RESEARCH PAPER

Residency Duration and Shelter Quality Influence Vibratory Signalling Displays in A Territorial Caterpillar

Jayne E. Yack, Sharleen Gill, Chris Drummond-Main & Thomas N. Sherratt

Department of Biology, Carleton University, Ottawa, ON, Canada

Abstract

Residents are more likely to win territorial disputes than intruders. One explanation for this prior resident advantage is that residents place a higher value on the resource and are therefore more motivated to win. Although value asymmetry models of animal contests often assume that contestants use information about resource value, information on the proximate cues affecting territorial behaviour is often lacking. We use a simple model system – territorial behaviour in the masked birch caterpillar (*Drepana arcuata*) to identify factors that affect territorial behaviour. Late instar caterpillars occupy solitary silken leaf shelters, which they defend against wandering conspecifics with a vibratory display. We evaluated how a caterpillar identifies itself as the owner and the factors that influence a resident's motivation to signal. To do so, we conducted three experiments between size-matched residents and intruders to assess how residency duration and shelter quality independently affected territorial displays during the early stages of a contest. Experiment 1 (Time Exp.) demonstrated that resident signalling rates increase with increased duration on the leaf prior to introducing the intruder. Residents also signal more than intruders after residency periods of 1–3 min and longer, demonstrating that residents gather information about resource value shortly after occupying a leaf. Experiment 2 (Squatter Exp.) aimed to disentangle the effects of time on the leaf and silk accumulation. Squatters (individuals in a shelter made by another) placed for 1–3 min on a leaf containing a full silk shelter signalled more to intruders than did caterpillars placed on a fresh leaf for 1–3 min. Experiment 3 (Shelter Removal Exp.) showed that residents whose shelters had been removed signal less than those occupying an intact shelter, despite an equal length of time investing in them. Our experiment is the first to covary both prior residency duration and territory quality, and we find that the motivation of caterpillars to signal is a function of both of these attributes.

Introduction

Prior residents are more likely to win territorial contests (Kokko et al. 2006). One explanation for their success is that the resident is larger, stronger and more aggressive than the opponent, which is why it succeeded in obtaining the territory in the first place; these residents are said to have greater resource-holding power (RHP) (Parker 1974; Maynard Smith & Parker 1976). However, even when an intruder has a fighting advantage over the resident, prior residents are still more likely to win (Arnott & Elwood 2008). These cases are better explained by the value asymmetry (VA) hypothesis, which postulates that residents are more motivated to fight and win because they have more to lose than intruders have to gain (Enquist & Leimar 1987; Bradbury & Vehrencamp 2011). If the value asymmetry increases with time spent on the property (for example, the resident invests in 'non-transferable' work such as negotiating

with neighbours or builds a shelter with a limited resource budget), then residents that are on a territory for longer periods should be even more motivated to fight than the average intruder. In support, in brown trout (*Salmo trutta*), replacements that were residents for 4 d were more aggressive and won more contests against original owners than those who were residents for only 2 d (Johnsson & Forser 2002). Similarly, in the green hairstreak butterfly (*Chrysozephyrus smaragdinus*), new residents fought longer as residence duration increased (Takeuchi 2006). Likewise, female iguanas (*Iguana iguana*) defend their nesting sites more aggressively and display more as their burrow deepens (Rand & Rand 1976).

While the adaptive significance (ultimate causation) of the 'prior residence effect' has been discussed at length (reviewed in Kokko et al. 2006), less is understood about the proximate factors mediating behavioural differences between contestants (Stockermans & Hardy 2013). In particular, what environmental (objective) cues do residents use to value a resource, and how is this information integrated with internal processes (subjective cues) to alter behaviour? Indeed, in many studies reporting VA, the proximate mechanisms associated with an individual's valuation of a territory are either assumed or mentioned in passing. Referring to the previously cited examples, it was argued that brown trout 'possess the cognitive ability to identify and remember their territory' (Johnsson & Forser 2002), that resident hairstreak butterflies fight longer because 'they have clear spatial memory' (Takeuchi 2006) and that in iguanas, 'the better the hole, the more energy the intruder is willing to expend in the attempt to gain it.' However, few studies focus on what information is gathered to assess value, and how it leads to a change in motivation (Arnott & Elwood 2008).

Proximate mechanisms underlying behavioural asymmetries are complex, and in many examples of VA, particularly those conducted in the field, it may not be easy to identify and disentangle the effects of multiple sensory cues, neurohormonal and cognitive factors involved. Identifying such proximate mechanisms would be facilitated by studying a simple model system that allows the experimenter to control factors that may contribute to value assessment (e.g. residency duration, territory quality, hunger levels), while allowing the experimenter to quantify agonistic behaviour. We propose that the masked birch caterpillar provides such an opportunity.

Masked birch caterpillars (*Drepana arcuata* (Drepanidae)) occur throughout North America where they reside on leaves of birch (*Betula* sp.) and alder (*Alnus* sp.). Late instar stages (3–5) live solitarily inside a silk leaf shelter that takes about 1–2 h to construct. A caterpillar first chooses a suitable leaf and then proceeds to lay a silk mat on the leaf surface and constructs the shelter by tying the leaf edges. Once an individual consumes the leaf (and its shelter), it will move on to another leaf and repeat the process until it pupates (Yack et al. 2001). Leaf shelters provide several benefits to caterpillars, including protection against predators, parasitoids and weather. In the masked birch caterpillar, a shelter with more silk strands tying the leaf edges together would provide greater protection. Therefore, we equate the amount of silk deposited with shelter quality. However, shelter construction also incurs costs, including time away from feeding and silk expenditure (Berenbaum et al. 1993; Lill & Marquis 2003). In the masked birch caterpillar, shelters can be usurped by wandering conspecifics, and contestants will engage in territorial displays that last from under a minute to a few hours (Yack et al. 2001). Territorial disputes begin when an intruder crawls upon the petiole of a resident's leaf. If a resident is feeding on the leaf outside of the shelter, it will return to its shelter. As the intruder approaches, the resident performs a vibratory display comprising three signal types: anal scraping, mandible drumming and mandible scraping (Fig. 1; see Video S1) (Yack et al. 2001; Scott et al. 2010; Guedes et al. 2012). Physically aggressive behaviours such as biting or pushing are absent in this species (Yack et al. 2001; Scott et al. 2010). During early stages of the encounter, the resident begins with low rates of anal scraping and mandible drumming, and as the encounter continues, signal rates increase and mandible scraping is added to the display. Signals therefore escalate in rate and type, providing the experimenter with a means of quantifying contestant motivation levels. In earlier staged contests, prior residents signalled more than did intruders and were more likely to retain their territory (Yack et al. 2001), but whether contestants are capable of assessing resource value, and adjust their behaviour accordingly, was not determined. Territorial behaviour in *D. arcuata* is an excellent model for studying factors that affect motivation for several reasons: first, the territory is simple (comprising a birch leaf and silk shelter) making it easy to manipulate and identify factors that contribute to value assessment. Second, contests can be set up in the laboratory, and territorial behaviours are easily documented with minimal disturbance, as the leaf shelters are open and do not conceal the contestants, and signalling behaviours can be simultaneously quantified using a microphone and video camera. Finally, signalling is a robust behaviour displayed by residents, allowing for a predictable
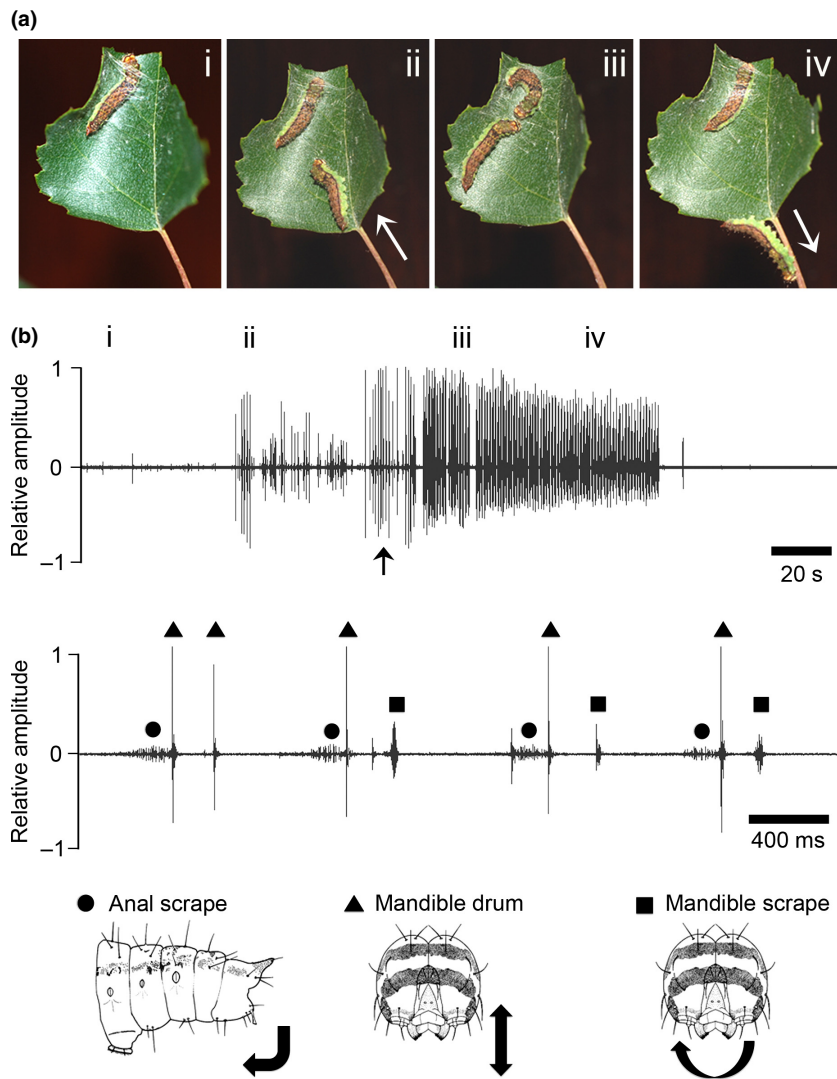
Fig. 1: Territorial encounter and vibratory display in the masked birch caterpillar. (a) Video frames from an encounter between a resident and intruder: (i) resident is inside its leaf shelter feeding at the leaf edge; (ii) intruder crawls upon the leaf in direction of arrow, and resident begins signalling; (iii) resident has turned around inside the shelter, and the two contestants interact; (iv) intruder leaves the leaf, and the resident continues to signal. (b) Waveform of the acoustic signals (recorded with a microphone) during the stages of the interaction (i-iv) shown in (a). The resident begins signalling when the intruder enters and continues for about 40 s after the intruder exits the leaf. A small black arrow below the trace indicates the time period expanded below, which shows the three types of signals, symbolized in the legend at the bottom.

and reliable way to quantify motivation levels. Our use of signalling rates to reflect an individual's relative motivation to retain or obtain a shelter directly utilizes one of the primary modes of communication among the caterpillars. Signals of preparedness to fight (or to persist) can in theory sometimes be bluff, but they must on average contain strategically important information about motivation to be maintained (Hurd & Enquist 2005; see Helgesen et al. 2013 for a recent discussion). Moreover, recent experimental work has revealed a surprising degree of honesty in carrying out threats on further provocation (Laidre 2009).

Our study tests whether cues associated with resource value influence territorial displays in caterpillars. In Experiment 1, we varied residence time prior to an encounter, predicting that during the initial stages of a contest, residents will signal more with increasing residency duration and will signal more than intruders. Experiment 2 keeps the shelter quality the same but alters the amount of time in the shelter. If signalling rates are affected by the shelter value, then a squatter (an individual placed on the shelter constructed by another) on a full silk shelter for 1–3 min should signal the same amount as a resident on a full shelter for 60–120 min. Experiment 3 keeps residency time the same but varies shelter quality by removing the shelter. If shelter quality is assessed, residents with intact shelters should signal more than those with shelters removed.

## Materials and Methods

Subjects

Gravid female *D. arcuata* moths were collected between May and Aug. (2006–2010) at mercury

vapour lights near Ottawa and Kingston, Ontario, Canada. Eggs from multiple females were laid on cuttings of paper birch (*Betula papyrifera*) and larvae reared indoors under an LD 16:8 photoperiod at 21–26°C. All larvae used for experiments were fourth or fifth instars chosen at random from broods of multiple (>20) wild-caught females. No individual was used twice in any experiment.

Experiment 1: The Effect of Residency Duration on Signalling

To determine the effect of residency on signalling, 144 encounters were staged between residents with varying residency durations and conspecific intruders. Contestants were matched with respect to their instar stage and size, based on head capsule width and length, respectively, to control for the effects of size asymmetry on behaviour (Yack et al. 2001). A twig bearing a birch leaf was cut to a length of 6–10 cm and placed in a water-filled vial through a hole in the lid. Fresh leaves were used for each experiment and were chosen on the basis of size (6–7 cm L × 4–5 cm W) and the absence of feeding scars or other visible damage. Before being used in an experiment, an individual was isolated in a separate container with a bare birch twig (no leaves) for 15–20 min. This was done to standardize the pre-trial experience of individuals. A caterpillar representing the 'resident' was placed onto a leaf for a period of 0 min (n = 29), 1–3 min (n = 30), 10 min (n = 33), 20 min (n = 21), or the duration of time it took to construct a full shelter (60–120 min) (n = 31) prior to introducing the 'intruder'. A full shelter was defined as one consisting of a silk mat and five or more silk strands connecting two sides of the leaf (Fig. 1). The intruder was transferred to the twig 2–3 cm below the leaf petiole. In 0-min trials, the intruder was placed on the twig just before the resident was placed on the leaf so that the two individuals would have more or less equal 'residency' times on the leaf. Thus, in 0-min trials, the resident was on the leaf anywhere from 0 to 30 s prior to the intruder's arrival. All trials were videotaped with a Sony Digital Handicam (TR7000; Sony Corp., Japan) and a remote Sony audio microphone (ECM-MS907) placed 1–2 cm behind the leaf. As the intention was to measure motivation levels of contestants at the beginning of a trial, contests were not monitored to completion. A schematic drawing of Experiment 1 is illustrated in Fig. 2a.

Videotapes were analysed to measure signalling rates in both residents and intruders. The mean rate of signalling was quantified in two ways: first, by count-
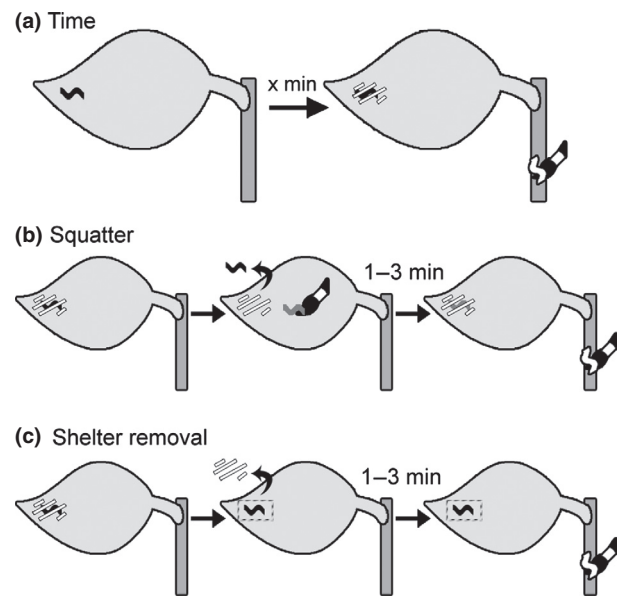
Fig. 2: Schematic representation of experimental methods. (a) Time (Exp. 1). A resident (black) is placed on a leaf and left undisturbed for a given time period (x min) before the intruder (white) is introduced to the twig with a paint brush. (b) Squatter (Exp. 2). An established resident (black) is removed from its shelter and replaced with a squatter (grey). After 1–3 min, an intruder (white) is introduced. (c) Shelter removal (Exp. 3). An established resident (black) has its shelter carefully removed before being introduced to an intruder (white).

ing the number of anal scrapes, mandible drums and mandible scrapes produced during each 5-s interval over the first 80 s of an encounter (trial 'began' when the intruder's head crossed the petiole–leaf junction), and second, by measuring rates over a 2-min interval flanking the time of closest contact (typically <2 cm) between contestants (i.e. 1 min before and 1 min after this time point). As intruders tend to crawl directly into the shelters, both measurements were usually made within the first few minutes of a trial. Rates were measured both ways because intruders approached residents at different speeds. Therefore, measuring signalling rates over a given time frame, as well as a given distance window, allowed us to capture different elements of the interaction.

Assessment of Silk Deposits Over Time

Silk deposits (either as a silk mat on the leaf surface or as part of the silk shelter) were examined at different time intervals to assess the relationship between time spent on the leaf and the relative amount of silk produced. Caterpillars were placed on leaves for 0, 3, 10, 20 or 60–120 min, corresponding to the residency time intervals used in Experiment 1. Caterpillars were

then removed from the leaves using a fine-tipped paintbrush, and the leaves photographed. Silk deposits were examined using scanning electron microscopy by cutting a section of leaf and mounting it on an aluminium stub. Leaf sections were dried, sputter coated with gold–palladium and examined with a JOEL scanning electron microscope (JSM-6400; Joel, Japan).

Experiment 2: Squatter on Full Shelters

To assess the effects of shelter quality on signalling, contests were staged between a 'squatter' and a size-matched intruder (n = 20). Full shelters were constructed by original residents on leaves for 60–120 min. The resident was then gently removed through the shelter entrance by rolling it onto a fine-tipped paint brush, taking care not to damage any silk strands. A second caterpillar referred to as the 'squatter' that had been isolated for 15–20 min (as described in Experiment 1) was then placed on the leaf adjacent to the shelter entrance. The squatter was on the leaf for 1–3 min (to provide time to establish itself inside the shelter), before the introduction of a new caterpillar (not the original resident), referred to as the intruder. Behaviours and signalling were recorded as described in Experiment 1. A schematic drawing of Experiment 2 is illustrated in Figure 2b. Videotapes were analysed for signal rates of residents as described in Experiment 1.

Experiment 3: Shelter Removal

To further test whether residents assess shelter quality, contests were staged between a full shelter resident and an intruder (n = 18) as previously described, but in this case, the resident's shelter was removed using tweezers and fine scissors 2–3 min prior to introducing the intruder. The bulk of the shelter was removed, although the silk mat on the leaf surface necessarily remained intact. A schematic drawing of Experiment 3 is illustrated in Figure 2c. Mean rates for each signal type between different conditions were compared using unpaired t-tests.

Statistical Analyses

Rates of signalling by residents in the different treatment groups (residency durations, treated as fixed factors) were compared using either one way analysis of variance (ANOVA) or the equivalent t-tests assuming equal variances when only two groups were being compared (t-tests based on unequal variances gave qualitatively identical results). Following standard model criticism and inspection of the residual plots, all of the signalling rate data were square-root-transformed to ensure both normality and homogeneity of variance in residual when conducting parametric tests. *Post hoc* comparisons following significant ANOVA were performed with Tukey's HSD. Due to the continued lack of normality of residuals after transformation and the fact that only a proportion of intruders signalled, intruder signalling rates in the different treatments were compared using a non-parametric Kruskal–Wallis test. The signalling rates of residents and intruders matched across contests were likewise compared using a non-parametric Wilcoxon signed ranks test.

Results

Experiment 1: Residency Duration

Resident signalling rates varied with the duration that a resident was on a leaf prior to an interaction (Fig. 3; Table 1, ANOVA: $F_{4,144} = 30.52$, p < 0.001). During the first 80 s of a contest, anal scrapes by 0-min residents were significantly lower than individuals with longer residency times (Tukey's HSD: all p from pairwise comparisons <0.001), while full shelter (60–120 min) residents signalled significantly more than those individuals with shorter residency times (Tukey HSD: all p ≤ 0.034). Mandible drumming also varied with the duration that a resident was on a leaf (ANOVA: $F_{4,143} = 1.06$, p < 0.001). Full shelter (60–120) residents produced significantly more mandible drums than did residents with shorter residency periods (Tukey HSD: all p ≤ 0.002), while low-duration residents (0 and 1–3 min) signalled at significantly lower rates than those residents that had been on the leaf for longer duration (Tukey's HSD: all p < 0.034). Finally, mandible scraping rates also varied with the duration that a resident was on a leaf (ANOVA: $F_{4,144} = 13.72$, p < 0.001), with 60- to 120-min residents signalling significantly more frequently than individuals with lower residency durations (Tukey HSD: all p ≤ 0.001).

Similar trends were observed when resident signalling rates were sampled during the closest 2 min (Fig. 3b; Table 1). Thus, the mean frequency of anal scraping within the closest 2 min varied with residency duration (ANOVA: $F_{4,109} = 28.09$, p < 0.001), with the signalling rates of 0-min residents significantly lower than those individuals with all other residency periods (Tukey HSD: all p ≤ 0.001) and 60- to 120-min residents signalling more than those with all
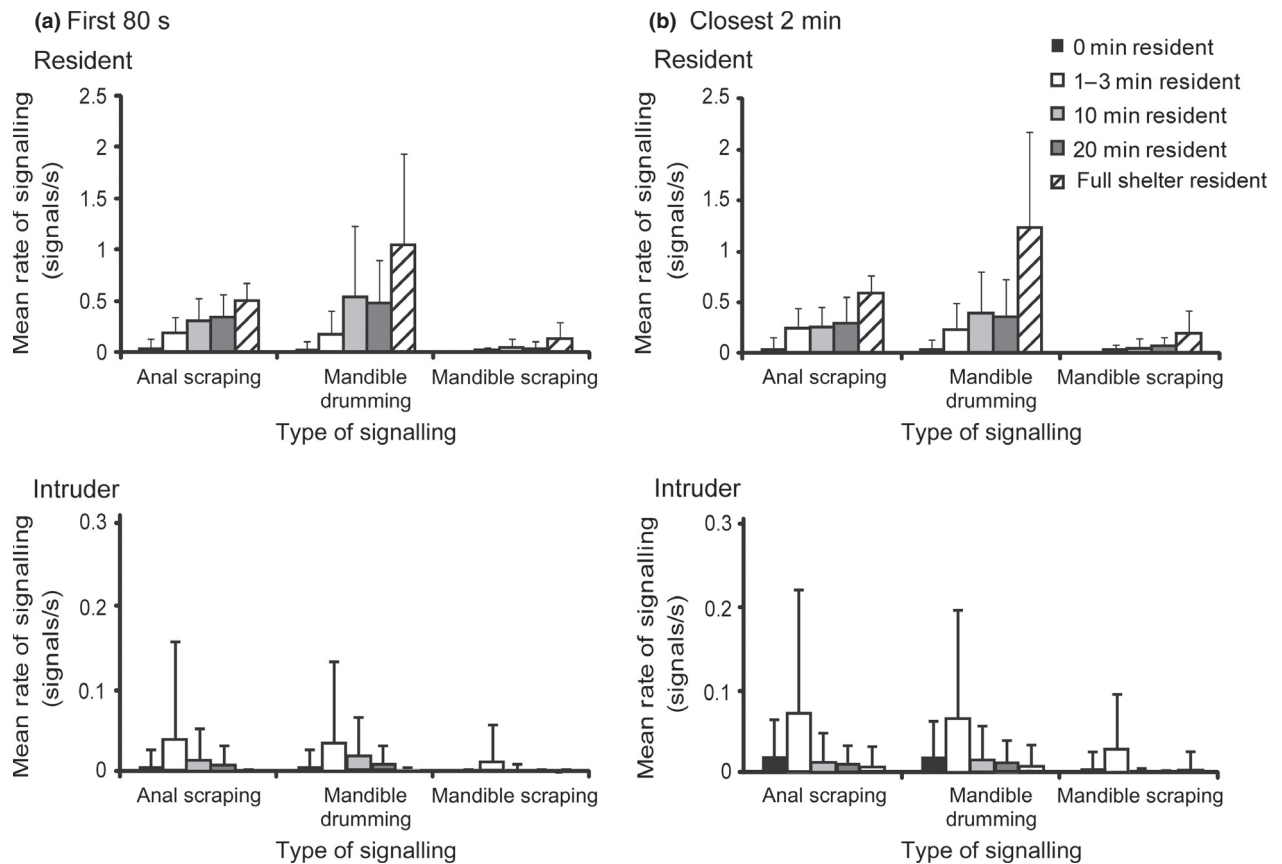
Fig. 3: Effect of time on resident and intruder signalling rates. (a) Resident (top panel) and intruder (bottom panel – note that y axis has been rescaled to better evaluate differences in intruder behaviour between conditions) signalling rates during the first 80 s of an encounter. (b) Signalling rates during the closest 2 min of an encounter (i.e. 1 min before and following the closest interindividual distance).

other residency periods (Tukey HSD: all p ≤ 0.001). Mandible drumming also varied with residency duration (ANOVA: $F_{4,109}$ = 33.12, p < 0.001), with 0-min residents signalling significantly less frequently than full nest residents (Tukey HSD: all p ≤ 0.039), and 60- to 120-min residents signalling more than those with shorter residency durations (Tukey's HSD: all p < 0.001). The frequency of mandible scraping during the closest 2 min also varied between treatments (ANOVA: $F_{4,109}$ = 9.60, p < 0.001) with 60- to 120-min residents signalling at significantly higher rates than the three lowest duration treatments (Tukey's HSD: all p from the three pairwise comparisons ≤0.001).

Residents signalled more than intruders under all conditions (Fig. 3a,b; Table 1). During the first 80 s of a contest, residents of all durations produced significantly higher rates of anal scraping (Wilcoxon signed rank tests: all p ≤ 0.031). The rate of mandible drumming was not significantly different in residents than intruders when comparing 0-min-duration resident

trials (Wilcoxon signed rank test: p = 0.021), but was significantly higher in all other residency duration treatments (Wilcoxon signed rank tests: all p ≤ 0.008). Mandible scraping rates were not significantly different in residents compared with intruders at residency durations of 0 and 10 min, but were significantly higher in the three remaining treatments (Wilcoxon signed rank tests: all p ≤ 0.031). Similar results were found when signals were sampled over the closest 2 min (Table 1).

When the signalling rates of intruders that faced residents of different residency periods were compared with each other, there were few discernable differences (Fig. 3; Table 1). Intruder anal scraping rates during the first 80 s did not differ significantly between conditions (Kruskal–Wallis test: X^2 = 5.471, p = 0.246), nor did mandible drumming (Kruskal–Wallis test: X^2 = 3.53, p = 0.474) or mandible scraping (Kruskal–Wallis test: X^2 = 3.10, p = 0.542). Likewise, during the closest 2 min of an encounter, no significant overall differences were observed in anal

Table 1: Signalling rates of residents and intruders as a function of the resident's prior residency time
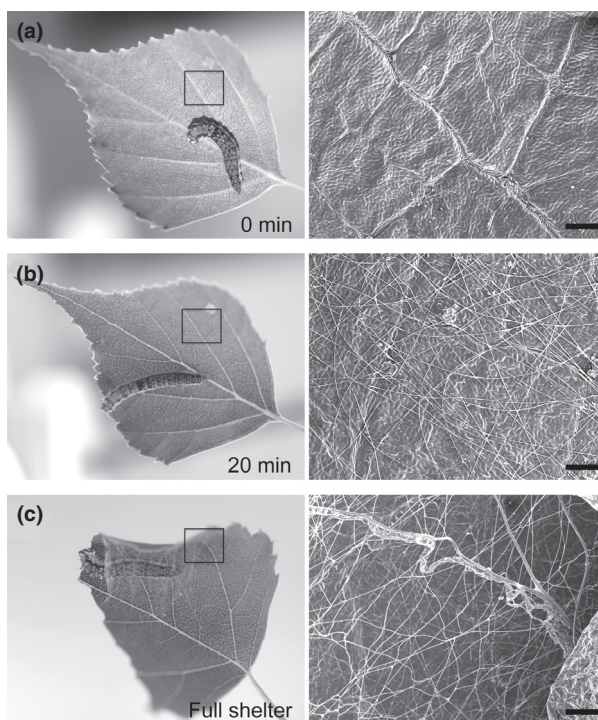
Residency time (min)	Anal scrapes		Mandible drums		Mandible scrapes	
	Mean # signals/s	SD	Mean # signals/s	SD	Mean # signals/s	SD
Signal rates over first 80 s of trial						
Resident						
0	0.038	0.086	0.027	0.073	0.003	0.012
1–3	0.179	0.162	0.173	0.223	0.018	0.031
10	0.308	0.221	0.541	0.692	0.039	0.097
20	0.340	0.227	0.478	0.416	0.031	0.070
60–120	0.504	0.168	1.042	0.889	0.132	0.162
Intruder						
0	0.004	0.019	0.004	0.019	0	0
1–3	0.036	0.113	0.032	0.094	0.010	0.043
10	0.012	0.036	0.016	0.045	0.001	0.005
20	0.006	0.022	0.006	0.021	0	0
60–120	0	0	0	0.002	0	0
Signal rates over 2 min of closest distance						
Resident						
0	0.051	0.106	0.042	0.091	0.004	0.013
1–3	0.245	0.192	0.238	0.254	0.033	0.049
10	0.261	0.198	0.391	0.411	0.042	0.102
20	0.293	0.256	0.351	0.377	0.069	0.095
60–120	0.593	0.170	1.241	0.938	0.191	0.225
Intruder						
0	0.020	0.044	0.020	0.043	0.006	0.019
1–3	0.072	0.150	0.066	0.131	0.026	0.069
10	0.013	0.035	0.016	0.041	0.001	0.004
20	0.012	0.022	0.014	0.025	0.001	0.002
60–120	0.007	0.024	0.008	0.025	0.004	0.022

Fig. 4: Resident silk deposits on leaf over time. Left panels show the leaf shelter construction, and right panels show scanning electron micrographs of silk on the leaf surface at different time intervals. Boxes on photographs indicate the general region of the shelter that is shown on the right. (a) Fifth instar caterpillar has just been placed on leaf (left), and there is no silk mat on the leaf surface (right). (b) After 20 min, the larva has laid down a silk mat on the leaf surface and is beginning to produce silk strands to form the shelter. (c) A full shelter has been created after 80 min, with the leaf edges drawn together with reinforcing silk strands.

scraping (Kruskal–Wallis test: X^2 = 3.93, p = 0.416), mandible scraping (Kruskal–Wallis test: X^2 = 7.68, p = 0.093) or mandible drumming (Kruskal–Wallis test: X^2 = 6.20, p = 0.185) between treatments.

Overall, the above results demonstrate that residents signal more as their prior residency time increases. As silk accumulates on the leaf over the first 1–2 h of residency (Fig. 4), the observed increase in signalling rates over time could be influenced by silk accumulation. Experiments 2 and 3 examine the effects of silk on resident signalling rates.

Experiment 2: Squatters

Squatters on a full shelter for 1–3 min generally signalled more than did original 1- to 3-min residents (Fig. 5). During the first 80 s of the encounter, squatters showed significantly higher mandible drumming rates (t-test: t_{48} = 4.48, p < 0.001) than 1- to 3-min residents, but they did not differ significantly in their rates of anal scraping (t-test: t_{48} = 1.48, p = 0.144) or mandible scraping (t-test: t_{48} = 0.24, p = 0.814).

Similar trends were observed when signals were analysed from the closest 2 min, with significantly higher mandible drumming rates in squatters (t-test: t_{40} = 4.67, p < 0.001), but non-significantly higher anal scraping (t-test: t_{40} = 1.85, p = 0.072) and mandible scraping (t-test: t_{40} = 0.16, p = 0.874). On the other hand, residents of full shelters (60–120 min) signalled significantly more than squatters (1–3 min) on full shelters, through anal scraping (t-test: t_{54} = 4.49, p < 0.001), mandible drumming (t-test: t_{54} = 4.67, p < 0.001) and mandible scraping (t-test: t_{54} = 3.39, p = 0.001) over the first 80 s of the trial (Fig. 5a). Similar results were obtained when signals were analysed over the closest 2 min, with squatters showing lower rates of anal scraping (t-test: t_{54} = 6.80, p < 0.001), mandible drumming (t-test: t_{54} = 5.16, p < 0.001) and mandible scraping (t-test: t_{54} = 3.21, p = 0.002) (Fig. 5b). Results from Experiment 2 suggest that caterpillars are assessing the value of the resource based on both time and silk cues.
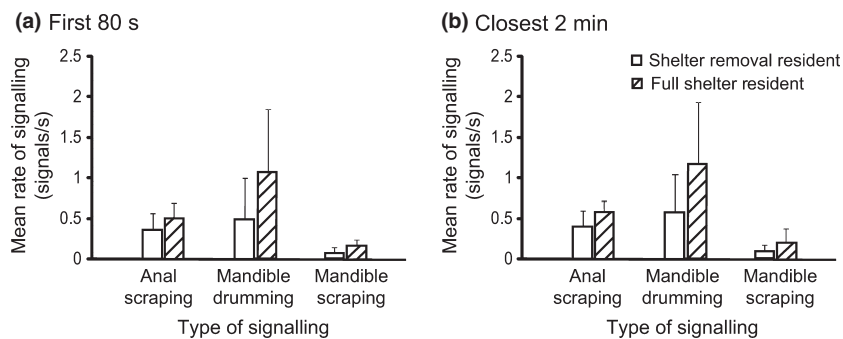
(a) First 80 s

(b) Closest 2 min

☐ Squatter
■ 1–3 min resident
▨ Full shelter resident
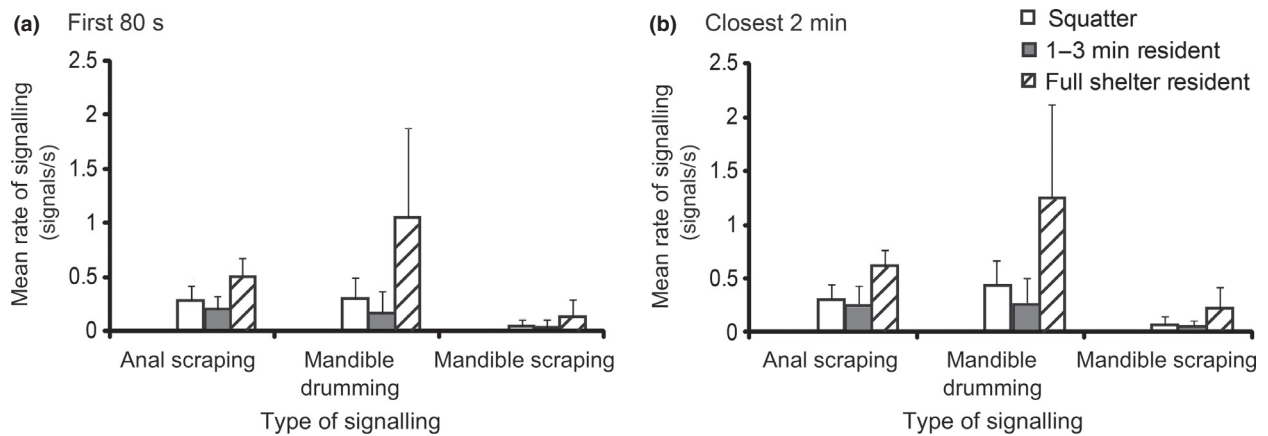
Fig. 5: Effect of the shelter from a previous resident on the signalling rates of a new (i.e. squatter) resident. (a) Signalling rates of squatters that have been on a leaf with a full shelter for 1–3 min, compared with 1–3 min original residents, and full shelter residents during the first 80 s of an encounter. (b) Signalling rates during the closest 2 min of an encounter (i.e. 1 min before and following the closest interindividual distance).

Experiment 3: Shelter Removal

If caterpillars are evaluating the shelter, we predicted that the removal of silk would result in decreased signalling. This prediction was supported (Fig. 6). During the first 80 s of a contest, residents whose shelters were removed had lower rates of anal scraping (t-test: $t_{52} = 2.80$, $p < 0.007$), mandible drumming (t-test: $t_{52} = 3.23$, $p = 0.002$) and mandible scraping (t-test: $t_{52} = 2.49$, $p = 0.016$) than residents on intact full shelters for the same duration. During the closest 2 min of an encounter, residents whose shelters were removed had significantly lower rates of anal scraping (t-test: $t_{52} = 3.50$, $p = 0.001$) and mandible drumming (t-test: $t_{52} = 3.62$, $p = 0.001$). Mandible scraping rates were also lower, but not significantly so (t-test: $t_{52} = 1.73$, $p = 0.090$). These results further support the hypothesis that residents are gathering information about the quality of their shelter.

Discussion

An assumption of VA models of animal contests is that individuals gather information about resource value; yet, there are few empirical studies that focus on identifying the proximate mechanisms involved (see Arnott & Elwood 2008; Elwood & Arnott 2012). Previous studies have demonstrated relationships between the intensity of agonistic behaviours and extrinsic cues of resource value such as site quality (e.g. spiders (Riechert 1984)), host size (e.g. parasitoid wasps (Stockermans & Hardy 2013)), female size (e.g. spiders (Bridge et al. 2000)) and residency period (e.g. tarantula hawk wasps (Alcock & Bailey 1997)), while other studies show how intrinsic cues such as reproductive state or contestant age (e.g. parasitoid wasps (Mohamad et al. 2013; Stockermans & Hardy 2013)) influence behaviour. Many such studies are either conducted in the field, where it is difficult to isolate

(a) First 80 s

(b) Closest 2 min

☐ Shelter removal resident
▨ Full shelter resident

Fig. 6: Effect of removing the leaf shelter on resident signalling. (a) A comparison of signalling rates between a resident that has had its shelter removed and a resident on a full intact shelter, during the first 80 s. (b) Signalling rates during the closest 2 min of an encounter (i.e. 1 min before and following the closest interindividual distance).

the many factors that could affect behaviour, or in the laboratory, where animals are removed from their natural environments. The advantage of the masked birch caterpillar model is that variables associated with both the territory and the contestants can be manipulated with minimal disturbance, while motivation levels can be quantified by monitoring signalling. This study is the first to show that in caterpillars, like for many other animals, prior ownership affects motivation to defend a territory. In addition, we take a first step in identifying objective cues that influence motivational behaviour.

Effects of Time and Silk on Signalling

Our results clearly indicate that resident caterpillars adjust signalling behaviour according to the time spent on the leaf. First, Experiment 1 shows that a resident's anal scraping and mandible drumming rates increase with residency duration. Mandible scraping deviated slightly from this pattern, as 0- to 30-s residents did not signal less than those on leaves for up to 20 min. Mandible scraping, however, occurs when the contest has escalated (Yack et al. 2001; Scott et al. 2010), and in the current study, we see that 60- to 120-min (full shelter) residents are more likely to mandible scrape than those with shorter residency durations. Thus, residents on leaves for long periods show increased motivation levels by producing higher signalling rates and more 'escalated' signals. Second, residents signalled at significantly higher rates than intruders under all residency times except for 0–30 s, where residents signalled more, but not significantly so. These results indicate that within 30 s there is ambiguity as to who is the 'resident' and who is the 'intruder', but by 1–3 min of prior residency, a caterpillar has already developed a 'sense' of ownership. As signalling was monitored only near the beginning of an encounter and intruders were never on leaves for more than a few minutes, they may not have had sufficient time to evaluate the territory. Similarly, in spiders, differences in fighting effort between residents and intruders over webs were attributed to differences in information available to contestants about the resource (Riechert 1984; Hack et al. 1997). Interestingly, when compared to one another, intruder signal rates were highest when residents had been on the leaf for 1–3 min and lowest when residents had been on the leaf for 60–120 min. This result suggests that intruders may somehow gauge the value of the resource as 'intermediate', and perhaps the resident might be willing to give it up more readily than one that it has invested in for a longer period. Experiment

2 provides a third way of looking at the effects of time: squatters residing on a full shelter for 1–3 min signal less than do 60- to 120-min original residents on a full shelter. These three lines of evidence indicate that over time, something happens to the resident that affects its motivation. Marking the passage of time is a complex process involving multiple senses and cognitive processes that presently are not well understood in insects (see Boisvert & Sherry 2006). One correlate of the passage of time in the masked birch caterpillar is silk accumulation (i.e. shelter development).

Our results suggest that silk assessment also plays a role in shelter valuation. Squatters (Exp. 2) residing on full shelters for 1–3 min signalled more than did 1–3 min original residents (with minimal silk), suggesting that caterpillars are assessing the quality of the shelter itself. Also, residents whose shelters were removed (Exp. 3) signalled less than those occupying a full intact shelter for the same time period; this indicates that residents place less value on a shelter of lower quality. Interestingly, it is not uncommon for an intruder, upon entering a resident's shelter, to cut some of the silk strands (Yack et al. 2001; J. E. Yack unpubl. data). We propose that this is analogous to 'vandalism' and hypothesize that it may function to reduce the value of the property, rendering the resident less motivated to defend the territory. Caterpillars may assess the quality of a shelter using chemical, visual or mechanosensory cues. Caddis fly larvae residents alter their aggressive response according to the value of the case they occupy (Englund & Otto 1991), which they assess by somehow measuring the case length and rigidity (Otto 1987), and spiders increase signalling rates when they occupy webs that are larger in area (Riechert 1984). However, it is not known what specific sensory cues are being used to gather this information.

Why might the masked birch caterpillar rely on both time and silk cues to assess resource value? Possessing the cognitive ability to measure the passage of time on a territory could be a reliable means of assessing investment, as typically a caterpillar is laying silk on the leaf for at least the first hour or two. However, assessing the passage of time alone may not always be a reliable indicator of resource value, as over time, the shelter could be damaged by weather, and the caterpillar consumes the leaf and the shelter. Also, a caterpillar could feasibly spend a long period of time on a leaf, but due to abiotic factors (low temperatures, wind or rain) or the presence of predators that may deter the caterpillar from moving (Guedes et al. 2012), shelter construction may be delayed. Also, in instances where intruders take over a shelter, the new

resident would benefit from not relying entirely on time cues to adjust its behaviour. Why then would a caterpillar not rely entirely on assessing the amount of silk? As there may be costs to wandering on a plant, such as being exposed to predators, a caterpillar that has already located a leaf may be better to remain on that leaf, even if it has not yet invested in silk. Finally, value is not simply the quality of the resource, but more generally the difference in pay-off from retaining it vs. losing a shelter. If a larva has spent a long time building a shelter, it may have fewer resources to build elsewhere, so the existing shelter becomes increasingly more valuable – not entirely as a result of the improving structure, but the heightened cost of building a structure elsewhere. Thus, both time and silk investment cues should contribute to value assessment.

Conclusions and Future Considerations

Our study demonstrates the prior residency effect in caterpillars, and, that for residents, motivation to defend the territory is influenced by time and silk investments. In nature, time and silk are unlikely to be the only cues that a caterpillar uses to evaluate resource value, however. Subjective cues including internal states influenced by hunger, developmental stage or fatigue could also potentially affect the perceived value of a resource (Arnott & Elwood 2008). In future studies, it would be interesting to examine how both objective and subjective cues of resource value might affect neural processes underlying signalling behaviour. Beyond resource value assessment, signalling rates in these insects may also be influenced by RHP, although in our study we minimized possible RHP effects by matching the sizes of contestants. As the encounter proceeds, contestants may also assess the RHP of their opponents and adjust their signalling accordingly. In a previous study, encounters were staged between contestants of unequal weights, and although residents signalled more and usually won encounters regardless of their opponent's size, in those in cases where intruders did win, the intruder was larger (Yack et al. 2001). A comparison of signalling rates between contestants of different sizes was not reported in that study, and it would be interesting to determine whether and how size asymmetry might affect the signalling rates of one or both contestants (see Taylor & Elwood 2003), or how signal characteristics might convey information about RHP. Spiders, for example, use vibratory cues to assess their opponent's weight relative to their own, which they then factor into a decision to retreat or to fight (Riechert

1984). Due to the simplicity of the masked birch caterpillar's territory, the robustness and reliability of the territorial display, and the ease at which signalling can be measured and quantified with minimal interference to the animal, we argue that this model system provides a special opportunity to identify proximate factors underlying motivation asymmetries in territorial systems.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article:

Video S1: Caterpillar Territorial Signals.