Ethology

Does Host Value Influence Female Aggressiveness, Contest Outcome and Fitness Gain in Parasitoids?

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Abstract

Intraspecific competition for resources is common in animals and may lead to physical contests. Contest outcomes and aggressiveness can be influenced by the resource holding potential of contestants but also by their perception of the resource value (RV). Competitors may assess resource quality directly (real RV) but may also estimate it according to their physiological status and their experience of the habitat quality (subjective RV). In this article, we studied contests between females of the solitary parasitoid *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) when exploiting simultaneously a host, a *Delia radicum* L. (Diptera: Anthomyiidae) pupa. We tested the effect of factors modifying host value on the occurrence of agonistic behaviours, contest outcomes and host exploitation. The factors tested were: the quality of the previous habitat experienced by females, female egg load, host parasitism status and the stage reached by the owner female in her behavioural oviposition sequence. Females successfully protected their host against intruders during its exploitation, but not after oviposition, and their aggressiveness did not seem to be influenced by their perception of the RV. The fact that the host is subsequently parasitized by the opponent females appears to mainly depend on the host selectiveness of females.

Introduction

When conspecifics look for and exploit similar resources, agonistic pairwise contests for indivisible resources are expected (Huntingford & Turner 1987; Archer 1988; Riechert 1998). Theoretical studies using game theory modelling and the concept of evolutionarily stable strategies (Maynard Smith 1982) suggest that the factors influencing the settlement of such conflicts can be divided into two main categories: the difference in contestants' competitive abilities (resource holding potential, RHP) and the value they place in the resource (resource value, RV; Parker 1974; Maynard-Smith & Parker 1976; Hammerstein 1981; Leimar & Enquist 1984). Contests are predicted to be won by the contestant with the highest RHP and/or RV (Parker 1974; Maynard-Smith & Parker 1976; Hammerstein 1981; Enquist & Leimar 1987).

Although this theoretical framework has been applied to a wide range of animal species (for a review, see Enquist & Leimar 1987 and Riechert 1998), it has seldom focused on insect parasitoids. Yet parasitoid adult females can compete for resources (i.e. the hosts from which their offspring develop) via direct agonistic behaviours, and the outcome of the competition will influence their fitness directly (Petersen & Hardy 1996; Field & Calbert 1999; Batchelor et al. 2005). Moreover, although aggressive behaviours have been reported in many parasitoid species (for a review, see Godfray 1994), studies have mainly focused on bethylid wasps (Hardy & Blackburn 1991; Petersen & Hardy 1996; Stokkebo & Hardy 2000; Goubault et al. in press; Humphries et al. 2006).

In bethylid wasps, body size (resource-uncorrelated RHP) and ownership status (resource-correlated RHP) have been shown to influence contest outcomes: larger females are more likely to win contests but owners usually out compete slightly larger intruder females (Petersen & Hardy 1996; Goubault et al. in press). This ownership advantage, usually seen as a resource-correlated RHP asymmetry, can also be an RV asymmetry as in *Goniozus nephantidis* (Stokkebo & Hardy 2000). In this species, the owners' advantage appears related to their larger egg loads (Stokkebo & Hardy 2000). Females with a higher egg load place a higher value into the host (subjective RV, Enquist & Leimar 1987) and are therefore more likely to win contests. Other RV asymmetries based on the real value of hosts (real RV, Enquist & Leimar 1987) can affect contest resolution: females defending larger hosts or more valuable brood usually have a higher probability of winning (Goubault et al. in press; Humphries et al. 2006).

Here, we investigated female aggressiveness and contest outcome in the pteromalid *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae). In this solitary parasitoid of dipteran pupae (Rueda & Axtell 1985), females can discriminate between parasitized and unparasitized hosts (Nell & van Lenteren 1982; Goubault et al. 2004a,b). Because the survival of the first egg deposited diminishes with time between ovipositions (Goubault et al. 2003) and because superparasitizing females also destroy eggs already present on hosts by piercing them with their ovipositor (i.e. ovicide; Nell & van Lenteren 1982; Goubault et al. 2004a), the second exploitation of hosts strongly reduces the first female's fitness gain.

We tested the effect of four different factors influencing females' subjective and real RV on female aggressiveness, contest outcome and fitness gain. We first explored the influence of the previous habitat quality. Females having experienced a habitat poor in hosts should place a higher value into the hosts than those having experienced high host availability. They should therefore be more motivated to fight and win contests more frequently (Enquist & Leimar 1987). In asymmetrical conflicts, females coming from a poor habitat are expected to take possession of the host while females from a rich habitat quickly abandon the contest (Maynard-Smith & Parker 1976; Hammerstein 1981; Leimar & Enquist 1984). Symmetrical contests, on the other hand, are predicted to be generally more violent and/or longer, especially between females coming from poor habitat (Maynard-Smith & Price 1973; Enquist & Leimar 1987). Second, we investigated the effect of female egg load. Host value for females having more eggs ready to lay should be higher and females with high egg load should consequently fight more violently and gain access to the hosts more frequently (Stokkebo & Hardy 2000). Third, we studied the influence of the host parasitism status (i.e. unparasitized vs. 24 h-parasitized hosts). Because the survival probability of eggs deposited in 24 h-parasitized hosts is considerably reduced (Goubault et al. 2003), the value of such hosts is low. Conflicts are therefore predicted to be less violent when females are competing for 24 h-parasitized than for unparasitized hosts. Finally, the RV asymmetry between contestants should increase as owners get more advanced in their behavioural oviposition sequence, as they are more likely to oviposit (and subsequently obtain offspring). Owners' value of hosts should therefore increase and owner females should be more likely to win contests.

Materials and Methods

Study Material

The host, *Delia radicum* L. (Diptera: Anthomyiidae), originated from pupae collected from cabbage fields (Le Rheu, Brittany, France) in 1994. It was reared on rutabaga roots (*Brassica napus* L.) at 20 ± 2°C, 60 ± 10% RH, L16:D8, according to the technique described by Neveu et al. (1996). The *P. vindemmiae* strain was collected in Rennes (Brittany, France) in 2000 from a population expected to experience high level of intraspecific competition (Goubault et al. 2004b) and was reared on pupae of *D. radicum* at 25 ± 1°C, 60 ± 10% RH, L16:D8. Adults were maintained in Petri dishes (diameter 8.5 cm; depth 2.7 cm) and supplied with honey, water and 150 host pupae that were replaced three times a week. Parasitized pupae were individually placed in gelatine capsules until adult emergence, preventing females from having any contact with other conspecific females. On the day of their emergence (d_0), *P. vindemmiae* females were mated, then isolated in tubes and supplied with water and honey. Hosts used during the experiments were 7- to 10-d-old *D. radicum* pupae.

Experimental Procedures

To investigate the influence of the previous habitat quality and female egg load on contest outcome and female aggressiveness, we generated three types of females. This was done by providing them with different numbers of *D. radicum* pupae from their emergence (at d_0) to the beginning of the experiment, 2 d later (d_2). The different female types were: (1) females that have experienced a poor habitat (called 'P'; they were supplied with a single unparasitized *D. radicum* pupa for 5 h at d_0), (2) females that have experienced a rich habitat (called 'R'; they were provided with five unparasitized pupae for 5 h at d_0 and five other unparasitized pupae for 5 h at d_1) and (3) females that have experienced a rich habitat and have a low egg load (called 'L'; they were given one unparasitized pupa for 5 h at d_0 and 10 other fresh pupae at d_2 for the 3 h preceding the beginning of the test). To test the effect of host quality, two types of hosts were used during the experiments: (1) unparasitized *D. radicum* pupae (called 'u') and (2) pupae that have been parasitized 24 h before the test by a conspecific female (only one egg was deposited on each pupa; these hosts were called 'p').

At the beginning of the experiment, either one or two females were introduced in an arena ($1.3 \times 1.3 \times 0.2$ cm) containing one host (see treatments below). The small arena was designed to keep both females in sharp focus under high magnification and it enabled us to detect small limb movements and subtle female–female interactions. It also simulated the galleries where *D. radicum* larvae, as well as those of other species attacked by *P. vindemmiae* (e.g. *Fannia canicularis* L., *Musca domestica* L. [Rueda & Axtell 1985]) naturally pupate. To observe contests, two females were simultaneously introduced and one of them was marked for identification. The marking was made at d_0 by depositing a small dot of acrylic white paint (Marabuwerke GmbH and Co., Tamm, Germany) on the dorsal surface of the thorax between the wing bases. When two females of different types were opposed, both types were alternatively marked. Preliminary experiments had shown that such marking did not influence the female behavioural responses.

During the experiment, we recorded all female behaviours with a behavioural data collection program (The Observer 4.0: Noldus, Wageningen, Netherlands). When two females were confronted, their behaviours were simultaneously recorded. In this case, host exploitation behaviours were recorded along with all the interaction behaviours between the competing females. As during a replicate both females may encounter each other several times, an 'encounter' was defined as starting when a female showed an interaction behaviour and ending when they both stopped all interaction behaviours for more than 10 s. Thus, several interaction behaviours may occur sequentially during an encounter. The ownership status of females was defined as follows: a female alone on the host for more than 10 s was considered as the 'owner', the other female was called 'intruder'. When both females simultaneously exploited the host up to the stage of drilling into the puparium, they were both considered as owners. Complete observations were stopped when the females did not contact the host nor interact with each other for more than 60 s. After each replicate, ovipositions were confirmed by dissecting the host. Each female was tested only once.

Table 1 presents the list of treatments generated by opposing different types of females and providing them with hosts of different quality. We first tested the influence of the presence of a competitor on host exploitation, by comparing (1) the mean number of eggs laid by each female, (2) the time from the beginning of the observation to the end of the first oviposition and (3) the total duration of each experiment, between the treatments where only one female (P-u) or two females (PP-u) simultaneously exploited an unparasitized host. Then, we assessed the effect of the previous habitat quality on host exploitation and female aggressiveness, by comparing situations where the contesting females had experienced the same (PP-u and RR-u) or different previous habitats (RP-u). The effect of female egg load was investigated by comparing situations where

Table 1: Experimental treatments. The block letters of the treatment names correspond to the type of females that were tested, two letters indicating that two females were confronted. The small letters indicate the type of host that was provided to the females

	Female 1		Female 2			
Treatment	Previous habitat[e]	Egg load	Previous habitat[e]	Egg load	Host quality	No. replicates
P-u[a]	Poor	High	–	–	unparasitized	10
PP-u[a,b]	Poor	High	Poor	High	unparasitized	10
RR-u[b,c,d]	Rich	High	Rich	High	unparasitized	10
RP-u[b]	Rich	High	Poor	High	unparasitized	39
LL-u[c]	Rich (3 h)	Low	Rich (3 h)	Low	unparasitized	9
RL-u[c]	Rich	High	Rich (3 h)	Low	unparasitized	18
RR-p[d]	Rich	High	Rich	High	parasitized	9

Small letters indicate that this treatment was used to investigate the effects of [a]the presence of a competitor, [b]quality of the previous habitat, [c]female egg load and [d]host quality on host exploitation, female aggressiveness and contest outcomes.

[e]'Previous habitat' corresponds to the quality of the habitat experienced by the females during the 2 d before the test, except in the situation 'rich (3 h)' where females experienced a rich habitat during the 3 h preceding the test.

the confronted females had similar (RR-u and LL-u) or different egg loads (RL-u). The influence of host quality was tested by comparing treatments where females were provided with an unparasitized host (RR-u) or with a 24 h-parasitized host (RR-p). In these three groups of treatments, we measured and compared: (1) the female aggressiveness level, estimated by the total number of bites per minute displayed during the whole experiment, (2) experiment durations, (3) number of ovicides committed per replicate, (4) proportions of replicates where either one or both females deposited an egg (in asymmetrical situations, we also noted which female oviposited first), (5) fitness gain rate of each female, estimated as the number of offspring potentially gained by female per hour. The fitness gain was calculated by taking into account that, in *P. vindemmiae*, eggs laid within a 4-h period have equal chances of winning the larval competition while an egg laid 24 h after another has only 40% chances of surviving (Goubault et al. 2003). Egg destruction via ovicide was also included in this measurement.

Finally, we measured the effect of the host exploitation stage reached by the owner female on female aggressiveness. Data of the three treatments RR-u, PP-u and RR-p (which gave similar results, see below) were pooled. When several ovipositions occurred during the same experiment, only the data recorded up to the end of the first oviposition were taken into account. The following parameters were compared according to the stage reached by the owner female in the behavioural oviposition sequence: (1) the number of female–female encounters per minute, (2) the number of bites per minute given by either both females together or by owner and intruder separately, (3) the proportion of encounters where the owner female kept the host.

Statistical Analyses

Data were analysed using generalized linear modelling with Genstat statistical package (Version 8; VSN International, Hemel Hempstead, UK). Our general approach was to use parametric analyses in which the assumed distribution of residuals was matched to the data rather than transforming data to fit standard Gaussian assumptions (Wilson & Hardy 2002). Log-linear analyses (suitable for 'small count' response variables, Crawley 1993) were used to investigate the effect of the treatments on the number of eggs laid and ovicides committed. Proportions were compared by logistic regressions. When the error variance of the data (such as bite rates and fitness gain per hour) did not conform to parametric assumptions, nonparametric Kruskal–Wallis tests were used. When significant, tests were followed by Mann–Whitney pairwise comparisons with Bonferroni correction. In the case of paired data, Wilcoxon signed-rank tests were used. Experiment durations were compared between treatments with log-rank tests.

Results

Effect of the Female Type on Female Egg Load

Poor and rich previous habitat did not affect female egg load: females P had 19.0 ± 0.7 ($\bar{x} \pm$ SE) eggs in their ovaries ($n = 10$) while females R had 19.8 ± 0.8 eggs ($n = 10$) (log-linear regression, $F_{1,19} = 0.49$, $p = 0.48$). In contrast, the Rich (3 h) habitat (i.e. females had access to 10 hosts during the 3 h preceding the test) considerably reduced female egg load: females L had only 9.2 ± 0.5 eggs ready to lay ($n = 10$; comparison with the egg load of females R: $F_{1,19} = 110.97$, $p < 0.001$).

Effect of the Presence of a Competitor

The behavioural oviposition sequence of single females was linear. They contacted the host with their antennae then with the tip of their abdomen, drilled a hole through the host puparium to insert their ovipositor (i.e. probing) and laid an egg. Some females interrupted the sequence to host-feed. In contrast, when two females were simultaneously present in the arena, the oviposition sequence was often interrupted by numerous behavioural interactions between them. These interactions showed different levels of aggressiveness, from non-aggressive contacts to attacks by biting the opponent (Goubault et al., 2005). Bite was the most aggressive behaviour and was considered as a full escalation because it usually happened at the end of encounters and led to the departure of the losing contestant from the close vicinity of the host. Females could bite wings or tarsi of their opponents, but no loss of body parts or any obvious injuries were observed. The presence of a competitor had consequently several effects on host exploitation. First, females laid fewer eggs than when alone (0.70 ± 0.13 vs. 1.20 ± 0.13, $F_{1,29} = 5.16$, $p = 0.023$). Second, it took more time to lay the first egg onto the host (2870 ± 290 s vs. 1780 ± 280 s; $\chi^2_1 = 5.11$, $p = 0.024$). Finally, as the host could be exploited by both females, one after the other, the complete experiments lasted longer (3470 ± 290 s vs. 1780 ± 280 s; $\chi^2_1 = 11.73$, $p = 0.0006$).

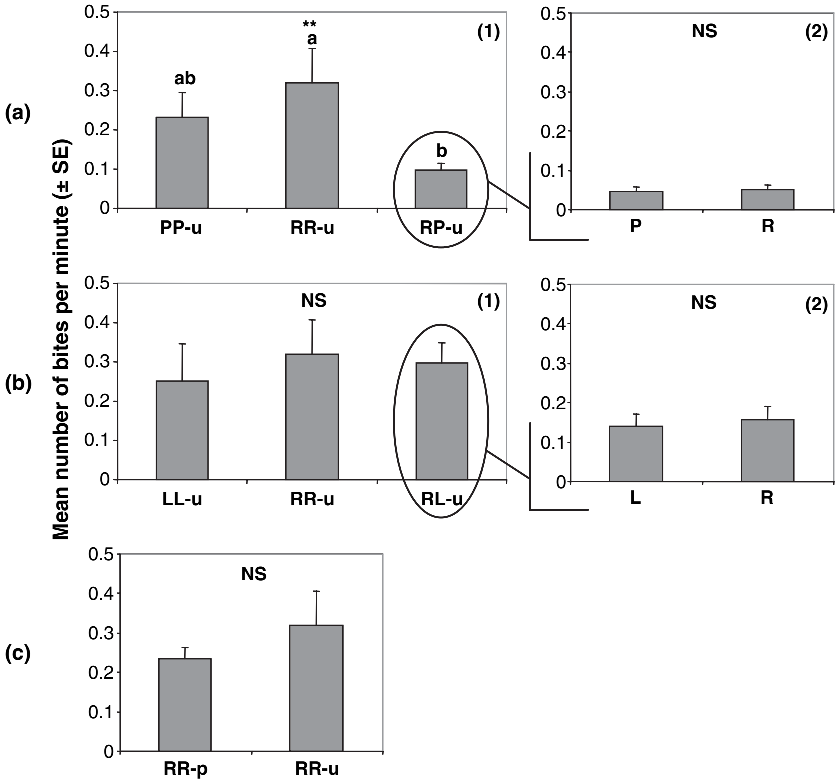

Fig. 1: Effect of (a) previous habitat quality, (b) female egg load and (c) host quality on female aggressiveness, in terms of biting rate. (1) Results of the overall effect of each treatment. (2) Results for each type of females in asymmetrical situations. NS: not significant, $^{**}p < 0.01$. Two different letters in each graph indicate a significant difference at $p < 0.017$ (i.e. $p < 0.05/3$ tests)

Quality of the Previous Habitat (PP-u vs. RR-u vs. RP-u)

Asymmetrical contests (RP-u) were less violent than symmetrical contests (PP-u and RR-u) ($H_2 = 12.71$, $p = 0.002$; Fig. 1a(1)) but the biting rates of females of both types (R and P) was not significantly different ($W_+ = 212.5$, $n = 29$, $p = 0.91$; Fig. 1a(2)). The duration of the experiment did not differ significantly in the three situations (Table 2). Fewer ovicides were committed when both females had experienced a rich habitat quality (RR-u) than in the two other situations (PP-u and RP-u) (Table 2). Females P performed as many ovicide as females R in asymmetrical experiments (binomial test: $p = 0.86$). The proportion of replicates where the two confronted females

Table 2: Effect of previous habitat quality, female egg load and host quality on host exploitation by females

Treatment	Mean experiment duration (s)	Mean number of ovicide	Proportion of replicates where both females oviposited (%)	Sample size
Quality of the previous habitat				
PP-u	3469 ± 291	0.30 ± 0.15 a	30.0 ± 15.3 ab	(10)
RR-u	2587 ± 313	0.00 ± 0.00 b	0.0 ± 0.0 a	(10)
RP-u	3353 ± 283	0.23 ± 0.08 a	50.0 ± 8.2 b	(39)
	$\chi^2_2 = 3.89$, $p = 0.14$	$F_{2,56} = 3.29$, $p = 0.045$	$G_2 = 5.84$, $p = 0.003$	
Female egg load				
LL-u	2055 ± 498	0.00 ± 0.00	44.4 ± 18.6 a	(9)
RR-u	2587 ± 313	0.00 ± 0.00	0.0 ± 0.0 b	(10)
RL-u	2992 ± 406	0.00 ± 0.00	16.7 ± 9.0 ab	(18)
	$\chi^2_2 = 2.66$, $p = 0.26$		$G_2 = 3.65$, $p = 0.03$	
Host quality				
RR-p	3761 ± 578	0.67 ± 0.24 b	44.4 ± 17.6 b	(9)
RR-u	2587 ± 313	0.00 ± 0.00 a	0.0 ± 0.0 a	(10)
	$\chi^2_1 = 2.88$, $p = 0.09$	$F_{1,17} = 19.96$, $p < 0.001$	$G_1 = 7.19$, $p = 0.007$	

For each effect tested, values ($\bar{x}$ ± SE) in the same column followed by different letters are significantly different at $p < 0.05$. The absence of letter means that there is no significant difference.

parasitized the host during the same test was significantly lower in RR-u than in RP-u (Table 2). In the asymmetric treatment, females R oviposited first more frequently than females P, i.e. in 26 of 39 replicates (binomial test: $p = 0.05$). Females that oviposited first always had a better fitness gain rate than their rival in all treatments (PP-u: $W_+ = 9$, $n = 10$, $p = 0.05$; RR-u: $W_+ = 0$, $n = 10$, $p = 0.005$; RP-u: $W_+ = 23$, $n = 27$, $p < 0.0001$; Fig. 2a(1)). First ovipositing females had a significantly higher fitness gain rate in RR-u than in RP-u and those from PP-u had intermediate results ($H_2 = 6.29$, $p = 0.04$). In the asymmetrical situation, the fitness gain rates of females of both types were not significantly different ($W_+ = 219.5$, $n = 27$, $p = 0.46$; Fig. 2a(2)).

Egg Load (LL-u vs. RR-u vs. RL-u)

Regardless of the treatment, female aggressiveness levels did not differ significantly ($H_2 = 0.79$, $p = 0.68$; Fig. 1b(1)). In asymmetrical situations, females of both types did not show significantly different biting rates ($W_+ = 46$, $n = 16$, $p = 0.26$; Fig. 1b(2)). Experiment duration was not affected by the treatment (Table 2). No ovicide was observed in any of these three treatments (Table 2). The proportion of replicates where the two females laid on the host was higher when at least one female L was involved in the contest (LL-u and RL-u) (Table 2). In the asymmetrical situation, females L were the first to oviposit in 10 of 18 replicates, a result which was not different from a 50:50 null hypothesis (binomial test: $p = 0.82$). First ovipositing females had a better fitness gain rate in all treatments (LL-u: $W_+ = 1$, $n = 6$, $p = 0.05$; RR-u: $W_+ = 0$, $n = 10$, $p = 0.005$; RL-u: $W_+ = 1$, $n = 16$, $p = 0.0005$; Fig. 2b(1)), and these gains did not vary between treatments ($H_2 = 0.34$, $p = 0.85$). In the asymmetrical situation, fitness gain rates of females R and L were not significantly different ($W_+ = 67$, $n = 16$, $p = 0.96$; Fig. 2b(2)).

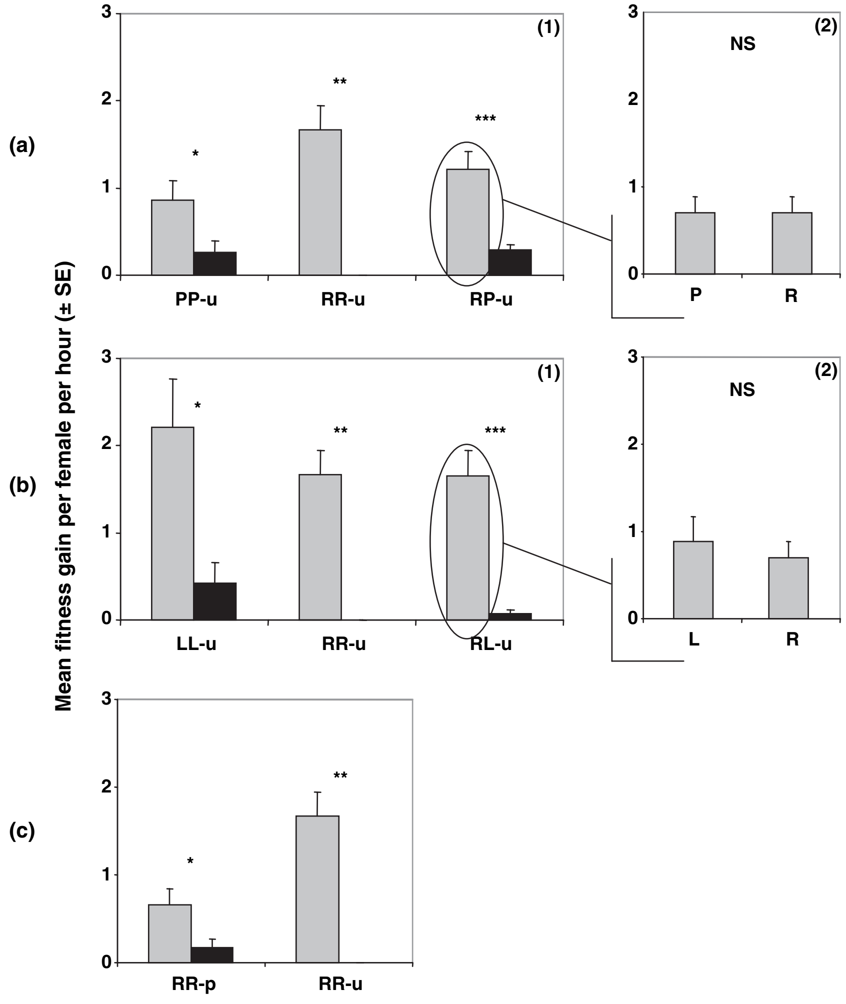

Fig. 2: Effect of (a) previous habitat quality, (b) female egg load and (c) host quality on the number of offspring potentially gained (i.e. fitness gain) per female per hour. Grey bars represent the mean fitness gain of the first ovipositing females and black bars that of the other females. (1) Results of the overall effect of each treatment. (2) Results for each type of females in asymmetrical situations. NS: not significant, $*p < 0.05$; $**p < 0.01$, $***p < 0.001$

Host Quality (RR-u vs. RR-p)

Host parasitism status did not influence female biting rates ($W_+ = 39$, $n = 19$, $p = 0.62$; Fig. 1c). Experiment duration was not affected by host quality (Table 2). Females performed more ovicides when confronted with 24 h-parasitized hosts (Table 2). The two females laid more often in succession when the host was already parasitized at the beginning of the experiment (Table 2). Females that oviposited first had a better fitness gain rate than their opponent (RR-p: $W_+ = 4$, $n = 8$, $p = 0.05$; RR-u: $W_+ = 0$, $n = 10$, $p = 0.005$; Fig. 2c), and this gain rate was significantly higher when the host was unparasitized at the beginning of the experiment ($W_+ = 12$, $n = 10$, $p = 0.007$).

Host Exploitation Stage

The number of female–female encounters per minute did not differ significantly along the behavioural oviposition sequence (Table 3). In contrast, the aggressiveness of female–female encounters (in terms of biting rates) varied with the host exploitation stage reached by the owner females ($H_5 = 24.79$, $p = 0.0002$, Fig. 3a). The further the owners in their oviposition sequence, the less violent the encounters. At each stage, biting rates showed by owners and intruders did not differ significantly (Table 3). The owner's probability of keeping the host after an encounter with an intruder was also affected by the host exploitation stage: it increased when the owner female started the abdominal examination of the host ($H_5 = 34.38$, $p < 0.001$, Fig. 3b).

Discussion

When encountering a conspecific during host exploitation, *P. vindemmiae* females displayed agonistic behaviours, from non-aggressive contacts to full attacks (i.e. bites), towards their opponent. These interactions considerably increased the oviposition duration by repeatedly interrupting the female oviposition behavioural sequence. Although escalations never led to fatalities or serious injuries, as observed in other fighting species (Lawrence 1981; Pérez-Lachaud et al. 2002), they usually led to the departure of the losing contestant from the close vicinity of the host. Females protected their host only during their exploitation. In contrast to bethylids (Griffiths & Godfray 1988; Hardy & Blackburn 1991; Goubault et al. in press), *P. vindemmiae* females did not show any brood guarding: after the owner had oviposited, an intruder female frequently took over and superparasitized the host, sometimes after having killed the owner's egg.

Quality of the Previous Habitat

In asymmetrical situations, females appeared less aggressive than in symmetrical contests, which is consistent with Contest Theory's predictions (Maynard-Smith & Parker 1976; Hammerstein 1981; Leimar & Enquist 1984). However, both types of females (from rich and poor habitats) showed similar low biting rates and the settlement of contests was not, as expected, in favour of females with the highest subjective RV (i.e. from poor habitat). In fact, females from rich habitat even appeared advantaged as they were more likely to oviposit first and first laying females had better fitness gains. Their greater host acquisition abilities may be related to *P. vindemmiae* females' capability to host-feed (Phillips 1993) and the fact that rich habitat females had more opportunities to do so. Host-feeding usually procures females' energy that they use during host searching and egg production (Jervis & Kidd 1986). As rich habitat females did not show increased egg

Table 3: Female–female encounter rates and biting rates of owner and intruder females ($\bar{x} \pm SE$) according to the host exploitation stage reached by the owners

			Biting rates		
Owner's host exploitation stage	n	Female–female encounter rate	Owners	Intruders	Owners' vs. intruders' biting rates
Antennal examination	25	1.08 ± 0.29	0.44 ± 0.12	0.49 ± 0.19	$W_+ = 85$, $n = 18$, $p = 0.98$
Abdominal examination	25	0.65 ± 0.15	0.19 ± 0.07	0.14 ± 0.05	$W_+ = 25$, $n = 11$, $p = 0.48$
Drilling	25	0.68 ± 0.22	0.11 ± 0.06	0.08 ± 0.03	$W_+ = 33$, $n = 11$, $p \approx 1$
Probing	23	0.53 ± 0.14	0.04 ± 0.02	0.04 ± 0.02	$W_+ = 4$, $n = 4$, $p = 0.72$
Host-feeding	20	0.39 ± 0.09	0.04 ± 0.02	0.04 ± 0.02	$W_+ = 11$, $n = 7$, $p = 0.61$
After the oviposition	25	0.49 ± 0.14	0.04 ± 0.02	0.25 ± 0.15	$W_+ = 50$, $n = 12$, $p = 0.39$
	$H_2 = 6.46$, $p = 0.26$				

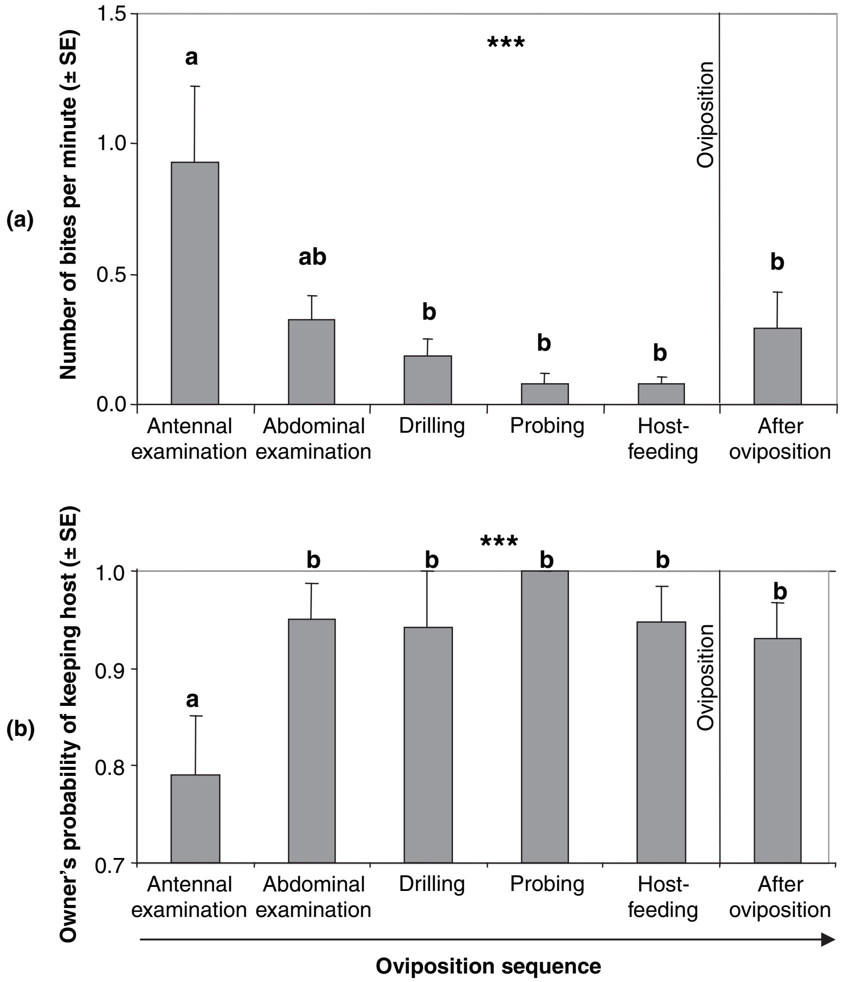

Fig. 3: Variation of (a) female aggressiveness and (b) owner's probability of keeping the host after an encounter with an intruder female, along the behavioural oviposition sequence. *** indicates a significant difference at $p < 0.001$. Two different letters indicate a significant difference at $p < 0.0036$ (i.e. $p < 0.05/14$ tests)

maturation, they might have converted this energy into host searching ability. Further experiments need to be carried out to conclude on this issue.

When poor habitat females were involved in the contests, we observed superparasitism more frequently. This might be related to their different host selection decisions. Females coming from a poor habitat had effectively experienced a longer (apparent) patch travel time (i.e. they had been deprived of hosts for a longer time); they should consequently be less selective and accept more often lower quality hosts (here, parasitized hosts; Mangel 1989; Sirot et al. 1997; Rosenheim 1999). Thus, in *P. vindemmiae*, the second exploitation of hosts would depend more on female selectiveness than on their ability to protect hosts.

Egg Load and Previous Experience

We generally did not observe any effect of egg load on *P. vindemmiae* female aggressiveness and contest outcome, in contrast to *G. nephantidis* (Stokkebo & Hardy 2000). However, in the latter species, the advantage of having a higher egg load was only detected when status asymmetry was absent (i.e. in owner–owner contests) which was not the case in our experiments. Moreover, our egg load manipulation may present some limitations. First, what we considered as a 'low' egg load might not have been low enough (i.e. females still had about nine eggs ready to be laid) to generate the expected difference in RV. Second, by manipulating egg load, we not only modified females' physiology but also their perception of host availability. The influences of these two factors on female host exploitation strategies can usually not be disentangled, as mentioned by Rosenheim & Rosen (1991). As a result, low egg load females may have been less selective (Mangel 1989; Sirot et al. 1997; Rosenheim 1999), leading hosts to be significantly more often parasitized by both females when a low egg load female was involved in the contest.

Host Quality

Pachycrepoideus vindemmiae females showed similar aggressiveness whether the host they were contesting for was parasitized or not. Yet, superparasitism was more frequent when the host was initially parasitized (low quality) than unparasitized (high quality host). Thus, despite a similar aggressiveness, host protection by the first owner female may have been more efficient when the host was of a higher quality, as observed in *G. nephantidis* (Goubault et al. in press; Humphries et al. 2006).

Host Exploitation Stage

As owners got farther in their behavioural oviposition sequence, the host value asymmetry with intruders increased, and owners were more likely to retain their host. Similar results were observed in *Trissolcus basalis*, where the asymmetry was obtained by introducing females at different time intervals. As owners exploited a patch, its value increased for them, and they won more frequently contests against intruders (Field & Calbert 1999). Moreover, we observed that aggressiveness of both females decreased along the oviposition sequence. For owners, this decrease may be due to their position on the host (such as when they were probing). For the intruders, it suggests that they were able to perceive where owners were in their behavioural oviposition sequence and adapt their agonistic behaviours accordingly. They might use visual cues, such as the characteristic position of owners when drilling the host puparium for instance, but also chemical information as the volatiles emitted by Bethylidae wasps (Gómez et al. 2005; Goubault et al. 2006). Further experiments would be required to identify the cues and signals involved.

Conclusions

In *P. vindemmiae*, host protection seemed only effective during host exploitation and no brood guarding was observed. Although the tested females came from a population expected to experience a high level of intraspecific competition (Goubault et al. 2004b), hosts may not be rare enough for such a time-consuming behaviour to have been selected for. In this particular population, females also tend to adopt time-limited rather than egg-limited strategies (Goubault et al. 2004b). They should consequently avoid wasting time in host protection and invest it in host searching.

Although resource density is known to influence the evolution of elaborate contests (Enquist & Leimar 1987), time limitation might also be a key factor favouring the selection of resource guarding behaviours and strategies in accordance with Contest Theory's expectations. Indeed, even when the resource is limited, the best strategy for short-lived individuals may be not to waste time in conflict but search for more resources. The study of different species or populations experiencing different levels of resource availability and time/egg limitation balance would be needed to conclude on this subject.

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