve as a mechanical latch in an ejection system driven by a localized increase in larval blood pressure (Caveney *et al.* 1998). The presence or absence of such anal structures is used as a larval taxonomic character across Lepidoptera (Gerasimov 1952; Mackay 1962; Stehr 1987).

Based on direct observations, published reports, or the presence of anal structures, I have determined that frass ejection occurs in at least 17 moth and butterfly families, distributed across nine superfamilies (Table 1). Within some families, larvae that inhabit leaf shelters eject their frass, while related free-ranging external feeders, stem borers, and other non-shelter-dwelling species generally do not (Stehr 1987; Scoble 1995). The scattered distribution of frass ejection behaviour both across and within taxa, and the correlation of the trait with a shelter-dwelling habit, suggests multiple independent evolutionary origins and/or losses of the behaviour and associated morphology, and implies an underlying selection pressure.

Despite the taxonomically widespread occurrence of frass ejection behaviour, and the use of associated anal structures as larval key characters, the phenomenon has been little studied. Frass ejection has been interpreted as a mechanism to remove olfactory cues for natural enemies, or to reduce exposure to pathogens (Frohawk 1892; Stehr 1987; Scoble 1995; Caveney *et al.* 1998); however, neither hypothesis has been evaluated experimentally. Nor have patterns of frass ejection across larval ontogeny been documented. In this

paper, I quantify frass ejection distances in all larval stages of the silver-spotted skipper, *E. clarus* (Cramer) (Hesperiidae), and then use this species as a model system to test the following three hypotheses:

Hypothesis 1 (Hygiene): Retention of frass in a larval shelter produces a microenvironment in which pathogens potentially harmful to larvae could flourish; frass ejection eliminates the problem.

Rationale: Caterpillars are susceptible to a range of bacterial, fungal, viral, and protozoan pathogens; indeed, many of these agents are used as 'microbial pesticides' to control pest Lepidoptera (NRC 2000). Opportunities for infection may be exacerbated by proximity to frass (Winter 2000). Naturally occurring infections of entomopathogenic fungi may be responsible for limiting insect populations, particularly in warm, moist environments (Hajek & St Leger 1994). Thus, ejection of frass may reduce the likelihood of pathogenic infection in shelter-dwelling larvae.

Hypothesis 2 (Crowding): Frass ejection behaviour is an evolved response to physical crowding of the shelter. If frass were not removed or ejected, a larva might be forced to build a new shelter sooner than would otherwise be necessary, an activity that could involve significant energetic or materials cost, as well as increased exposure to visually oriented enemies.

Rationale: Energetic and materials costs of building a new shelter can be significant for lepidopteran larvae that use a great deal of silk in the construction of their structures (Ruggiero & Merchant 1986; Berenbaum *et al.* 1993). In addition, larvae outside of shelters are more vulnerable to predation than are those inside (Damman 1987; Jones *et al.* 2002), and moving larvae are particularly vulnerable to

Table 1 Distribution of frass ejection behaviour across Lepidoptera. Frass ejection, determined by reports of the behaviour (FE), or by presence of associated anal structures (AS), occurs in at least nine superfamilies and 17 families within Lepidoptera. Anal structures are not necessarily homologous across groups

Superfamily	Family	Reference
Tineoidea	Tineidae	Dominguez-Romero (1996) (AS) and M.R.W., pers. obs. (AS, FE)
Gelechioidea	Oecophoridae	Scoble (1995) (AS, FE)
	Gelechiidae	Stehr (1987) (AS), Scoble (1995) (AS, FE) and C. Loeffler, pers. comm. (FE)
Tortricoidea	Tortricidae	Mackay (1962) (AS), Stehr (1987) (AS) and J. Brown, pers. comm. (AS, FE)
Zygaenoidea	Megalopygidae	Epstein (1996) (AS, FE)
	Somabrachyidae	Epstein (1996) (AS, FE)
	Zygaenidae	Epstein (1996) (AS, FE)
	Aididae	Epstein (1996) (AS, FE)
	Limacodidae	Stehr (1987) (AS, FE) and Epstein (1996) (AS, FE)
	Dalceridae	Stehr (1987) (AS, FE) and Epstein (1996) (AS, FE)
Drepanoidea	Drepanidae	Scoble (1995) (AS, FE)
Hedyloidea	Hedyliidae	Scoble (1995) (AS, FE)
Hesperioidea	Hesperiidae	Frohawk (1892, 1913) (AS, FE), Caveney <i>et al.</i> (1998) (AS, FE) and M.R.W., pers. obs. (AS, FE)
Papilionoidea	Pieridae	Stehr (1987) (AS), D. Harvey, pers. comm. (FE) and K. Wolfe, pers. comm. (FE)
	Nymphalidae	D. Harvey, pers. comm. (FE) and K. Wolfe, pers. comm. (FE)
Noctuoidea	Notodontidae	M.R.W., pers. obs. (AS, FE)
	Arctiidae	Rawlins (1984) (FE)

AS, Presence of anal structure; FE, report of frass ejection.

attack by visually oriented predators (Bernays 1997). Thus, larvae that eject frass from shelters may limit both costs of shelter reconstruction and exposure to predators.

Hypothesis 3 (Natural enemies): Frass ejection behaviour eliminates cues that could signal the location of the larva to potential enemies.

Rationale: Numerous laboratory studies have demonstrated that hymenopteran parasitoids respond to the odour of host frass (e.g. Nordlund & Lewis 1985; Takabayashi & Takahashi 1989; Agelopoulos & Keller 1994; Mattiacci & Dicke 1995; Steidle & Fischer 2000). Comparatively less is known about the response of predators to frass; however, some predatory beetles have been shown to respond to frass and frass volatiles (Wainhouse *et al.* 1992; Royer & Boivin 1999). Thus, ejection of frass from a shelter in which a larva spends most of its time could eliminate potential cues for enemies.

MATERIALS AND METHODS

Study organism

Epargyreus clarus is a large skipper that is common through much of the continental United States, and uses herbaceous

and tree species in the pea family (Leguminosae) as hosts (Scott 1986). Larvae of all instars construct and inhabit shelters of cut or folded leaves and silk, successively abandoning shelters and building new ones approximately five times across five instars (Lind *et al.* 2001). They spend most of their time resting on the 'ceiling', and venture out only to feed for brief periods (Lind *et al.* 2001).

Epargyreus clarus larvae were obtained from eggs of butterflies caught on the Georgetown University campus, placed in a 2 m³ outdoor flight cage, and provided with freshly cut *Pueraria lobata* (kudzu) leaves for oviposition. Leaves were taken from a large kudzu patch on the Georgetown campus. Larvae were housed in covered plastic shoeboxes (0.3 × 0.2 × 0.1 m³) and provided with freshly cut kudzu leaves as needed until pupation; boxes were cleaned daily.

Frass ejection distance

To quantify the distance that *E. clarus* larvae eject their frass pellets, I placed individual sheltered larvae on large sheets of clean white paper (first to third instars) or on a clean, light-coloured floor (fourth and fifth instars). Every

15 min for 4 h, I checked the paper or floor for fresh pellets, and measured the linear distance from the edge of the originating shelter to the pellet. Pellets largely maintain their shape when they land, and generally do not bounce or roll; they contain about 67% water, by weight [mass of six fresh fifth instar pellets (mean \pm SE) = 24 ± 1.6 mg, $n = 10$ groups of pellets; mass of six dried fifth instar pellets = 7.84 ± 0.3 mg, $n = 10$ groups of pellets]. I plotted the mean of at least three frass shots per larva, for 12 larvae of each instar, and determined the best linear fit for the data.

Tests of hypotheses: hygiene

To assess the effect on *E. clarus* of extended exposure to frass and its associated microorganisms, I reared caterpillars from hatching through pupation on kudzu leaves, each inside small ($5 \times 15 \times 20$ cm) covered plastic boxes from which frass was either removed daily or left to accumulate for the entire 30-day larval period. Hatchling larvae were placed individually on freshly cut kudzu leaves inserted into 'aqua-piks' and were randomly assigned to either the 'clean' or 'frassy' treatment. I removed the frass from the 'clean' boxes daily, using paper towels to lightly brush the frass from leaf and box surfaces. The leaves in the frassy treatment were also lifted each day to control for disturbance. Every 5 days, all larvae were removed from their shelters and weighed on a Mettler Toledo AB54 balance, and were then replaced on fresh, field-collected leaves. Pupae were weighed 3 days after pupation. The experiment was conducted in 1998 with 61 larvae and repeated in 1999 with 50 larvae.

In 1998, I compared the growth trajectories of larvae in clean and frassy treatments using repeated measures ANOVA on larval and pupal mass. In 1999, I sexed the pupae, and so used repeated measures ANOVA with sex and treatment as independent variables. In both years, I used two-tailed *t*-tests to compare number of days to pupation, and I assessed survival to pupation with a chi-square test of independence. Fungal samples collected from the frassy treatment were identified by Dr Richard Humber, a USDA mycologist specializing in entomopathogenic species.

Tests of hypotheses: crowding

In preliminary studies, I found that when I inserted large quantities of frass into an occupied shelter, such that the interior volume was reduced by about half, the larva quickly left, and moved on to build a new shelter. Thus, to assess the crowding hypothesis, I measured the relative energetic cost of building additional shelters. I removed larvae from their shelters periodically so that they were forced to build new shelters, either 50, 100 or 200% more often than their

respective controls, and compared the performance of each pair of treatments and controls. For each level of disturbance, newly emerged caterpillars were randomly assigned to a treatment or control group. The 50% increase group (tested in 1998) was made to build new shelters every 4 days; the 100% group (tested in 1999) was made to build new shelters every 3 days, and the 200% group (tested in 1999) was made to build new shelters every day.

On disturbance days I opened the shelters of the treatment caterpillars, gently removed the larva with a paintbrush or by hand, and replaced it on another leaflet of the kudzu leaf. After the first instar, control larvae were gently poked inside their shelters with a paintbrush to control for disturbance. Fresh leaves were provided to all larvae in both treatments every 5 or 6 days. Larvae were weighed on leaf change days until they became pre-pupae, and then again 3 days after pupation.

Within each pair of treatments and controls, I used two-tailed *t*-tests to compare overall larval weight gain from days 6–31 (days 7–25 for the 50% increase group), pupal weight, and days to pupation. Survival to pupation in each set of treatments and controls was compared using a chi-square test of independence.

Relative risk of predation for sheltered vs. unsheltered caterpillars by visually oriented predators was assessed in another study (Jones *et al.* 2002).

Tests of hypotheses: natural enemies

To determine whether predatory *Polistes fuscatus* wasps respond to the odour of *E. clarus* frass, I affixed either six pellets of fresh frass (mean \pm SE = 24 ± 1.6 mg) or an equivalent number of visually similar black glass beads to a small piece of scotch tape, and inserted it into one of two shelters recently made by third instar larvae on a kudzu leaf (see Fig. 4). The larvae were first removed from the shelters, and neither frass pellets nor beads were visible from without. I placed the leaf assembly inside a mesh or plexiglas cage ($0.5 \times 0.5 \times 1.5$ m³) containing an actively foraging *P. fuscatus* colony, and recorded the number of visits to and time spent by individually marked foragers on the shelters and leaflets.

To determine whether *E. clarus* caterpillars were more vulnerable to predation in the presence of frass, I offered individual *P. fuscatus* wasps a choice of two similarly sized kudzu leaflets, each bearing a sheltered second or third instar caterpillar, and either frass or a visual control. Shelters were partially opened, but the larvae were hidden from view. About 25 pellets of fresh fifth instar frass or a similar number of black matte glass beads (1-mm diameter) were placed in the centre of each kudzu leaflet; leaflets were placed flat on the floor of the cage, approximately 30-cm apart. Only one wasp was tested at a time; other foragers in the colony were collected in

small plastic cups and removed from the cage. I recorded which caterpillar the wasp discovered first, the amount of time it took the wasp to discover the caterpillar, and which caterpillar the wasp killed first. I conducted 17 trials, alternating leaflet positions each trial. Trials lasted for 5 min, or until the first caterpillar was killed. Results were assessed with a chi-square goodness-of-fit test.

RESULTS

Frass ejection

Epargyreus clarus larvae of all stages forcefully eject their faecal pellets, whether inside or outside of the shelter; those

inside exert the end of their abdomens through a small opening in the shelter before firing a pellet. Larvae eject their frass pellets considerable distances (Fig. 2); the longest distance observed was 153 cm, for a fifth instar larva. The ratio of shot distance to larval length ranges from a low of about seven to a high of about 39, with an average for all instars of about 19 times body length (Fig. 2).

Tests of hypotheses: hygiene

Fungal hyphae were clearly visible and abundant on the accumulated waste in the frassy treatment by days 15–20, and at no point were hyphae seen in the clean treatment. Nevertheless, the two groups of larvae performed similarly

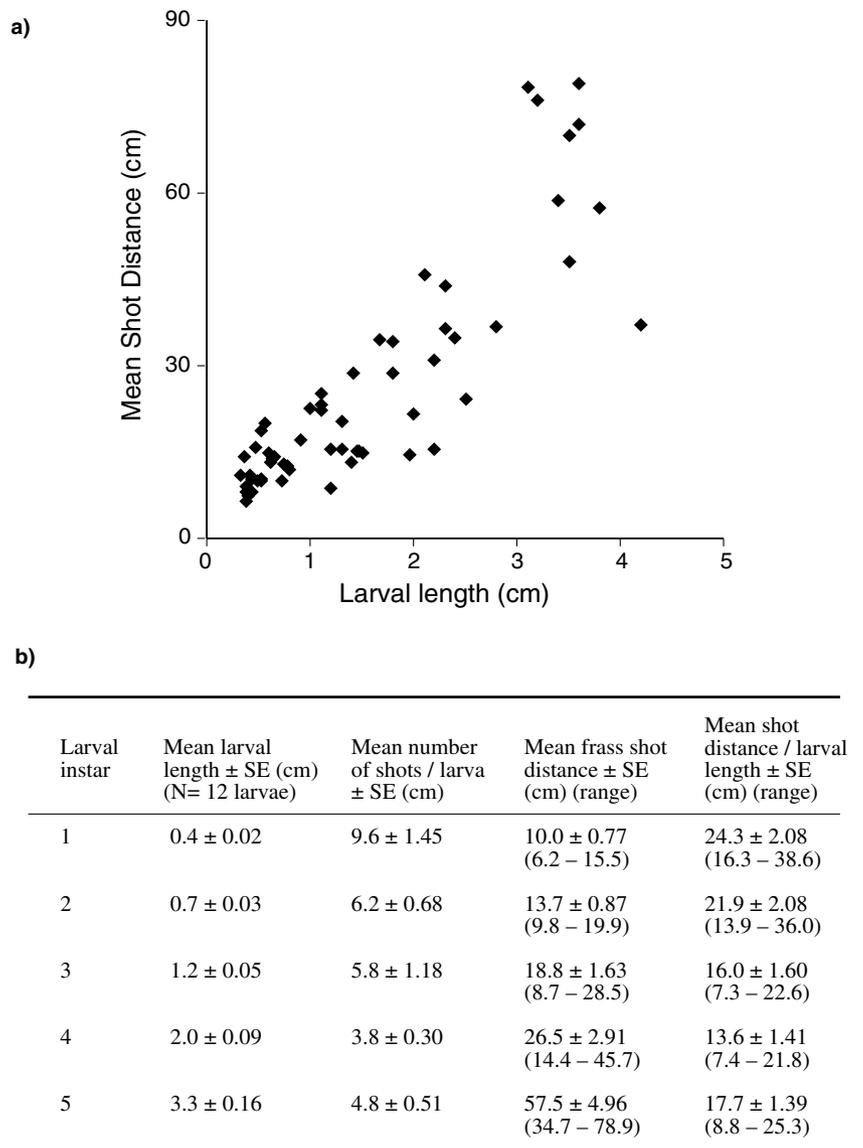


Figure 2 (a) Mean frass shot distance increases with larval length. Each data point represents the mean of at least three frass shots made by an individual larva within 4 h. Twelve larvae of each stadium (1–5) are included. Linear fit: mean shot distance = $2.05 + 15.44$ larval length; $P < 0.001$, $r^2 = 0.74$. (b) Frass shot distance data.

in both years, as indicated by repeated measures ANOVAS on larval and pupal mass (1998: time \times treatment, $F_{5,28} = 0.8508$, n.s.; 1999: time \times treatment, $F_{6,27} = 1.370$, n.s.) (Fig. 3). The interaction between pupal sex, treatment, and time, included in the model for 1999, was not significant ($F_{6,27} = 0.3334$, n.s.).

Larvae did not differ in days to pupation in either year (1998: two-tailed t -test, $t_{44} = 1.7676$, n.s.; 1999: $t_{39} = -0.484$, n.s.). Survival to pupation was significantly higher for the clean treatment in 1998 ($\chi^2 = 4.584$, $P < 0.05$), but did not differ between treatments in 1999 ($\chi^2 = 0.1165$, n.s.).

Fungi growing on frass were identified as non-entomopathogenic, generalist species, including *Scopulariopsis* sp., *Penicillium* sp., and a mucoralean zygomycete (R. Humber, pers. comm.).

Tests of hypotheses: crowding

Treatment and control groups for all disturbance levels differed significantly in number of shelters constructed

(Table 2). Days to pupation did not differ between treatments for any of the disturbance levels, and larval weight gain and pupal mass differed only in the most extreme (200%) treatment, when caterpillars were made to build 32 shelters, rather than nine (Table 2). In none of the three trials was there a significant difference between the proportion of control and treatment larvae that survived to pupation (Table 2).

Tests of hypotheses: natural enemies

Polistes fuscatus foragers ($n = 10$) visited the leaflets with shelters containing frass significantly more than they did those containing beads (mean percentage of visits to bead vs. frass shelter and leaflet, $t_2 = 6.417$, 9 d.f., $P < 0.001$); they also spent a significantly larger percentage of time on the frassy shelters and leaflets (mean percentage of time spent on bead vs. frass shelter and leaflet, $t_2 = 7.518$, 9 d.f., $P < 0.001$) (Fig. 4). Wasps that visited the shelters included both 'experienced' foragers, which had previously killed an

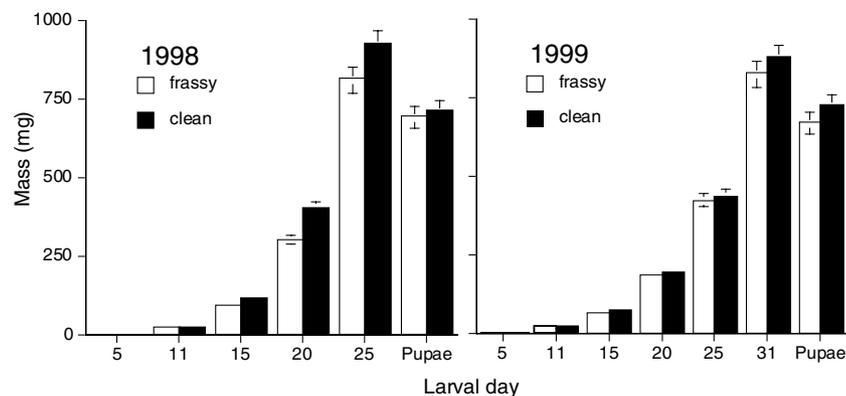


Figure 3 *Epargyreus clarus* larvae reared under clean or 'frassy' conditions did not differ significantly in growth trajectory in 1998 or 1999. Repeated measures ANOVA, time \times treatment: 1998: $F_{5,28} = 0.8508$, n.s.; 1999: $F_{6,27} = 1.37$, n.s.

Table 2 Cost of increased shelter building. Larvae were made to build increased numbers of shelters relative to their respective control groups. For all disturbance levels, treatment groups made significantly more shelters than did controls (50% increase: $t = 17.003$, 30 d.f., $P < 0.001$; 100% increase: $t = -13.065$, 38 d.f., $P < 0.001$; 200% increase: $t = 22.5537$, 30 d.f., $P < 0.001$). Larval weight gain and pupal mass differed between treatments and controls only in the 200% increase group (larval weight gain: $t_2 = 2.619$, 33 d.f., $P = 0.0132$; pupal mass: $t_2 = 3.784$, 30 d.f., $P < 0.001$)

	50%		100%		200%	
	Control ($n = 15$ larvae)	Increase ($n = 17$ larvae)	Control ($n = 19$ larvae)	Increase ($n = 21$ larvae)	Control ($n = 15$ larvae)	Increase ($n = 17$ larvae)
No. of shelters constructed ($\bar{X} \pm$ SE)	8.67 \pm 0.34	11.94 \pm 0.32**	8.84 \pm 0.38	15.76 \pm 0.37**	9.20 \pm 0.73	31.89 \pm 0.69**
Days to pupation ($\bar{X} \pm$ SE)	33.07 \pm 1.16	33.52 \pm 1.09	44.58 \pm 0.95	44.24 \pm 0.91	43.67 \pm 0.91	44.50 \pm 0.86
Larval weight gain, days 6–31 (g) ($\bar{X} \pm$ SE)	0.76 \pm 0.09	0.78 \pm 0.09	0.78 \pm 0.07	0.64 \pm 0.06	0.81 \pm 0.07	0.56 \pm 0.07*
Pupal mass (g) ($\bar{X} \pm$ SE)	0.72 \pm 0.02	0.74 \pm 0.02	0.83 \pm 0.03	0.78 \pm 0.03	0.83 \pm 0.02	0.70 \pm 0.02**
Survived to pupation	15 of 17	17 of 19	19 of 33	21 of 33	15 of 33	17 of 34

Asterisks indicate significant differences between control and increase treatments within a disturbance level. * $P < 0.05$; ** $P < 0.001$.

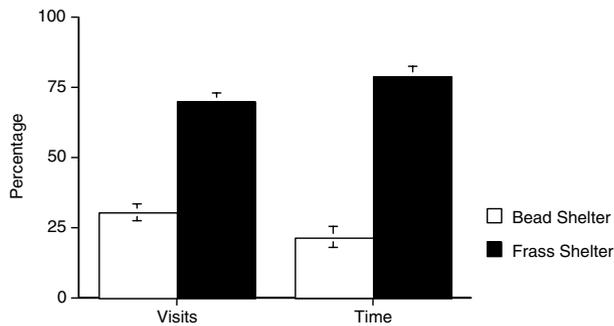


Figure 4 *Polistes fuscatus* foragers ($n = 10$ wasps) responded preferentially to shelters containing frass pellets rather than beads. Mean percentage of visits to bead shelter and leaflet vs. frass shelter and leaflet, $t_2 = 6.417$, 9 d.f., $P < 0.001$; mean percentage of time spent on bead shelter and leaflet vs. frass shelter and leaflet, $t_2 = 7.518$, 9 d.f., $P < 0.001$.

E. clarus larva, and 'naïve' foragers, which had not; t -tests showed no difference in behaviour between the groups.

Larvae on frass-bearing leaflets were significantly more likely to be killed during a 5-min trial than were larvae with beads on their leaflets. Only three of the 17 frass-associated larvae survived a 5-min trial with a foraging wasp, compared to 14 of the bead-associated larvae ($\chi^2 = 7.12$, 1 d.f., $P < 0.01$). Again, both naïve and experienced wasps participated in these trials, and showed no difference in behaviour.

DISCUSSION

Natural enemies

Of the three hypotheses tested here to account for the occurrence of frass ejection behaviour in *E. clarus* larvae, my experimental results provide the strongest support for the natural enemies hypothesis, and only weak support for the hygiene and crowding hypotheses. I found that predatory *Polistes* wasps are attracted to the odour of larval frass, and that larvae in close proximity to frass are more vulnerable to wasp attack. Thus, because presence of frass odours negatively impacts larval survival, removal of such olfactory cues is likely to have been a driving force behind the evolution of frass ejection behaviour.

Predation is a significant source of mortality for *E. clarus*, even in the absence of frass-associated cues. About 30% of sheltered fourth instar larvae placed out in a large kudzu patch on the Georgetown campus and censused twice daily were taken by *Polistes* and other predators within 2 days; parasitism rates of larvae collected from the same site were very low (unpublished data). In the neotropics, shelter-building caterpillars as a group are particularly vulnerable to parasitoid attack (Gentry & Dyer 2002). Presumably,

mortality from predation or parasitism in all systems would be even higher if frass pellets in or near a shelter provided additional cues for natural enemies.

Although hymenopteran parasitoids are well known to respond to chemical cues from frass and other odour sources, few studies have examined the response of predatory insects to such odours (Vet & Dicke 1992; also see references in 'Rationale' for Hypothesis 1, above). I have found that *Polistes* wasps respond to the odour of *E. clarus* frass whether or not they have had prior experience with *E. clarus* larvae; they also respond to the frass of other prey species reared on different host plants (unpublished data). Because *Polistes* wasps are widespread generalist predators (Raveret Richter 2000), the attraction to frass reported here suggests that such olfactory cues may contribute to *Polistes*' foraging strategy for a range of prey items.

Hygiene

Avoidance of exposure to pathogens is an obvious explanation for frass ejection behaviour (Scoble 1995), and was, in fact, the first interpretation offered for the phenomenon: Frohawk (1892), describing frass ejection in a skipper caterpillar, concluded that the behaviour by the larva "undoubtedly is a means to prevent fouling its domicile." It appears, however, that prolonged exposure to frass and its associated fungal or other colonists is not an important morbidity or mortality factor for *E. clarus*, at least under my experimental conditions, as larvae in clean and frassy treatments did not differ in growth trajectory or days to pupation, and survival to pupation was higher for the clean treatment in only one of the 2 years.

The fungi growing on the frass pellets were found to be generalist, non-entomopathogenic taxa. Because leaves used in the experiment were collected from the field site and were not washed, it is likely that they carried on their surfaces a representative sample of the natural microflora so that the colonists in the boxes represented a sub-sample of the population that was able to survive transit through the larval gut (R. Humber, pers. comm.). Bacteria and viruses are almost certain to be present in the frass, but as performance of the clean and frassy treatments did not differ significantly, I presume that those present in this study were not pathogenic to larvae.

Finally, though many shelter-dwelling larva eject their frass, some species that live in shelters or other enclosed spaces, e.g., some pyralids (Ruehlmann *et al.* 1988) and tent caterpillars (Fitzgerald 1995) do not, lending further support to the idea that close contact with faecal material is not necessarily harmful in and of itself. It is, of course, possible that these larvae have developed ways to mediate or tolerate any adverse effects resulting from close association with their own frass.

Crowding

Results of this study lend little support to the crowding hypothesis. The cost of shelter construction seems unlikely to have been a driving force behind the evolution of faecal firing in this system, as a fitness effect was not apparent even when treatment larvae built double the number of shelters made by their controls. A cost was evident only in the most extreme treatment, when larvae constructed over three times as many shelters as did their controls, a situation that seems unlikely to occur in nature. The increased exposure that would result from any additional shelter building, however, could make larvae more vulnerable to predation. When *Polistes* wasps were offered a choice of a sheltered vs. an unsheltered *E. clarus* caterpillar, the unsheltered caterpillar was discovered and killed before the sheltered caterpillar in 17 out of 17 trials (Jones *et al.* 2002). Though I cannot rule out the importance of increased exposure to visual predators, the time required by *E. clarus* for shelter construction varies over six-fold among individuals of a given larval instar (unpublished data), arguing against strong selection to minimize exposure to enemies.

Why shoot so far?

Epargyreus clarus and other skipper larvae shoot their frass pellets up to nearly 40 body lengths away, at speeds of 1.3 m s^{-1} or more (Caveney *et al.* 1998). For *E. clarus*, however, a shot distance of a few centimetres or so would generally be sufficient to propel the pellet off the leaflet surface. Why then shoot so far and so fast? It is possible that these extremes are unrelated to the adaptive significance of the behaviour, and may instead be a by-product of larval physiology. In caterpillars belonging to at least 13 families, haemolymph pressure in the last abdominal segment is raised considerably during defecation, perhaps as a means of moving haemocytes over lung-like 'aerating tracheae' located there (Locke 1998). This transient elevation in haemolymph pressure may have served as a 'pre-adaptation', which, in conjunction with the evolution of a rigid anal comb, could then have come to power a projectile mechanism to discharge frass pellets (Caveney *et al.* 1998). Thus, while removal of frass from close proximity to the larva may be an adaptation to reduce locational cues for natural enemies, the speed and distance of frass ejection in skipper larvae may represent a co-option of available larval physiology.

CONCLUSIONS

Predatory and parasitic insects depend to varying degrees on visual and olfactory cues to locate insect prey. Such cues may originate from a number of sources, including the prey itself, its food, or interactions between prey and food, such

as feeding damage and waste production (Witz 1989; Vet & Dicke 1992; Gross 1993). Interestingly, some parasitoids do not respond to the odour of the host insect itself, but respond strongly to the odour of its frass (e.g. Steidle & Schöller 1997), presumably because selection would act against emission of host volatiles that could provide locational information to parasitoids, but would not directly affect emission of volatiles from feeding damage or defecation (Vet & Dicke 1994). Frass ejection behaviour allows larvae to distance themselves from olfactory cues that might provide information to their enemies, producing an olfactory crypsis for sheltered larvae. Analogously, mobile (non-shelter-building) caterpillars can snip off partially eaten leaves, or rest at a distance from an unfinished leaf, to reduce the availability or reliability of visual cues for enemies (Heinrich 1979).

Further investigation is necessary to determine whether the results of these studies can be generalized to other systems. However, based on the attractiveness of frass to generalist parasitoids and predators, it is possible that frass ejection behaviour could reduce vulnerability to natural enemies not just for *E. clarus*, but for other frass-ejecting taxa as well. Additional selection pressures could be important in other systems, depending on factors such as climate, larval susceptibility to pathogens, and larval behaviour. Infrequent outbreaks of uncommon pathogens could also be important for *E. clarus* and other larvae. It is also possible that in some frass-ejecting taxa the trait serves no current function, and its presence in a taxon reflects the phylogenetic history of the group.

ACKNOWLEDGEMENTS

I am grateful to Eric Lind, Meg Jones, Erin Wilson, Melissa Conrad, Regina Ramirez, Stephanie Hsia, Linda Aucoin, Jeremy Long, John McClure, Jen Maupin, and Ignacio Castellanos for help with the experiments and field work; Richard Humber at the USDA-ARS Collection of Entomopathogenic Fungal Cultures, Ithaca, NY, for identification of fungal species; Carol von Dohlen, Neal Williams, Pedro Barbosa, John Weiss, Anurag Agrawal, Elizabeth Bernays, Josh Rosenthal, the GU Ecology, Evolution, and Behavior Group, and three anonymous reviewers for helpful comments on the manuscript; and to the USDA CSREES and Georgetown University for funding.

REFERENCES

- Agelopoulos, N.G. & Keller, M.A. (1994). Plant–natural enemy association in the tritrophic system, *Cotesia rubecula*–*Pieris rapae* Brassicaceae (Cruciferae): I. Sources of infochemicals. *J. Chem. Ecol.*, 20, 1725–1734.

- Berenbaum, M.R., Green, E.S. & Zangerl, A.R. (1993). Web costs and web defense in the parsnip webworm (Lepidoptera: Oecophoridae). *Environ. Entomol.*, 22, 791–795.
- Bernays, E.A. (1997). Feeding by lepidopterous larvae is dangerous. *Ecol. Entomol.*, 22, 121–123.
- Boonstra, R., Krebs, C.J. & Kenney, A. (1996). Why lemmings have indoor plumbing in summer. *Can. J. Zool.*, 74, 1947–1949.
- Brown, G.E., Chivers, D.P. & Smith, R.J.F. (1996). Effects of diet on localized defecation by northern pike, *Esox lucius*. *J. Chem. Ecol.*, 22, 467–475.
- Caveney, S., McLean, H. & Surry, D. (1998). Faecal firing in a skipper caterpillar is pressure-driven. *J. Exp. Biol.*, 201, 121–133.
- Damman, H. (1987). Leaf quality and enemy avoidance by the larvae of a pyralid moth. *Ecology*, 68, 88–97.
- Dominguez-Romero, M. (1996). Sistemática i ecològica dels tineïds de la Península Ibèrica (Lepidoptera). Tesi Doctoral, Universitat de València.
- Eisner, T. & Eisner, M. (2000). Defensive use of fecal thatch by a beetle larva (*Hemisphaerota cyanea*). *Proc. Natl. Acad. Sci. USA*, 97, 2632–2636.
- Epstein, M.E. (1996). Revision and phylogeny of the Limacodid-Group families, with evolutionary studies on slug caterpillars (Lepidoptera: Zygaenoidae). *Smithsonian Institution Contributions to Zoology*, 582.
- Fitzgerald, T.D. (1995). *The Tent Caterpillars*. Cornell University Press, Ithaca, NY.
- Frohawck, F.W. (1892). Life history of *Carterocephalus palaemon*. *The Entomologist*, 25, 256.
- Frohawck, F.W. (1913). Faeces ejector of lepidopterous larvae. *The Entomologist*, 46, 200–202.
- Gentry, G.L. & Dyer, L.A. (2002). On the conditional nature of neotropical caterpillar defenses against their natural enemies. *Ecology*, 83, 3108–3119.
- Gerasimov, A.M. (1952). *Insects – Lepidoptera. Part 1, Caterpillars*. Zoological Institute of the Academy of Sciences USSR, Fauna S.S.S.R., 56, 1–338. (In Russian).
- Gross, P. (1993). Insect behavioral and morphological defenses against parasitoids. *Ann. Rev. Entomol.*, 38, 252–273.
- Hajek, A.E. & St Leger, R.J. (1994). Interactions between fungal pathogens and insect hosts. *Ann. Rev. Entomol.*, 39, 293–322.
- Hart, A.G. & Ratnieks, F.L.W. (2001). Task partitioning, division of labour and nest compartmentalisation collectively isolate hazardous waste in the leaf-cutting ant *Atta cephalotes*. *Behav. Ecol. Sociobiol.*, 49, 387–392.
- Heinrich, B. (1979). Foraging strategies of caterpillars: leaf damage and possible predator avoidance strategies. *Oecologia*, 42, 325–337.
- Hölldobler, B. & Wilson, E.O. (1990). *The Ants*. Belknap Press of Harvard University Press, Cambridge, MA.
- Jones, M.T., Castellanos, I. & Weiss, M.R. (2002). Do leaf shelters always protect larvae from invertebrate predators? *Ecol. Entomol.*, 27, 753–757.
- Kenagy, G.J. & Hoyt, D.F. (1980). Reingestion of feces in rodents and its daily rhythmicity. *Oecologia*, 44, 403–409.
- Lind, E.M., Jones, M.T., Long, J.D. & Weiss, M.R. (2001). Ontogenetic changes in leaf shelter construction by larvae of *Epar-gyreus clarus* (Hesperiidae), the silver-spotted skipper. *J. Lepid. Soc.*, 54, 77–82.
- Locke, M. (1998). Caterpillars have evolved lungs for hemocyte gas exchange. *J. Insect Physiol.*, 44, 1–20.
- Mackay, M.R. (1962). Larvae of the North American Tortricinae (Lepidoptera: Tortricidae). *Can. Entomol. Suppl.*, 28, 1–182.
- Mattiacci, L. & Dicke, M. (1995). The parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae) discriminates between first and fifth larval instars of its host *Pieris brassicae* on the basis of contact cues from frass, silk and herbivore-damaged leaf tissue. *J. Insect Behav.*, 8, 485–498.
- Nordlund, D.A. & Lewis, W.J. (1985). Response of females of the braconid parasitoid *Microplitis demolitor* to frass of larvae of the noctuids, *Heliothis zea* and *Trichoplusia ni* and to 13-methylhentriacontane. *Entomol. Exp. Appl.*, 38, 109–112.
- NRC (National Research Council) (2000). *The Future Role of Pesticides in US Agriculture*. National Academy Press, Washington, DC.
- Ralls, K. (1971). Mammalian scent-marking. *Science, New Series* 171, 443–449.
- Ravert Richter, M. (2000). Social wasp (Hymenoptera: Vespidae) foraging behavior. *Ann. Rev. Entomol.*, 45, 121–150.
- Rawlins, J.E. (1984). Mycophagy in Lepidoptera. In: *Fungus-Insect Relationships* (eds Wheeler, Q. & Blackwell, M.). Columbia University Press, New York, pp. 382–423.
- Royer, L. & Boivin, G. (1999). Infochemicals mediating the foraging behaviour of *Aleochara bilineata* Gyllenhal adults: source of attractants. *Entomol. Exp. Appl.*, 90, 199–205.
- Ruehlmann, T.E., Matthews, R.W. & Matthews, J.R. (1988). Roles for structural and temporal shelter-changing by fern-feeding lepidopteran larvae. *Oecologia*, 75, 228–232.
- Ruggiero, M.A. & Merchant, H.C. (1986). Estimated energy budget for a population of eastern tent caterpillars (Lepidoptera: Lasiocampidae) in Maryland. *Environ. Entomol.*, 15, 795–799.
- Schoener, T.W. (1971). Theory of feeding strategies. *Ann. Rev. Ecol. Syst.*, 2, 369–404.
- Scoble, M.J. (1995). *The Lepidoptera: Form, Function and Diversity*. Oxford University Press, Oxford.
- Scott, J.A. (1986). *The Butterflies of North America: a Natural History and Field Guide*. Stanford University Press, Stanford.
- Stehr, F.W. (1987). *Immature Insects*. Kendall/Hunt, Dubuque, IO.
- Steidle, J.L.M. & Fischer, A. (2000). Quantity does matter: how feces are used for host stage selection by granary weevil parasitoid *Lariophagus distinguendus*. *J. Chem. Ecol.*, 26, 2657–2664.
- Steidle, J.L.M. & Schöller, M. (1997). Olfactory host location and learning in the granary weevil parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *J. Insect Behav.*, 10, 331–342.
- Stewart, P.D., Macdonald, D.W., Newman, C. & Cheeseman, C.L. (2001). Boundary faeces and matched advertisement in the European Badger (*Meles meles*): a potential role in range exclusion. *J. Zool.*, 255, 191–198.
- Takabayashi, J. & Takahashi, S. (1989). Effects of host fecal pellet and synthetic kairomone on host-searching and post-oviposition behavior of *Apanteles kariyai*, a parasitoid of *Pseudaletia separata*. *Entomol. Exp. Appl.*, 52, 221–227.
- Troyer, K. (1982). Transfer of fermentative microbes between generations in a herbivorous lizard. *Science*, 216, 540–542.

- Vet, L.E.M. & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Ann. Rev. Entomol.*, 37, 141–172.
- Wainhouse, D., Beech-Garwood, P.A., Howell, R.S., Kelly, D. & Orozco, M.P. (1992). Field response of predator *Rhizophagus grandis* to prey frass and synthetic attractants. *J. Chem. Ecol.*, 18, 1693–1705.
- Winter, W.D. (2000). *Basic Techniques for Observing and Studying Moths and Butterflies*. The Lepidopterists' Society, Los Angeles.
- Witz, B.W. (1989). Antipredator mechanisms in arthropods: a twenty year literature survey. *Fla. Entomol.*, 73, 73–99.

- Wotton, R.S. & Malmqvist, B. (2001). Feces in aquatic ecosystems. *Bioscience*, 51, 537–544.

Manuscript received 3 October 2002
First decision made 12 November 2002
Second decision made 13 January 2003
Manuscript accepted 28 January 2003