Reduction of oviposition time and enhanced larval feeding: two potential benefits of aggregative oviposition for the viburnum leaf beetle

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Abstract. 1. Although aggregation in non-social arthropods is common, its adaptive value is not always clear. Oviposition behaviour of the viburnum leaf beetle (*Pyrrhalta viburni*) is aggregative, with females preferring to lay eggs on twigs already infested by conspecifics. We previously showed that aggregative oviposition aided in overcoming host plant defences. Here we explore two additional benefits of this behaviour: reduction of time investment in oviposition; and improvement of host use by larval group feeding.

2. In video-recorded trials, the presence of conspecific egg masses did not have an impact on the time associated with oviposition, but it did affect females' pre-oviposition behaviour: the length of foraging movements was 21% shorter on infested than on non-infested twigs, and was concentrated near the infested area.

3. In two separate studies of insect performance, larvae were reared to maturity in groups of different densities (five or 10, and five or 20, respectively) on several hosts (four and 17 *Viburnum* spp., respectively). Independent of host species, adults were > 13% heavier (groups of 10 vs. five), and pupation success was 27% higher (groups of 20 vs. five) at higher larval density.

4. Two additional effects were dependent on host species: larval survivorship (groups of 10 vs. five), and adult mass (groups of 20 vs. five). These effects were not associated with plant defensive traits (i.e. trichomes or leaf toughness), but adult mass was associated with host nutritional quality (i.e. foliar nitrogen content).

5. These results show that several traits of *P. viburni* larval performance are positively density-dependent, and suggest that benefits of group feeding could have played a role in the evolution of oviposition behaviour in *P. viburni*. Our results also demonstrate that plant quality can mediate benefits of aggregation, underscoring the importance of ecological context in understanding the feeding strategies of insects.

Key words. Benefits of group feeding, feeding strategies, improved host use, nutritional ecology, oviposition behaviour, social stimulation, subsocial insects.

Introduction

Gregarious behaviours (i.e., active aggregations of conspecifics) are common in non-social arthropods and their adaptive value has received considerable attention (Stamp, 1980; Prokopy & Roitberg, 2001; Costa, 2006). For immature insects, the documented benefits of aggregations fall into two main categories: benefits associated with protection from natural enemies via enhanced aposematism (Sillén-tullberg, 1990; Gamberale & Tullberg, 1996), active group defences (Stamp, 1980) or enemy dilution (Turchin & Kareiva, 1989; Lawrence, 1990), and benefits improving insect performance (feeding facilitation), which can occur through improved host location (Weed, 2010) or resource use (Denno & Benrey, 1997; Fordyce, 2003). Mechanisms of improved resource use often rely on collectively overcoming specific plant defences (Young & Moffett, 1979; Nahrung *et al.*, 2001), or inducing

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a physiological change in the plant allowing optimal feeding efficiency (Fordyce & Nice, 2004). Consequently, the effects of group feeding on insect performance are expected to be host-dependent and associated with specific plant traits. For example, variation in secondary chemistry and nutritional content can have an impact on the benefits of gregariousness (Young & Moffett, 1979; Lawrence, 1990), and has been proposed to explain the evolution of egg clustering in some butterflies (Stamp, 1980; Chew & Courtney, 1991). Despite this notion, few studies investigating aggregation have compared the benefits of gregariousness on more than a few host species (Lawrence, 1990; Reader & Hochuli, 2003; Fordyce & Nice, 2004).

The viburnum leaf beetle (VLB) [*Pyrrhalta viburni* (Paykull)] is a univoltine chrysomelid native to Eurasia and invasive in north-eastern North America (Weston *et al.*, 2007). Females lay their eggs in masses (approx. eight eggs per mass) inside round cavities excavated through the bark and the pith of terminal twigs of their host plants. Cavities filled with eggs are then covered with a 'cap' made of a frass-like secretion (length 2–4 mm) (Weston *et al.*, 2008). We recently discovered that VLB oviposition is aggregative: females prefer to lay eggs on twigs already infested by conspecifics, and position their egg masses adjacent to existing egg masses, forming aligned clusters along infested twigs (Desurmont & Weston, 2011). Consequently, larvae often occur at high densities on the leaves near egg masses: although they do not feed gregariously in the strict sense, they do tend to form loose feeding groups on the undersides of leaves (G. A. Desurmont, pers. obs.). In its introduced range, dense egg aggregations often lead to complete defoliation of susceptible North American *Viburnum* shrubs, often resulting in shrub death after 2–4 years of repeated infestation (Weston *et al.*, 2007). After completing their development, third-instar larvae crawl to the ground and burrow into the soil where they form individual pupation chambers made of soil particles within 3 cm of the surface (Weston & Desurmont, 2008).

One realised fitness benefit of the aggregative oviposition behaviour for VLB is to overcome a plant defensive reaction: the production of plant wound tissue that crushes or expels eggs from their cavities (Desurmont & Weston, 2011). Heavily infested twigs often die, circumventing their capacity to produce a wound response, and egg survivorship is consequently positively associated with egg mass density. This correlation was consistent across 16 *Viburnum* species from Europe, Asia, and North America (Desurmont *et al.*, 2011), with the exception of *V. tinus*, a European species (Desurmont *et al.*, 2012). Here, we explore two potential additional benefits of aggregative oviposition. The first one is reduction of oviposition time: choosing twigs already infested may reduce the time spent by females searching for favourable oviposition sites, and laying egg masses adjacent to existing ones may facilitate boring through the bark of a presumably 'weakened' twig. The second benefit we explore is group feeding for larvae.

In order to investigate the effect of aggregative oviposition on time costs associated with oviposition, we estimated the duration of oviposition events (i.e. time to complete one oviposition) on infested and non-infested twigs, testing the hypothesis that oviposition duration is shorter on previously infested wigs. To test the hypothesis that insect performance increases with insect density, we reared VLB larvae at different densities on 17 *Viburnum* species from Europe, Asia, and North America and measured correlates of insect fitness (i.e. larval survivorship, development duration, pupation success, and adult mass). In addition, we assessed whether benefits or costs of group feeding were related to defensive traits (i.e. trichome density and leaf toughness) or nutritional quality (i.e. percentage of foliar nitrogen) of the hosts.

Material and methods

Insects and plant material

All insects used for the experiments were collected in the area of Ithaca, NY (USA), on unmanaged *Viburnum* spp. plants. VLB adults used for the oviposition time experiment were collected in the weeks preceding the trials and kept on *Viburnum opulus* L. and *Viburnum dentatum* L. shoots in rectangular plastic containers (30 × 22 × 10 cm) with vented screen lids. Adults were used only once in experiments. VLB larvae used for the group feeding experiment came from egg-infested twigs of *V. dentatum* and *V. opulus* collected in the field during the winter preceding the experiment. Eggs were held in the refrigerator until needed, and then transferred to an incubator maintained at 17 °C for 3 days, and finally to a chamber held at 22 °C until hatching. Newly hatched larvae were then transferred to one of the *Viburnum* test species. All plant material used for the experiments came from a common garden in Ithaca, NY, containing unmanaged mature shrubs of all the test species. Twigs used in the experiments were cut 72 h before and kept with the cut ends in water-filled floral tubes. Insect colonies, plant material, and experiments were kept in incubators at 22 °C under an LD 15:9 h photoperiod.

Description of VLB oviposition behaviour

In order to quantify the time associated with an oviposition event on infested and non-infested twigs, the oviposition behaviour of VLB females was video-recorded. Because females are thought to be more active during the night (Weston *et al.*, 2008), trials were recorded under red light in a darkened room. Females were taken from the laboratory colony immediately prior to the trials and were placed on the leaves of a *V. opulus* twig in a clear cylindrical container (height 21 cm, diameter 11 cm) with a vented screen lid. Twigs were selected to be homogeneous in length (12 cm) and were 2.5–3.0 mm in diameter. Each twig was placed leaning against one side of the container to present a clear upper- and underside to females, which typically prefer to lay eggs on the underside of twigs (Weston *et al.*, 2008). Two leaves were left on each twig for food. Female behaviour was then video-recorded with a Sony TRV67 Handy Cam using the

NightShot mode (which records infrared wavelengths of light produced by the camera's built-in IR illuminator). If a female was in the process of laying eggs at the end of the recording period, the trial was extended by 8 h. Videos of successful trials were then monitored and the behaviour of each female was analysed (discussed later). In total, 37 trials (21 on non-infested twigs and 16 on infested twigs) were monitored between July and September 2007. Infested twigs contained one to five egg masses that were deposited by conspecifics in the laboratory within 24 h prior to the trial.

We divided the oviposition process into three consecutive steps that consistently corresponded to the behaviour of females. Prior to oviposition, females make successive back-and-forth movements along the twig. These movements were divided into two categories: runs (continuous straight walking movement of a female along a twig) and turns (female executing a 180° turn on a twig). If a female stopped for more than 5 s during a run, the run was considered over. We defined this first step of the oviposition process as 'searching'. The length of each run, the total number of runs, and the total searching duration were recorded for each oviposition event. After searching, females place their body parallel to the twig, head facing down, and start chewing the egg cavity through the bark. Females usually do not interrupt chewing until engaging in egg-laying. This second step was defined as 'chewing', and the total time spent chewing the cavity was recorded. After chewing, females rotate 180° to start the process of egg-laying and producing the protective cap with their heads facing up. The frass-like protective secretion is produced and smeared over the egg cavity by females via repeated back-and-forth movements of the terminal abdominal segments. Females were never observed actively adding chewed bark to the secretion with their mouthparts in the process. We defined this step as 'egg-laying/cap making' (EC). The total duration of both activities combined was recorded because they could not be distinguished on the recordings. Once the egg cap is finished, females sometimes rotate again, apparently to inspect the fresh

egg cap by repeated contacts with the mouthparts and antennae for 1–10 min before walking away. This last aspect of the oviposition process was not included in the analysis.

Analysis of oviposition time on infested and non-infested twigs

The effect of previous infestation by conspecifics on the behavioural parameters measured was analysed by one-way ANOVA (JMP 8, SAS Institute Inc., Cary, North Carolina). Data were transformed using square root transformations to meet the assumptions of the model. An additional analysis was performed for trials where VLB laid more than one egg mass during the recording period. In such cases, data from the first oviposition event were included in the main analysis, and a separate analysis compared data from the first oviposition with data from the following oviposition event(s) in a paired *t*-test (one trial = one pair) (JMP 8).

Larval group-feeding experiments

Two separate experiments were conducted in June 2007 and May 2009, with unrelated insects randomly selected from our laboratory colony. In the first experiment, third-instar VLB larvae initially reared on leaves of *Viburnum trilobum* (syn: *V. opulus* var. *americanum*) were transferred and reared until the adult stage in groups of five and 10 on four *Viburnum* spp. (Table 1); in the second experiment, first-instar larvae were reared from egg hatch to the adult stage in groups of five or 20 on 17 *Viburnum* spp. (Table 1). For both experiments, five replicates of each larval density were exposed to each host species. Insects and plants were kept in clear, cylindrical plastic containers (8.5 × 8.5 cm) with a vented lid. Young shoots of the test plants were kept in floral tubes inside the containers and changed when needed. Once larvae reached the third instar, a thin layer (*c*. 3 cm) of potting mix was added to the

Table 1. *Viburnum* species used for the larval group-feeding experiments in 2007 and 2009.

2007	Origin	2009	Origin
V. carlesii Hemsl.	Asia	*V.* × *bodnantense* Aberc. ex Stearn	Asia
V. lentago L.	North America	*V. carlesii* Hemsl.	Asia
V. sieboldii Miq.	Asia	*V. dentatum* L.	North America
V. trilobum Marsh.	North America	*V. dilatatum* Thunb.	Asia
		V. lantana L.	Europe
		V. lentago L.	North America
		V. macrocephalum	Asia
		V. opulus L.	Europe
		V. plicatum var. *tomentosum* Thunb.	Asia
		V. prunifolium L.	North America
		V. rafinesquianum Shult.	North America
		V. rhytidophyllum Hemsl.	Asia
		V. sargentii Koehne	Asia
		V. setigerum Hance	Asia
		V. sieboldii Miq.	Asia
		V. tinus L.	Europe
		V. trilobum Marsh.	North America

containers as a pupation substrate. Newly emerged adults were sexed and weighed in the 24 h following emergence. For both experiments, the following parameters were measured: larval survivorship (% of larvae reaching pupation), pupation success (% of pupae reaching adult stage), and adult mass (male and female). For the second experiment, we also measured larval development duration (total number of days between egg hatch and pupation) and adult development duration (total number of days between egg hatch and adult stage). Effects of host species and larval density on the parameters measured were analysed using two-way ANOVA (JMP 8). Survivorship data were arcsine-transformed to meet the assumptions of the model. In the analyses of the second experiment, replication at the species level varied from seven to 17, based on the number of *Viburnum* hosts that had at least three replicates. For example, on some suboptimal *Viburnum* hosts with low larval survivorship, there was emergence of adult males and/or females in less than three replicates: the parameters male and/or female mass were thus not included in the general model for these hosts.

For the second experiment, the association between defensive or nutritional plant traits of the 17 hosts and the effects of larval density were investigated as follows: for each significant effect of larval density or the interaction between larval density and host species on insect performance, the difference between the species mean of insect performance parameters (i.e. survivorship, development duration, pupation success, adult mass) at high larval density and at low density was calculated. This difference was then plotted against the species mean of the plant traits and analysed using linear regression (JMP 8). In these analyses, species means of adult mass were weighted in the analysis by the number of individuals produced for each species to account for variation in sample size.

Measures of defensive traits and nutritional quality of the hosts

Two defensive traits were measured for the 17 hosts used in the second larval group-feeding experiment: leaf trichomes and leaf toughness. Trichome density was assessed by counting trichomes from the top and bottom of leaf discs (diameter 6 mm) under a dissecting microscope. Leaf toughness was measured in the field with a force-gauge penetrometer that measures the grams of force needed to penetrate a surface (Type 516, Chatillon Corp., New York, New York). Two measures of leaf toughness were taken per leaf and averaged to a single value. Nutritional quality of the hosts was evaluated by measuring percentage leaf nitrogen using a CHN elemental analyzer (Carlo Erba NC2500 elemental analyzer; Thermo Electron, Waltham, Massachusetts). All plant traits were measured from five field-collected leaves per species in May 2009. Typically, the youngest fully expanded leaves of a shoot were used for the measures, which corresponds both to the leaves used in the group-feeding experiments and to the typical feeding preferences of VLB larvae in the field (Weston *et al.*, 2007).

Results

Analysis of oviposition time on infested and non-infested twigs

The majority of ovipositing females (81%) produced a single egg mass in trials, and the rest produced two or more (maximum: four egg masses). The duration of pre-oviposition searching was 17.6 ± 1.9 min (mean $\pm$ SE) and females made 14.3 ± 2.2 runs while searching, covering a total distance of 61.1 ± 10.9 cm. There was no effect of previous infestation on the duration of searching ($F_{1,36} = 0.17$, $P = 0.68$), the total number of runs made by a female during searching ($F_{1,34} = 1.76$, $P = 0.19$), or the total distance covered by females ($F_{1,32} = 0.03$, $P = 0.84$). However, runs were 21% shorter on infested than on non-infested twigs (3.0 vs. 3.8 cm per run, $F_{1,34} = 4.54$, $P = 0.04$). Females engaged in searching on non-infested twigs usually inspected the entire length of the twig, repeatedly making extensive back-and-forth runs before choosing an oviposition site, whereas females searching on infested twigs usually concentrated most of their searching in the area of the twig already infested. Chewing egg cavities took the longest of the steps during oviposition (103.9 ± 5.6 min), while producing the egg cap took 15.6 ± 0.8 min. Previous infestation had no effect on the duration of chewing ($F_{1,31} = 2.88$, $P = 0.10$) or producing the egg cap ($F_{1,31} = 0.15$, $P = 0.70$). Females laying eggs on a previously infested twig consistently (80.9%) deposited new egg masses immediately adjacent (i.e. < 0.5 cm) to the existing mass(es) on the distal portion of the twig. Females never deposited an egg mass further than 2.5 cm away from existing egg mass(es).

A separate analysis performed for trials where more than one egg mass had been laid (seven trials in total) gave similar results: the duration of activities did not differ between the first egg mass laid and the following one(s) for pre-oviposition searching ($t = 0.60$, $P = 0.57$), chewing ($t = 0.57$, $P = 0.59$), and egg cap production ($t = 0.13$, $P = 0.90$).

Larval group-feeding experiments

In the 2007 experiment (groups of five and 10 larvae on four *Viburnum* hosts), the highest larval density increased male ($F_{1,23} = 8.59$, $P < 0.01$) and female mass ($F_{1,25} = 7.95$, $P < 0.01$) by $> 13\%$, but did not affect the other indicators of performance (Table 2, Fig. 1b). Host species had an effect on all aspects of insect performance except pupation success, and the interaction between larval density and host species was significant for larval survivorship, with highly divergent effects of density on survivorship among the *Viburnum* species ($F_{3,32} = 3.43$, $P = 0.03$, Table 2; Fig. 1a).

In the 2009 experiment (groups of five and 20 larvae on 17 hosts), three *Viburnum* species were found to be unsuitable for larval development (*V. dilatatum*, *V. plicatum* var. *tomentosum*, and *V. tinus*). Because no females emerged at low density for *V. bodnantense*, *V. carlesii*, and *V. lentago*, and because no males emerged at low density for *V. carlesii* and *V. prunifolium*, these species were excluded from analyses of female and male mass, respectively.

Table 2. ANOVA for effects of *Viburnum* host species and larval density on several indicators of viburnum leaf beetle performance during two separate experiments (2007 and 2009).

Insect performance measure		Year					
		2007			2009		
		d.f.	F	P	d.f.	F	P
Larval survivorship (%)	Species	3	6.72	**<0.01**	16	21.90	**<0.0001**
	Density	1	0.6	0.44	1	1.18	0.28
	Species × density	3	3.43	**0.03**	16	1.06	0.89
	Error	32	–	–	137	–	–
Pupation success (%)	Species	3	2.23	0.10	11	7.55	**<0.01**
	Density	1	0.27	0.61	1	17.25	**<0.0001**
	Species × density	3	0.23	0.87	11	0.85	0.59
	Error	32	–	–	92	–	–
Development larva (days)	Species	–	–	–	11	65.24	**<0.0001**
	Density	–	–	–	1	1.45	0.23
	Species × density	–	–	–	11	0.6	0.82
	Error	–	–	–	92	–	–
Development adult (days)	Species	–	–	–	9	16.51	**<0.0001**
	Density	–	–	–	1	0.04	0.84
	Species × density	–	–	–	9	0.72	0.68
	Error	–	–	–	74	–	–
Male mass (mg)	Species	3	9.46	**<0.001**	6	16.86	**<0.0001**
	Density	1	8.59	**<0.01**	1	3.83	0.06
	Species × density	3	1.14	0.35	6	1.33	0.26
	Error	23	–	–	63	–	–
Female mass (mg)	Species	3	13.67	**<0.001**	8	23.97	**<0.0001**
	Density	1	7.95	**<0.01**	1	1.53	0.22
	Species × density	3	1.37	0.27	8	2.39	**0.02**
	Error	25	–	–	47	–	–

Bold values indicate significant effects ($P < 0.05$).
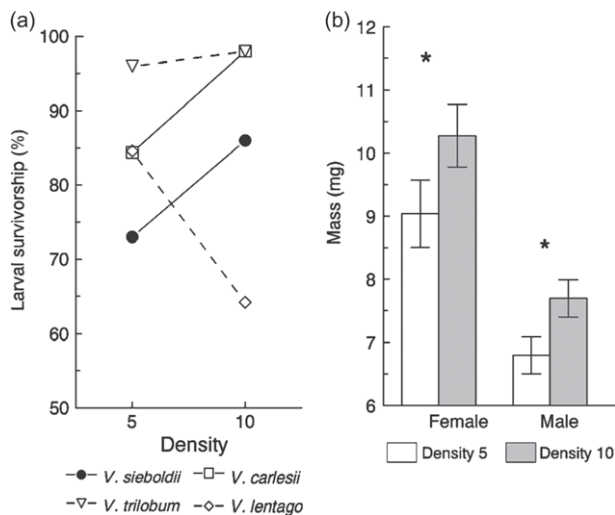
Fig. 1. Performance of viburnum leaf beetle (VLB) larvae reared in groups of five and 10 on four *Viburnum* hosts under laboratory conditions (2007 experiment). (a) Larval survivorship; solid lines indicate Asian hosts and dotted lines indicate North American hosts. (b) Mean (±SE) male and female mass of VLB across the four *Viburnum* species. Shown is the grand mean (±SE) because there was no interaction with plant species. Asterisks represent significant effects (ANOVA, $P < 0.05$).

Pupation success averaged $27 \pm 3\%$ higher across species when larvae were reared in groups of 20 rather than in groups of five ($F_{11,92} = 17.25$, $P < 0.0001$; Table 2, Fig. 2a). Host species had a strong effect on all indicators of insect performance ($P < 0.001$), but the interaction between host species and larval density was significant only for female mass (Table 2, Fig. 2b). There was no association between the effect size of larval density on female mass (i.e. mean female mass at density 20–mean female mass at density 5) or defensive traits (leaf trichome density, $F_{1,10} = 0.10$, $P = 0.76$; leaf toughness, $F_{1,10} = 2.57$, $P = 0.14$). However, the effect of larval density on adult mass was negatively correlated with host percentage leaf nitrogen for both females ($F_{1,10} = 10.55$, $P = 0.01$; Fig. 3a) and males ($F_{1,11} = 7.05$, $P = 0.02$; Fig. 3b). In other words, a positive effect of larval density on adult weight was only apparent on low-nitrogen host species.

Discussion

Aggregative oviposition for viburnum leaf beetles is a rare case of communal oviposition among non-eusocial insects (New, 1985; McCall & Cameron, 1995; Reed, 2005). Because of the high degree of effort conferred to each egg mass (>2 h), each oviposition event is expected to have substantial time and energy costs, which may be decreased by aggregating egg
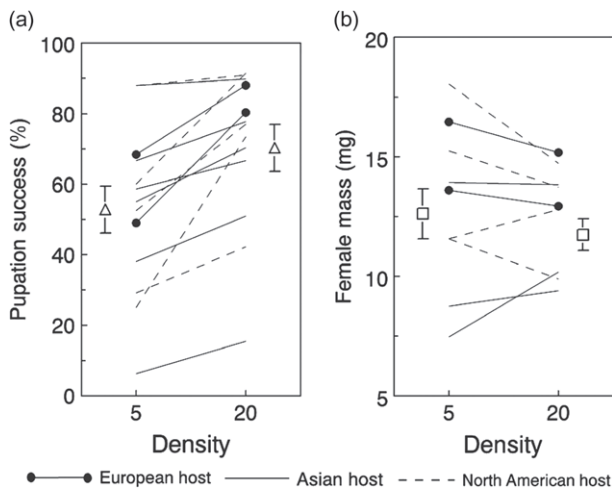
Fig. 2. (a, b) Pupation success (a) and female mass (b) of viburnum leaf beetles reared in groups of five and 20 larvae on 17 *Viburnum* species of different origins under laboratory conditions (2009 experiment). Hosts with fewer than three replicates are not shown. Overall means ± SE are shown beside reaction norms, and differ significantly for pupation success ($P < 0.05$) but not for female mass ($P = 0.22$).
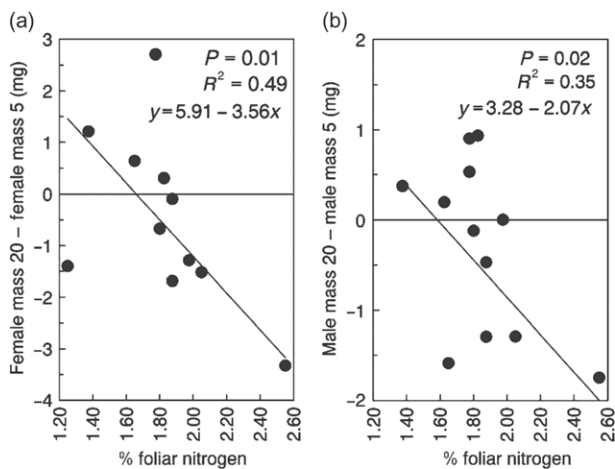
Fig. 3. Association between host nutritional quality (percentage foliar nitrogen) and viburnum leaf beetle response to density in female mass (a) and male mass (b) on several *Viburnum* hosts ($n = 11$ species for females, and $n = 12$ species for males). The *y*-axis was calculated as the mean mass reached in groups of 20 minus the mean mass reached in groups of five (one dot = one species).

masses. Additionally, because high densities of eggs result in high larval densities on leaves, larvae may benefit from feeding in groups. Our results did not show evidence of a reduction of oviposition time on infested twigs: the duration of every step of the oviposition process (searching, excavating the cavity and making the egg cap) was unaffected by the presence of conspecifics' egg masses. Nonetheless, females shortened their runs and concentrated them near the infested area on infested twigs. We speculate that this behaviour may correspond to host-marking for future oviposition events (Nufio & Papaj,

2001), or to an evaluation of the defensive capacity of the twig prior to egg-laying (Desurmont & Weston, 2010).

Our experiments on larval group feeding revealed two general (i.e. consistent for all host species tested) benefits of increased larval densities on insect performance: heavier adult mass (2007 experiment; Fig. 1b) and higher pupation success (2009 experiment; Fig. 2a). However, these two benefits were not consistent between the two experiments; differences in experimental design between 2007 and 2009 may have caused this discrepancy. In particular, the use of third-instar larvae initially reared on *V. trilobum* in 2007 implies that the effects of host switching may have interacted with the effects of larval density and affected insect performance differently compared with the 2009 experiment. While the mechanisms behind this lack of consistency are open to conjecture, our findings illustrate some sensitivity to environmental variables for the benefits of aggregations.

What causes larvae to do better in dense groups? Because experiments were conducted under laboratory conditions, we can exclude mechanisms related to protection from natural enemies (Breden & Wade, 1987), thermoregulation (Bryant *et al.*, 2000) and host location efficiency (Weed, 2010). Benefits resulting from inducing a physiological change in the plant are also unlikely because experiments were conducted with excised shoots (Denno & Benrey, 1997). Improvement of host use due to feeding facilitation, on the other hand, remains a viable hypothesis. Feeding facilitation (or 'social stimulation') may have occurred via increased stimulation to feed, as has been demonstrated for several species (Wade & Breden, 1986; Chang & Morimoto, 1988; Clark & Faeth, 1997; Nahrung *et al.*, 2001). However, feeding facilitation typically benefits early instars (Reader & Hochuli, 2003; Inouye & Johnson, 2005), resulting in increased larval survivorship or faster development (Lawrence, 1990; Nahrung *et al.*, 2001; Fordyce, 2003; Weed, 2010), which we did not consistently observe. Nonetheless, production of heavier adults (Fig. 1b) may be a consequence of a more efficient feeding due to optimal stimulation during larval development.

The generalised enhanced pupation success in denser groups (Fig. 2a) may be related to the specifics of VLB pupation behaviour. Previous research suggests that the ability to break the surface of the soil plays a role in pupation success (Weston & Desurmont, 2008). While larvae were never observed failing to penetrate the pupation substrate in our experiment, we cannot rule out the possibility that pupation behaviour is facilitated by the presence of conspecifics for VLB larvae.

Two effects of larval group feeding were mediated by the host plant on which larvae developed: larval survivorship (2007 experiment) and female mass (2009 experiment). The effect of group feeding on larval survivorship was not observed on *V. trilobum*, which was the host on which larvae were reared before the start of the experiment (egg hatch to third instar) (Fig. 1a), and was absent in 2009, when larvae were reared on their respective hosts from egg hatch. Thus, it is possible that this particular effect on performance was a consequence of host switching (Stoyenoff *et al.*, 1994) rather than group feeding. The effect of host plant on female mass observed in 2009 (Fig. 2a) was not associated with the defensive traits of the

hosts. Nonetheless, the benefits of aggregation were strongest on nitrogen-poor hosts, and the density effect was reversed (i.e. higher density was detrimental) on more nutritious hosts (Fig. 3a,b). Although insects were never food-limited, crowding could have prevented larvae from reaching optimal feeding rates on highly nutritious hosts when reared in groups of 20, while larvae still benefited from increased feeding efficiency on poor hosts at the same larval density. Several studies have shown that the benefits of gregariousness are reduced when larvae feed on higher-quality host plants or tissues (Tsubaki, 1981; Lawrence, 1990; Reader & Hochuli, 2003). In light of our 2007 and 2009 results, we advance the idea that group feeding has a beneficial effect on viburnum leaf beetle adult mass, but that this benefit may be outweighed by costs after a certain larval density threshold, and that this threshold is directly dependent on the nutritional value of the host. The fact that the effects of aggregation on adult mass were not affected by the origin of the host (Fig. 2), and thus were consistent on hosts with which VLB does not share a history of coexistence (i.e. North American and Asian hosts), reinforces the idea that a universal plant trait such as foliar nitrogen content may be critical for density-dependent effects.

Recent research has demonstrated that a realised fitness benefit of viburnum leaf beetle aggregative oviposition is to overcome a defensive egg-crushing wound response from the plant (Desurmont & Weston, 2011). However, aggregative oviposition did not necessarily initially evolve to overcome plant defences. For example, in mass-attacking bark beetles targeting live trees, adult aggregations allow beetles to overcome host physical defences and successfully colonise host trees (Byers, 1989; Raffa, 2001). However, it has been hypothesised that gregarious behaviour in bark beetles initially evolved in species attacking dead trees, and that the primary benefit of this behaviour was originally related to mate-finding (Costa, 2006). If larval group feeding does have an adaptive value for viburnum leaf beetle, as our results suggest, two evolutionary scenarios are possible. Either aggregative oviposition evolved as a means to overcome twig wound response and group-feeding-related benefits arose later, or aggregative oviposition initially evolved to increase larval performance, and these aggregations allowed females to oviposit on live twigs and overcome their defences. For *Pyrrhalta esakii*, an Asian congener sharing the same host plants (*Viburnum* spp.) and mode of oviposition, eggs are primarily laid in the dead tips of twigs (Satoh, 2002). We speculate that ancestral *Pyrrhalta* could have targeted dead or less defended twigs for egg-laying, and that aggregative oviposition may have allowed them to colonise well-defended live twigs.

In conclusion, our study reveals that several aspects of viburnum leaf beetle performance are enhanced when larvae are feeding in dense groups. Because both host-dependent and host-independent effects were observed, several mechanisms may underlie these effects. On the one hand, general benefits independent of leaf quality are probably associated with mechanisms such as feeding facilitation, and on the other, host nutritional content directly affected the benefits of density for mass at adulthood. Thus, our study constitutes a strong example of the host-specific benefits of gregariousness. Although it remains to be tested whether group feeding presents a realised fitness benefit under field conditions, we suggest that larval group feeding could have played a role in the evolution of oviposition behaviour. Future investigations of the evolutionary history of oviposition in Asian *Pyrrhalta* spp. could clarify the selective forces favouring aggregative oviposition in the viburnum leaf beetle.

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