

# Insect gladiators II: Competitive interactions within and between bethylid parasitoid species of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae)

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## Abstract

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) causes substantial reductions in coffee production. It originates from Africa but is now present in almost all of the major coffee producing countries. Classical biological control attempts around the world, including South and Central America, the Caribbean, Indian sub-continent, Indonesia, and Polynesia, including introductions of the African bethylid wasps *Prorops nasuta* Waterston and *Cephalonomia stephanoderis* Betrem, have not yet proved sufficiently successful. In Mexico, a bethylid wasp indigenous to North America and Europe, *Cephalonomia hyalinipennis* Ashmead, has naturally started to attack the borer. The presence of multiple species of natural enemies has the potential to disrupt biological control via negative interspecific interactions. We evaluate both inter- and intraspecific competition among these three bethylids in the laboratory, focussing on pairs of adult females competing directly for hosts. In interspecific contests, the loser is frequently killed. *C. stephanoderis* is the most successful species, while *P. nasuta* females never killed their opponents. Intraspecific interactions often involved fighting behavior but were non-fatal. We discuss the implications of the differing strengths of such inter- and intraspecific interactions for the coexistence of these natural enemies of the coffee berry borer and for biological control.

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## 1. Introduction

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), continues to cause substantial reductions in coffee production throughout the world (Baker, 1999; Baker et al., 2002). Native to Africa, it is now present in almost all of the major coffee producing countries, including Mexico. *H. hampei* was first observed in the Soconusco region (Chiapas) in 1978 and has since become a major pest throughout Mexico (Baker, 1984; Infante et al., 2001a). As part of a biological

control program, releases of two parasitoid natural enemies from Africa, *Prorops nasuta* Waterston and *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylinidae) commenced in Chiapas in 1988 (Barrera et al., 1990a). *C. stephanoderis* has established but is rare (Damon, 1999; Damon and Valle, 2002) and *P. nasuta* has never persisted longer than 15 months (Infante et al., 2001a); neither species has achieved economically significant pest control. A new association has also developed in Mexico where *Cephalonomia hyalinipennis* Ashmead, a bethylid wasp indigenous to North America and Europe (Gordh and Móczár, 1990), has been found naturally attacking *H. hampei* in the Soconusco region of Chiapas (Pérez-Lachaud, 1998).

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These three bethylid species share many similar life-history traits. All three species are synovigenic and need to host-feed before they can mature eggs. Adult females feed on all developmental stages of the borer, preferentially consuming eggs, small larvae and adults (Barrera et al., 1990b; Lauzière et al., 2001a), with the possible exception of *C. hyalinipennis*, which does not appear to attack adult borers. Females preferentially parasitize prepupae, pupae, and occasionally large larvae of the CBB (Abraham et al., 1990; Barrera et al., 1989; Infante and Luis, 1993; Lauzière et al., 2001b; Pérez-Lachaud and Hardy, 1999, 2001). Individual females usually oviposit within only one berry.

Negative interactions between these bethylids are known to occur. Intraguild predation occurs between *P. nasuta* and *C. stephanoderis* in the laboratory via consumption of allospecific eggs and larvae (Infante et al., 2001b). *C. hyalinipennis* has recently been identified as a facultative hyperparasitoid of several bethylid parasitoid species, including *C. stephanoderis* and *P. nasuta*, in the laboratory (Pérez-Lachaud et al., 2004). In addition dyadic contests for hosts occur between adult females. After entering a berry containing CBB, females exhibit host guarding (Pérez-Lachaud et al., 2002), a behavior observed in many bethylid species (Griffiths and Godfray, 1988; Hardy and Blackburn, 1991; Petersen and Hardy, 1996). Guarding females attempt to repel con- and allo-specific intruders entering the berry. The contests that result can involve a series of chases, biting and, at full escalation, attempts to attack and paralyze each other with their stingers (modified ovipositors), which house venom to induce paralysis in hosts prior to oviposition. Direct interspecific contests frequently result in the paralysis and death of the defeated female (Pérez-Lachaud et al., 2002), demonstrating the potency of the venom when used on allospecifics. The outcome of dyadic contests is, in general, expected to be related to the costs incurred (Enquist and Leimar, 1990; Payne, 1998), differences in competitive ability between individuals (Grafen, 1987; Hammerstein, 1981; Maynard-Smith and Parker, 1976), prior ownership (Papaj and Messing, 1998; Petersen and Hardy, 1996) and to the resource value (Dugatkin and Biederman, 1991; Field and Calbert, 1998; Wells, 1988) to the competitors.

Pérez-Lachaud et al. (2002) documented dyadic contests between the abovementioned bethylid species that are parasitoids of the CBB. They placed emphasis on contests involving *C. stephanoderis* and investigated 7 of the 12 possible combinations of owner versus naïve or experienced intruder for these three species in the laboratory. Here we present data on the remaining five combinations of interspecific contests. Further, we extend these investigations to intraspecific contests for CBB hosts. We discuss these findings in the contexts of the evolution of fatal fighting and the relative importance of intra- and interspecific interactions in relation to species

coexistence and their implications for biological control of the CBB.

## 2. Materials and methods

Parasitoids were reared at El Colegio de la Frontera Sur (ECOSUR, Tapachula, Chiapas, Mexico). The *C. stephanoderis* stock was derived from insects collected in Togo in 1988, and the *P. nasuta* stock from insects reared in Brazil in 1992. The *C. hyalinipennis* culture was initiated in 1997 from material collected in the Soconusco region (Mexico). Pupae and prepupae of the coffee berry borer were removed directly from naturally attacked coffee berries collected from various localities around Tapachula.

The experimental procedure followed that of Pérez-Lachaud et al. (2002) in which full methodological details are given. Briefly, we observed behavioral interactions during encounters between pairs of adult female bethylids. In all interspecific, and most intraspecific, experiments, one female was a prior 'owner' of a batch of 10 hosts, which had been paralyzed and fed upon by that female. Owners had already oviposited in the majority of these contests. We refer to the non-owner females as the 'intruder.' Intruders were either 'naïve' (had not previously encountered hosts) or 'experienced' (had host fed and oviposited prior to the experiment). We studied those combinations of owner versus naïve or experienced intruder not addressed by Pérez-Lachaud et al. (2002), such that, by combining these studies all 12 possible combinations of interspecific contests were observed (Table 1). We also enhanced the sample size of two of the combinations previously studied by Pérez-Lachaud et al. (2002). Intraspecific contest experiments involved either an owner and an intruder (Table 2) or two naïve non-owners (Table 3), of known body size (head width), with methodology otherwise identical to that used for interspecific contests.

Interactions between females were observed within the apparatus used by Pérez-Lachaud et al. (2002) consisting of an opaque plastic block with three chambers linked by a slot (through which the parasitoids studied can pass) and covered with clear Plexiglas. This apparatus was modified from that originally developed by Petersen and Hardy (1996), who provide a detailed description and illustration. Due to the relatively small size of bethylids attacking the coffee berry borer, the apparatus used in this study, and by Pérez-Lachaud et al. (2002), was made to a smaller scale than the original, such that the dimensions of the chambers were 10 mm diameter and 5 mm depth, with 10 mm between each chamber and a slot of 1 mm in width. Barriers can be pushed into the slot to isolate one or more chambers, and be withdrawn to connect the chambers. This apparatus allows interactions between females to occur within a confined space, similar to in a coffee berry, with

Table 1  
Outcomes of dyadic interspecific interactions

Row	Owner	Intruder	Intruder state	n	Contests won by prior owners				Contests won by intruders			Mean time to contest resolution (h)
					Total	P <sup>c</sup>	Intruder killed	Intruder not killed	Total	Owner killed	Owner not killed	
1 <sup>a</sup>	<i>C. stephanoderis</i>	<i>C. hyalinipennis</i>	Naïve	12	11	0.006	6	5	1	0	1	20.7
2 <sup>b</sup>			Experienced	10	9	0.021	8	1	1	0	1	24.0
3 <sup>a</sup>	<i>P. nasuta</i>		Naïve	10	10	0.002	10	0	0	0	0	16.8
4 <sup>b</sup>			Experienced	10	10	0.002	10	0	0	0	0	12.9
5 <sup>a</sup>	<i>C. hyalinipennis</i>	<i>C. stephanoderis</i>	Naïve	14	12	0.013	10	2	2	1	1	40.2
6 <sup>a</sup>			Experienced	11	1	0.012	0	1	10	8	2	8.6
7	<i>P. nasuta</i>		Naïve	10	9	0.021	6	3	1	0	1	20.7
8			Experienced	10	8	0.109	5	3	2	0	2	79.1
9 <sup>a</sup>	<i>P. nasuta</i>	<i>C. stephanoderis</i>	Naïve	13	4	0.267	0	4	9	8	1	28.1
10			Experienced	10	3	0.344	0	3	7	6	1	41.7
11	<i>C. hyalinipennis</i>		Naïve	10	3	0.344	0	3	7	5	2	81.8
12			Experienced	10	1	0.021	0	1	9	6	3	83.6

<sup>a</sup> Data collected by Pérez-Lachaud et al. (2002).

<sup>b</sup> Data are a combination of results obtained by Pérez-Lachaud et al. (2002) and the current study.

<sup>c</sup> Probabilities of  $\geq$  observed number of wins by owner (binomial test).

Table 2  
Outcomes of dyadic intraspecific interactions

Row <sup>a</sup>	Owner	Intruder state	n	Contests won by prior owners			No winner	Contests won by intruders		Mean time to contest resolution (h)
				Total	Intruder killed	Intruder not killed		Owner killed	Owner not killed	
1	<i>C. stephanoderis</i>	Naïve	5	5	0	5	0	0	0	7.2
2		Experienced	5	5	0	5	0	0	0	7.2
3	<i>C. hyalinipennis</i>	Naïve	5	5	0	5	0	0	0	3.0
4		Experienced	5	2	0	2	0	0	3	3.0
5	<i>P. nasuta</i>	Naïve	5	1	0	1	3	0	1	120.0

<sup>a</sup> Experiments were performed on 5 of the 6 possible combinations of owner versus naïve or experienced intruder for three species.

Table 3  
Outcomes of dyadic intraspecific non-owner contests

Row	Species	n	Contests won by		Contests involving same sized individuals	Contests with no winner	Mean time to contest resolution (h)
			Larger female	Smaller female			
1	<i>C. stephanoderis</i>	10	4	2	2	2	37.1
2	<i>C. hyalinipennis</i>	10	6	1	2	1	70.3
3	<i>P. nasuta</i>	10	2	4	1	3	82.9

each female free to enter and leave the immediate vicinity of the hosts. Observations were made from above, through a binocular dissection microscope. Environmental conditions were  $28 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH under natural lighting with an approximately 12:12 (L:D)h photoperiod.

Interactions between prior owners and intruders were set up as follows: a newly emerged mated female (>2 days old) was placed in a glass tube (dimensions 1.5 cm  $\times$  7.3 cm, sealed with gauze) and presented with a batch of 10 host pupae and prepupae for 3–6 days prior to the experiment and moved, with any resulting progeny, into an observation chamber in the apparatus one day before the experiment. On transfer, the owner was

presented with a further five fresh hosts. Thus owner females were aged from 4 to 7 days old. An ‘intruder’ female was placed into an adjoining chamber the day before the observations, with the chambers isolated by barriers. Naïve intruders were aged 4–7 days and had been fed on concentrated honey solution. Experienced intruders were of a similar age as owners and had been presented with hosts during the first 4–5 days of adult life, but neither their resulting offspring nor the hosts’ remains were present in the apparatus.

Intraspecific interactions between two naïve non-owners were set up and observed in a similar manner to owner–intruder experiments, except that a female aged 2–5 days, that had been fed on honey was placed into

each of the two extreme chambers in the apparatus, and 10 hosts were placed in the central chamber.

In all experiments, the barriers were withdrawn during the day following set-up, allowing both females access to all three chambers and the hosts. Behaviors of both females were observed for 3 h following barrier withdrawal and the locations of the females within the apparatus were recorded every 15 min (scan sampling). Individual females were used in experiments only once to avoid confounding variables arising from prior experience of contests (Mesterton-Gibbons, 1999). A total of 130 interspecific owner-intruder interactions were observed (Table 1): 57 of these observations were made in this study and 73 derived from the prior work of Pérez-Lachaud et al. (2002). The current study also provided a total of 55 intraspecific interactions: 25 owner-intruder interactions (Table 2) and 30 naïve non-owner interactions (Table 3).

When direct interactions (pursuit and attempts to sting or bite) occurred during the 3 h observation period, the outcome of interactions was scored on the basis of the number of encounters won by each female. The female that won the majority of encounters (and possessed the hosts) or eventually paralyzed and/or killed the other female, was scored as the winner. However, direct aggressive interactions frequently did not occur during the observation period. In these cases, we continued the experiment overnight scoring the position and/or behavior at 24 h (or for every subsequent 24 h until an outcome was evident) after the beginning of the experiment.

As in Pérez-Lachaud et al. (2002), the influences of experience and species were explored using log-likelihood ratio tests (*G*-tests) of data in  $2 \times 2$  contingency tables for comparing between rows in Table 1 or Table 2 and influence of ownership status was explored using two-tailed binomial tests for small samples with the null hypothesis that owners and intruders win equal numbers of contests (comparisons within rows of Table 1 or Table 2). The influence of body size in intraspecific non-owner interactions was studied using two-tailed binomial tests. The incidence of fatal fighting in inter- and intraspecific contests was compared using *G*-tests.

### 3. Results

#### 3.1. Interspecific interactions

Interactions between previously unobserved species combinations comprised similar behaviors to those reported by Pérez-Lachaud et al. (2002). Owners generally remained near their hosts prior to barrier removal. About half of the intruders that entered following barrier withdrawal fed upon the hosts they encountered (30/57). Owner-intruder encounters generally involved no

immediate agonistic response, but these often occurred during the observation period. Experienced intruders engaged more readily in direct contests with owners than did naïve intruders. Agonistic interactions consisted of pursuit, biting and stinging. Stinging often resulted in paralysis and death.

*Cephalonomia stephanoderis* was the most aggressive species, and *P. nasuta* the least. When *C. stephanoderis* had prior experience of hosts (i.e., when females were owners or experienced intruders), after the initial encounter, females immediately became more active and commonly attacked and attempted to paralyze their opponents within 10 min (12/17 interspecific contests, 5/10 intraspecific contests). *C. hyalinipennis* females with prior experience of hosts generally inspected allospecifics several times before chasing, biting and attempting to sting them. Although initially less aggressive, once agonistic interactions commenced, *C. hyalinipennis* appeared to be as aggressive as *C. stephanoderis*. Both *C. stephanoderis* and *C. hyalinipennis* owners usually chased intruders throughout the apparatus, while *P. nasuta* owners usually did not (5/7, 14/20, and 6/30 replicates respectively, based only on results collected in this study). Experienced *C. stephanoderis* and *C. hyalinipennis* had usually laid eggs prior to the experiment, but experienced *P. nasuta* (owners and intruders) had generally paralyzed some hosts but not oviposited (43/57 replicates). We noticed that the least aggressive species, *P. nasuta*, has less flexibility of abdominal movement than the two *Cephalonomia* species and that it often adopted a static threatening/defensive posture, standing with its abdomen curved under its thorax with the stinger pointing forwards, but appeared unable to mount an active attack.

#### 3.1.1. Outcome of contests

The outcomes of interspecific contests are summarized in Table 1. Our analysis combines data collected during this study with those reported by Pérez-Lachaud et al. (2002). In half of the 12 experimental scenarios, owners won significantly more contests than did intruders (Table 1, rows 1–5 and 7) and in two (rows 6 and 12) intruders won significantly more than owners.

In scenarios in which the owner was *C. stephanoderis*, owners won all, or nearly all, of the contests (Table 1, rows 1–4). When the owner was *C. hyalinipennis*, owners were significantly advantaged against naïve but not experienced *C. stephanoderis* or *P. nasuta* intruders (Table 1, rows 5–8), and lost significantly against experienced *C. stephanoderis* intruders (Table 1, row 6). In contrast, *P. nasuta* owners had no significant ownership advantage in individual contests (Table 1, row 9–12), and lost significantly against experienced *C. hyalinipennis* (Table 1, row 12).

When all contests involving a particular owner species were combined, *C. stephanoderis* and *C. hyalinipennis*

*nis* owners won significantly more contests than did the intruders (*C. stephanoderis* won 40: lost 2, binomial test,  $P < 0.001$ ; *C. hyalinipennis* won 30: lost 15, binomial test,  $P = 0.036$ ) but *P. nasuta* owners lost significantly more contests than they won (won 11: lost 32, binomial test,  $P = 0.002$ ).

Overall, when acting as intruders, both *C. stephanoderis* and *C. hyalinipennis* won and lost similar numbers of contests (*C. stephanoderis* won 28: lost 20, binomial test,  $P = 0.312$ ; *C. hyalinipennis* won 18: lost 24, binomial test,  $P = 0.441$ ) but *P. nasuta* intruders lost significantly more contests than they won (won 3: lost 37, binomial test,  $P < 0.001$ ).

We explored the influence of intruder species on contest outcome by comparing across rows of Table 1. Experienced *C. stephanoderis* intruders were significantly more successful against *C. hyalinipennis* owners than were experienced *P. nasuta* intruders (comparison of rows 6 and 8:  $G = 11.97$ ,  $df = 1$ ,  $P < 0.001$ ). Comparisons of other scenarios indicated that the species of the intruder had no significant influence on contest outcome, whether intruders were naïve (rows 1 and 3:  $G = 1.25$ ,  $df = 1$ ,  $P > 0.05$ ; rows 5 and 7:  $G = 0.10$ ,  $df = 1$ ,  $P > 0.05$ ; and rows 9 and 11:  $G < 0.01$ ,  $df = 1$ ,  $P > 0.05$ ) or experienced (rows 2 and 4:  $G = 1.44$ ,  $df = 1$ ,  $P > 0.05$ ; rows 10 and 12:  $G = 1.30$ ,  $df = 1$ ,  $P > 0.05$ ): in these cases *C. stephanoderis* and *C. hyalinipennis* owners tended to win, and *P. nasuta* owners tended to lose.

The influence of intruder experience was explored by comparing across rows of Table 1. One comparison indicated an effect of intruder experience on contest outcome: experienced *C. stephanoderis* won significantly more often than naïve females when intruding on *C. hyalinipennis* owners (rows 5 and 6:  $G = 16.432$ ,  $df = 1$ ,  $P < 0.001$ ). In all five other comparisons, no effects of intruder experience were detected (rows 1 and 2:  $G < 0.01$ ,  $df = 1$ ,  $P > 0.05$ ; rows 3 and 4:  $G < 0.01$ ,  $df = 1$ ,  $P > 0.05$ ; rows 7 and 8:  $G = 0.40$ ,  $df = 1$ ,  $P > 0.05$ ; rows 9 and 10:  $G < 0.01$ ,  $df = 1$ ,  $P > 0.05$ ; and rows 11 and 12:  $G = 1.29$ ,  $df = 1$ ,  $P > 0.05$ ).

When both *C. stephanoderis* and *C. hyalinipennis* had prior experience of hosts, *C. stephanoderis* won more often, as both owner and intruder and hence ownership did not influence contest outcome (comparison of rows 2 and 6:  $G < 0.01$ ,  $df = 1$ ,  $P > 0.05$ ). Similarly, contests between experienced individuals of *C. hyalinipennis* and *P. nasuta* showed no influence of ownership as *C. hyalinipennis* generally won (rows 8 and 12:  $G = 0.40$ ,  $df = 1$ ,  $P > 0.05$ ). In contrast, experienced *C. stephanoderis* achieved greater success against experienced *P. nasuta* when in the role of owner (rows 4 and 10:  $G = 4.69$ ,  $df = 1$ ,  $P < 0.05$ ).

*Cephalonomia stephanoderis* thus appears to be the most successful competitor in interspecific interactions: this species nearly always won contests when in the role of owner, defeated *C. hyalinipennis* owners when experi-

enced and generally defeated *P. nasuta* owners irrespective of experience. *P. nasuta* is the least successful competitor as it usually lost contests, whether in the role of owner or intruder.

### 3.2. Intraspecific interactions

The suite of behaviors observed in intraspecific interactions involving at least one experienced female were the same as in interspecific interactions, with the exception that attempted stinging did not result in paralysis and death. In accord with observations of interspecific interactions, contests between *C. stephanoderis* dyads were generally the most aggressive and those between pairs of *P. nasuta* the least. Both *C. stephanoderis* and *C. hyalinipennis* owners usually chased intruders throughout the apparatus, while *P. nasuta* owners did not (8/10, 9/10, and 0/5 replicates, respectively). Experienced *C. stephanoderis* and *C. hyalinipennis* (owners and intruders) had usually laid eggs prior to the experiment (15/15 and 14/15 replicates, respectively), but while experienced *P. nasuta* (owners) had generally paralyzed some hosts, they had only oviposited in 1/5 replicates. Experienced intruders of *C. hyalinipennis* chased owners throughout the apparatus in 3/5 replicates; on 2/3 of these occasions the contest began with the owner chasing the intruder.

#### 3.2.1. Outcome of intruder–owner contests

In *C. stephanoderis*, owners won all contests against conspecific intruders (Table 2), constituting a significant advantage associated with ownership (rows 1 and 2 combined: binomial test:  $P = 0.002$ ); hence there was no significant effect of size or intruder experience on contest outcome (rows 1 and 2:  $G < 0.01$ ,  $df = 1$ ,  $P > 0.05$ ).

*Cephalonomia hyalinipennis* owners won all intraspecific contests against naïve intruders, but lost 3/5 contests against experienced intruders (Table 2), indicating that experience is a significant advantage to intruders (comparison of rows 3 and 4:  $G = 5.49$ ,  $df = 1$ ,  $P < 0.05$ ). On each of the three occasions that an experienced intruder defeated a conspecific owner, the intruder was the larger individual (head width). *C. hyalinipennis* owners won two contests against experienced conspecific intruders: on one occasion the owner was larger and on the other the competitors were the same size. When intruder experience was ignored, the size difference between contestants had a (marginally) significant effect on contest outcome ( $G = 3.85$ ,  $df = 1$ ,  $P \approx 0.05$ ), larger owners tending to win against smaller intruders. In *C. hyalinipennis* there was no overall advantage to ownership (rows 3 and 4 combined: binomial test:  $P = 0.344$ ), due to the loss of three contests against experienced intruders.

*Prorops nasuta* owners won one and lost one contest of five against a naïve conspecific intruder (Table 2). The

other three contests had no discernable winner, neither female excluding its competitor from the hosts after 168 h: in these cases agonistic interactions were not observed. No results on contests involving experienced *P. nasuta* intruders were collected.

### 3.2.2. Outcome of contests between naïve females

Agonistic behavior was relatively rare (*C. stephanoderis* 5/10 contests, *C. hyalinipennis* 3/10 contests, and *P. nasuta* 0/10 contests) during the 3 h observation period. Individuals tended to explore the apparatus, inspect each other with their antennae without aggression, and inspect, paralyze, and feed on hosts. There was not always a discernable winner in intraspecific contests (Table 3), but most contests were ultimately resolved (Table 3). In contests that were resolved, there was no significant advantage associated with being the larger contestant in any of the three species (Table 3: *C. stephanoderis*,  $n=7$ , binomial test:  $P=0.688$ ; *C. hyalinipennis*  $n=8$ , binomial test:  $P=0.125$ ; and *P. nasuta*  $n=7$ , binomial test:  $P=0.687$ ).

### 3.3. Comparison of inter- and intraspecific interactions

Fatal fighting was not observed in any of the 55 intraspecific contests but did occur in 89/130 (68.5%) interspecific contests: this overall difference is highly significant ( $G=94.13$ ,  $df=1$ ,  $P<0.001$ ). There were no fatalities in any of the contests, whether intra- or interspecific, won by *P. nasuta*. The significance of the overall difference is due to the high prevalence of fatalities in contests won by *C. stephanoderis* and *C. hyalinipennis* (comparisons of fatalities in intra- and interspecific contests won by *C. stephanoderis*,  $G=28.87$ ,  $df=1$ ,  $P<0.001$ , and *C. hyalinipennis*,  $G=13.96$ ,  $df=1$ ,  $P<0.001$ ).

Since survival during conspecific and allospecific contests differed within *C. stephanoderis* and *C. hyalinipennis*, we compared the number of attacks made by owners in each type of contest, but were unable to analyze these differences statistically due to small and unequal sample sizes. Using this criterion, *C. hyalinipennis* was about ten times more aggressive in intra- than interspecific contests (mean and range: intra- 15.1, 0–40,  $n=10$ ; interspecific 1.7, 0–16,  $n=20$ ). In *C. stephanoderis* the difference was much smaller (mean and range: intra- 11.9, 0–42,  $n=10$ ; interspecific 8.42, 0–26,  $n=7$ ). There was also little difference for *P. nasuta* (mean and range: intra- 0, 0,  $n=5$ ; interspecific 1.0, 0–8,  $n=30$ ).

## 4. Discussion

Extending investigations to all combinations of owner–intruder interspecific interactions between *C. stephanoderis*, *C. hyalinipennis*, and *P. nasuta* dyads has confirmed that these species actively and directly

compete for hosts, with the death of the loser a common event. Also as found by Pérez-Lachaud et al. (2002), *C. stephanoderis* is generally the most successful competitor. Examination of intraspecific interactions revealed that although agonistic contests often occur, fatalities do not. We discuss these results first in terms of contest behavior and then in terms of the consequences of such agonistic interactions for the coexistence of natural enemy species and biological control.

Direct aggressive interactions between females have now been documented in several bethylid species (Hardy and Blackburn, 1991; Pérez-Lachaud et al., 2002; Petersen and Hardy, 1996; Stokkebo and Hardy, 2000) and in a few other hymenopterans (e.g. Field and Calbert, 1998, 1999; Moore and Greeff, 2003; Tarpay and Fletcher, 2003). While behavioral defense against allospecifics occurs (Hardy and Blackburn, 1991; Mills, 1991; Pérez-Lachaud et al., 2002), most reports concern intraspecific interactions. Within species belonging to the Parasitica, female–female contests generally do not result in escalation involving attack and retaliation. For example, *Pachycrepoides vindemmiae* Rondani (Hym: Pteromalidae) females generally have very brief interactions with minimal physical contact and only occasional biting (Goubault et al., 2005) and Moore and Greeff (2003) report that contests in the fig wasp *Platyscapa awekei* Wiebes (Hym.: Agaonidae) do not result in injurious fighting. Contests are generally decided by size difference, the larger individuals prevailing by lifting their competitors off the surface using their mandibles whilst ovipositing. In contrast, in aculeate species the literature suggests that contests are generally more aggressive. Full escalation, e.g., fights involving grappling, is common between conspecific females of *Anoplius viaticus* (L.) (Hym.: Pompilidae, Field, 1992), *Goniozus nephantidis* (Musebeck) (Hym.: Bethyridae, Petersen and Hardy, 1996), *C. stephanoderis*, and *C. hyalinipennis* (this study). In *C. stephanoderis* and *C. hyalinipennis* attempts to sting opponents are common yet there were no fatalities from intraspecific fighting. In *G. nephantidis*, fatal fighting was long thought to be absent, but one instance has recently been observed (A. Hebblethwaite and I.C.W.H., unpublished data), while in contests between honey bee queens (*Apis mellifera* L., Hym.: Apidae) fatalities are common (Tarpay and Fletcher, 2003). It may be that venom injection does not generally occur when conspecifics attack each other with their stingers or that, in some species, conspecifics are immune to each other's venom.

In contrast to intraspecific interactions, fatalities are common in interspecific interactions between *C. stephanoderis*, *C. hyalinipennis*, and *P. nasuta*. Evolutionary game theory predicts that fatal fighting will occur only when resources are severely limiting (Enquist and Leimar, 1990; Grafen, 1987; Mesterton-Gibbons, 1992; Pérez-Lachaud et al., 2002), under other conditions the risk of injury

leads individuals to avoid direct contests or settle them without aggression. If, however, some individuals are able to engage in aggressive contests with little risk of injury while fighting with and killing their competitors, fatal fighting may commonly occur even when resource limitation is not severe (Pérez-Lachaud et al., 2002). The lack of fatal fighting between conspecifics suggests that resources are not an important limiting factor (e.g., due to low parasitoid population densities relative to the CBB, Damon, 1999). This is in contrast to the suggestions of Pérez-Lachaud et al. (2002), who only studied interspecific contests. It now seems likely that fatal fighting between *C. stephanoderis* and *P. nasuta* females is due to *P. nasuta* females being unable to injure or kill *C. stephanoderis*, while *C. stephanoderis* can kill *P. nasuta* relatively easily (see below). The interpretation of fatal fighting between *C. stephanoderis* and *C. hyalinipennis* is hampered by the fact that these species have been geographically separated for much of their evolutionary history.

The success of *C. stephanoderis* in interspecific contests could result from several factors, such as temperature (Infante et al., 2001b; Pérez-Lachaud et al., 2002), egg maturation state and morphology. In our experiments, *P. nasuta* often did not lay eggs on hosts. *Prorops nasuta* tends to have a longer pre-oviposition period than the two *Cephalonomia* species (Abraham et al., 1990). We observed that *P. nasuta* exhibits distinctly different paralysis and oviposition behavior from the two *Cephalonomia* species. These *Cephalonomia* have abdomens that can be flexed in several places while *P. nasuta* appears only able to bend the tip of the abdomen near the stinger and the narrowest part of the abdomen (the ‘waist’). When paralyzing a host, both *Cephalonomia* species mount it whilst bending their abdomen around to sting it (*C. stephanoderis*: Lauzière et al., 2000; *C. hyalinipennis*: T.P.B. pers. obs.). In contrast, *P. nasuta* does not mount its hosts prior to stinging: it stands near to the host and then bends its abdomen forwards beneath its thorax before advancing towards the host and attempting to insert its stinger (T.P.B. pers. obs.). Within a coffee berry, stinging may be facilitated by the surrounding bean tissue keeping the host in position. In the apparatus used in our experiments, where hosts are not fixed in position, this method often resulted in unsuccessful attempts at paralysis during which *P. nasuta* females pushed the host around the arena. These differences in morphology and behavior are likely to lead to a weaker distinction between experienced and inexperienced females in *P. nasuta* than in *Cephalonomia* in our experiments, and thus provide a candidate explanation for the lack of an influence of ‘ownership’ in *P. nasuta*. Differences in abdominal morphology are also likely to affect the ability to sting opponents during agonistic interactions, which likely accounts for the lack of fatalities inflicted by *P. nasuta* females, the low levels of aggression exhibited by this species and its overall low level of success in contests.

The occurrence of fatal fighting between allospecifics but not conspecifics implies that interspecific competition is stronger than intraspecific competition, and thus that coexistence of these species may be unlikely (Hassell, 2000, p. 120). If contest behavior were the primary determinant of the strength of competitive interactions, we would expect *C. stephanoderis* eventually to exclude *P. nasuta* and *C. hyalinipennis* from the coffee agroecosystem. Indeed, in our laboratory, *C. stephanoderis* commonly invades *P. nasuta* cultures, but not vice versa (T.P.B. and J.F.B., pers. obs.). However, a range of factors may act to diminish the importance of interspecific contest behavior, and thus promote coexistence. In Africa, the natural ranges of *C. stephanoderis* and *P. nasuta* may not greatly overlap (Murphy and Moore, 1990). Where they have been found together, *C. stephanoderis* is more prevalent (Barrera, 1991; Borbon-Martinez, 1989; Komlan, 2004; Moore and Prior, 1988; Vega et al., 1999). Even when multiple species co-occur and exploit the same resource, such as a population of CBB, species-independent aggregation of individuals over resource units (Shorrocks and Sevenster, 1995) or a metapopulation structure (Hassell, 2000; Lei and Hanski, 1998) may allow competitively inferior species to persist.

Persistence of *P. nasuta* in nature or agroecosystems may also be promoted by contest avoidance. Once inside a berry, *P. nasuta* females have been observed to block the entrance with the bodies of dead adult borers (Infante et al., 2001b). While such blockages may prevent competitors from entering berries and thus reduce the incidence of contests, they may also prevent the successful emergence of maturing parasitoids. During investigations involving dissections of coffee berries, we have on several occasions found large numbers of dead adult *P. nasuta* inside their natal berry; emergence may not have been possible due to such a blockage (T.P.B., I.C.W.H., and J.F.B., unpublished data). These companion studies, conducted in parallel and in exactly the same environmental conditions as this contest work, suggest that *P. nasuta* cultures have the highest net population growth rates of the three species.

Persistence of *C. hyalinipennis*, despite being inferior to *C. stephanoderis* in behavioral contests, is likely to be promoted by the fact that it has the ability to develop as a hyperparasitoid of pupal *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud et al., 2004), and by producing larger clutch sizes than *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud and Hardy, 2001). In New World coffee agroecosystems falling within its native range, *C. hyalinipennis* populations may also be able to persist via the exploitation of alternative hosts (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 2001).

To date, only one theoretical study has modeled the population dynamics of the CBB and its parasitoids (Gutierrez et al., 1998). This model considered

the regulation of CBB populations afforded by *C. stephanoderis*, *P. nasuta* and the Eulophid endoparasitoid *Phymastichus coffea* LaSalle, but not *C. hyalinipennis*. Only *P. coffea* was predicted to exert significant control on CBB. Due to poor numerical responses, *C. stephanoderis* and *P. nasuta* were each predicted to exert poor control when released alone. According to this model, releasing these two species together would lead to even less control due to interspecific competition (although releasing either or both in conjunction with *P. coffea* may improve the control exerted by this Eulophid alone). Competition between *C. stephanoderis* and *P. nasuta* was, however, assumed to be symmetrical: our results indicate that asymmetrical competition is more likely, *C. stephanoderis* being the stronger competitor. Incorporating such observations, and the consideration of *C. hyalinipennis*, into this modeling framework would likely affect the predicted dynamics and perhaps generate different strategic recommendations.

In summary, this study indicates that coexistence of *C. stephanoderis*, *C. hyalinipennis*, and *P. nasuta* in coffee agroecosystems may be compromised by aggressive interspecific interactions, particularly because negative interspecific interactions are stronger than intraspecific interactions. The occurrence of interference competition via contest behavior is likely to lead to the long-term establishment of at most one of these species as a biological control agent, unless the relative importance of interspecific interactions is reduced by factors such as lack of host limitation, alternative host use, contest avoidance or differences in parasitoid reproductive strategy. This conclusion contrasts with that of Infante et al. (2001b), which was based on medium-term population trends in laboratory microcosms, without observation of female–female interactions in the immediate vicinity of hosts. Although the species we studied showed the full behavioral repertoire associated with agonistic contests, and fatalities were common, the importance of interspecific interactions in the field remains unevaluated and population-dynamic models that incorporate asymmetric interspecific competition between these species have yet to be developed.

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### References

- Abraham, Y.J., Moore, D., Godwin, G., 1990. Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera, Bethyilidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera, Scolytidae). Bull. Entomol. Res. 80, 121–128.
- Baker, P.S., 1984. Some aspects of the behavior of the coffee berry borer in relation to its control in southern Mexico (Coleoptera, Scolytidae). Folia Entomol. Mex. 61, 9–24.
- Baker, P.S., 1999. The coffee berry borer in Colombia. Final Report of the DFID-Cenicafé-CABI Bioscience IPM for coffee project, 143 pp.
- Baker, P.S., Jackson, J.A.F., Murphy, S.T., 2002. Natural Enemies, Natural Allies. The Commodities Press, Cali, Colombia.
- Barrera, J.F., 1991. Report of activities undertaken during a study period in Kenya on *Prorops nasuta* W. a parasite of the coffee berry borer. Misc. Soc. Colombiana Entomol., 1–23.
- Barrera, J.F., Gómez, J., Infante, F., Castillo, A., de la Rosa, W., 1989. Biologie de *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyilidae) en laboratoire. I. Cycle biologique, capacité d'oviposition et émergence du fruit du caféier. CaféCacao Thé 33, 101–108.
- Barrera, J.F., Moore, D., Abraham, Y.J., Murphy, S.T., Prior, C., 1990a. Biological control of the coffee berry borer, *Hypothenemus hampei*, in Mexico and possibilities for further action. In: Brighton Crop Protection Conference, Pests and Diseases—1990, Vol. 1. British Crop Protection Council, Thornton Heath, UK, 391–396.
- Barrera, J.F., Baker, P.S., Valenzuela, J.E., Schwarz, A., 1990b. Introduction of two African parasitoid species to Mexico for biological control of the coffee borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). Folia Entomol. Mex. 79, 245–247.
- Borbon-Martinez, O., 1989. Bioécologie d'un ravageur des baies de caféier, *Hypothenemus hampei* Ferr. (Coleoptera: Scolytidae) et de ses parasitoïdes au Togo. PhD thesis, Université Paul-Sabatier, Toulouse, France, 184 p.
- Damon, A., 1999. Evaluation of current techniques and new approaches in the use of *Cephalonomia stephanoderis* (Hymenoptera: Bethyilidae) as a biological control agent of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae), in Chiapas Mexico. DPhil. dissertation, University of London, Wye College, 327pp.
- Damon, A., Valle, J., 2002. Comparison of two release techniques for the use of *Cephalonomia stephanoderis* (Hymenoptera: Bethyilidae), to control the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae) in Soconusco, southeastern Mexico. Biol. Control 24, 117–127.
- Dugatkin, L.A., Biederman, L., 1991. Balancing asymmetries in resource holding power and resource value in the pumpkinseed sunfish. Anim. Behav. 42, 691–692.
- Enquist, M., Leimar, O., 1990. The evolution of fatal fighting. Anim. Behav. 39, 1–9.
- Field, J., 1992. Intraspecific parasitism and nest defence in the solitary pompilid wasp *Anoplius viaticus* (Hymenoptera: Pompilidae). J. Zool. Lond. 228, 341–350.
- Field, S.A., Calbert, G., 1998. Patch defence in the parasitoid wasp *Trissolcus basalis*: when to begin fighting? Behaviour 135, 629–642.
- Field, S.A., Calbert, G., 1999. Don't count your eggs before they're parasitized: contest resolution and the tradeoffs during patch defense in a parasitoid wasp. Behav. Ecol. 10, 122–127.
- Gordh, G., Móczár, L., 1990. A catalog of the world Bethyilidae (Hymenoptera: Aculeata). Mem. Am. Entomol. Inst. 46, 1–364.
- Goubault, M., Outreman, Y., Poinot, D., Cortesero, A.M., 2005. Patch exploitation strategies of parasitic wasps under intraspecific competition. Behav. Ecol. in press.
- Grafen, A., 1987. The logic of divisively asymmetric contests—respect for ownership and the desperado effect. Anim. Behav. 35, 462–467.

- Griffiths, N.T., Godfray, H.C.J., 1988. Local mate competition, sex-ratio and clutch size in bethylid wasps. *Behav. Ecol. Sociobiol.* 22, 211–217.
- Gutierrez, A.P., Villacorta, A., Cure, J.R., Ellis, C.K., 1998. Tritrophic analysis of the coffee (*Coffea arabica*)—coffee berry borer [*Hypothenemus hampei* (Ferrari)]—parasitoid system. *Annu. Soc. Entomol. Brasil* 27, 357–385.
- Hammerstein, P., 1981. The role of asymmetries in animal contests. *Anim. Behav.* 29, 193–205.
- Hardy, I.C.W., Blackburn, T.M., 1991. Brood guarding in a bethylid wasp. *Ecol. Entomol.* 16, 55–62.
- Hassell, M.P., 2000. *The Spatial and Temporal Dynamics of Host-parasitoid Interactions*. Oxford Univ. Press, Oxford.
- Infante, F., Luis, J.H., 1993. Estadísticos demográficos de *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyidae) a temperaturas constantes. *Folia Entomol. Mex.* 87, 61–72.
- Infante, F., Mumford, J., Baker, P., Barrera, J., Fowler, S., 2001b. Interspecific competition between *Cephalonomia stephanoderis* and *Prorops nasuta* (Hym., Bethyidae), parasitoids of the coffee berry borer, *Hypothenemus hampei* (Col., Scolytidae). *J. Appl. Entomol.* 125, 63–70.
- Infante, F., Mumford, J., Mendez, I., 2001a. Non-recovery of *Prorops nasuta* (Hymenoptera: Bethyidae), an imported parasitoid of the coffee berry borer (Coleoptera: Scolytidae) in Mexico. *Southwest. Entomol.* 26, 159–163.
- Komlan, W., 2004. Contribution a la gestion agroécologique des scolytes *Hypothenemus hampei* Ferr. (Coleoptera: Scolytidae) dans les caféières du Togo. PhD thesis. Université Toulouse III- Paul Sabatier, France. 148 p.
- Lauzière, I., Brodeur, J., Pérez-Lachaud, G., 2001a. Host stage selection and suitability in *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyidae), a parasitoid of the coffee berry borer. *Biol. Control* 21, 128–133.
- Lauzière, I., Pérez-Lachaud, G., Brodeur, J., 2000. Behavior and activity pattern of *Cephalonomia stephanoderis* (Hymenoptera: Bethyidae) attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *J. Insect Behav.* 13, 375–395.
- Lauzière, I., Pérez-Lachaud, G., Brodeur, J., 2001b. Importance of nutrition and host availability on oogenesis and oviposition of *Cephalonomia stephanoderis* (Hymenoptera: Bethyidae). *Bull. Entomol. Res.* 91, 185–191.
- Lei, G.C., Hanski, I., 1998. Spatial dynamics of two competing specialist parasitoids in a host metapopulation. *J. Anim. Ecol.* 67, 422–433.
- Maynard-Smith, J., Parker, G.A., 1976. The logic of asymmetric contests. *Anim. Behav.* 24, 159–175.
- Mesterton-Gibbons, M., 1992. Ecotypic variation in the asymmetric hawk-dove game: when is bourgeois an evolutionary stable strategy?. *Evol. Ecol.* 6, 198–222.
- Mesterton-Gibbons, M., 1999. On the evolution of pure winner and loser effects: a game-theoretic model. *Bull. Math. Biol.* 61, 1151–1186.
- Mills, N.J., 1991. Searching strategies and attack rates of parasitoids of the ash bark beetle (*Leperisinus varius*) and its relevance to biological control. *Ecol. Entomol.* 16, 461–470.
- Moore, D., Prior, C., 1988. Present status of biological control of the coffee berry borer *Hypothenemus hampei*. Brighton Crop Protection Conference Vol. 3. British Crop Protection Council, Thornton Heath, Surrey, UK, 1119–1124.
- Moore, J.C., Greeff, J.M., 2003. Resource defence in female pollinating fig wasps: two's a contest, three's a crowd. *Anim. Behav.* 66, 1101–1107.
- Murphy, S.T., Moore, D., 1990. Biological control of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera, Scolytidae): previous programmes and possibilities for the future. *Biocontrol. News Inform.* 11, 107–117.
- Papaj, D.R., Messing, R.H., 1998. Asymmetries in physiological state as a possible cause of resident advantage in contests. *Behaviour* 135, 1013–1030.
- Payne, R.J.H., 1998. Gradually escalating fights and displays: the cumulative assessment model. *Anim. Behav.* 56, 651–662.
- Pérez-Lachaud, G., 1998. A new bethylid attacking the coffee berry borer (Coleoptera: Scolytidae) in Chiapas (Mexico) and some notes on its biology. *Southwest. Entomol.* 23, 287–288.
- Pérez-Lachaud, G., Batchelor, T.P., Hardy, I.C.W., 2004. Wasp eat wasp: facultative hyperparasitism and intra-guild predation by bethylid wasps. *Biol. Control* 30, 149–155.
- Pérez-Lachaud, G., Hardy, I.C.W., 1999. Reproductive biology of *Cephalonomia hyalinipennis* (Hymenoptera: Bethyidae), a native parasitoid of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae), in Chiapas, Mexico. *Biol. Control* 14, 152–158.
- Pérez-Lachaud, G., Hardy, I.C.W., 2001. Alternative hosts for bethylid parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biol. Control* 22, 265–277.
- Pérez-Lachaud, G., Hardy, I.C.W., Lachaud, J.-P., 2002. Insect gladiators: Competitive interactions between three species of bethylid wasps attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biol. Control* 25, 231–238.
- Petersen, G., Hardy, I.C.W., 1996. The Importance of being larger: parasitoid intruder-owner contests and their implications for clutch size. *Anim. Behav.* 51, 1363–1373.
- Shorrocks, B., Sevenster, J.G., 1995. Explaining local species-diversity. *Proc. R. Soc. Lond. Ser. B* 260, 305–309.
- Stokkebo, S., Hardy, I.C.W., 2000. The importance of being gravid: egg load and contest outcome in a parasitoid wasp. *Anim. Behav.* 59, 1111–1118.
- Tarpy, D.R., Fletcher, D.J.C., 2003. “Spraying” behavior during queen competition in honey bees. *J. Insect Behav.* 16, 425–437.
- Vega, F.E., Mercadier, G., Damon, A., Kirk, A., 1999. Natural enemies of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Togo and Cote d’Ivoire, and other insects associated with coffee beans. *African Entomol.* 7, 243–248.
- Wells, M.S., 1988. Effects of body size and resource value on fighting behaviour in a jumping spider. *Anim. Behav.* 36, 321–326.