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# **Keep calm, we know each other: kin recognition affects aggressiveness and conflict resolution in a solitary parasitoid**

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# Keep calm, we know each other: kin recognition affects aggressiveness and conflict resolution in a solitary parasitoid

**Abstract** - Intraspecific competition for indivisible resources can trigger the expression of agonistic behaviour in individuals of many animal species. Aggressiveness and conflict resolution may be influenced by the value individuals place in the resource (subjective resource value) but also by genetic relatedness between competitors. The ability to differentiate genetically related from unrelated individuals (i.e., kin recognition) can play a key role in the dynamics of agonistic interactions between individuals. In this context, the theory of kin selection predicts that competitors should display fewer aggressive behaviours towards closely related individuals. Recognition of kin conspecifics can be driven by the perception of (1) genetically-linked phenotypic cues (phenotypic matching) and/or (2) environmental cues (familiarity). In the hymenopteran solitary parasitoid *Eupelmus vuilleti*, individuals develop on larvae and pupae of their host, *Callosobruchus maculatus*, which infest cowpea seeds (*Vigna unguiculata*). *Eupelmus vuilleti* females can fight for the host on which they lay their eggs. Here, we investigated the effect of genetic relatedness (genotype) and familiarity (the seed containing the host on which they develop as juvenile) on aggressiveness and contests outcome over hosts in *E. vuilleti* females. We first demonstrated that the probability of a conflict escalating was affected by the interaction between genetic relatedness and familiarity among females. We then found that familiarity alone affected the likelihood of contest resolution. The occurrence of escalated conflicts was reduced between related and familiar females, and contests were more likely to be clearly resolved when occurring between familiar competitors.

Our results highlighted a parasitoid wasp's abilities to identify and discriminate kin, showing for the first time that two kin recognition components can interact in mediating competition avoidance for resource access in a solitary insect species.

**Key words:** *Eupelmus vuilleti*, familiarity, genetic relatedness, phenotype matching, subjective resource value.

## INTRODUCTION

Intraspecific competition for an indivisible resource can lead to direct interactions between individuals (i.e., conflicts, Hardy & Briffa, 2013). To prevent other individuals from accessing the resource, animals often exhibit agonistic behaviour during contests (Hack et al., 1997; Riechert, 1998). Winning a conflict can improve access to sexual partners (West-Eberhard, 1979), food resources (Vogel, 2005), territories (Festa-Bianchet et al., 1990) or dominance status (Chase, 1974), but being aggressive can potentially be costly in terms of energy expenditure (Maynard Smith, 1974), physical injuries (Palombit, 1993; Innocent et al., 2011), or increased predation risk (Jakobsson et al., 1995). Animals should therefore evaluate the costs and benefits of exhibiting agonistic behaviours and adjust their behaviour accordingly (Maynard Smith & Price, 1973). In the case of dyadic interactions, game theory predicts that contest outcome should be influenced by the asymmetries that exist between contestants in terms of their intrinsic ability to obtain and hold access to a resource ('Resource Holding Potential', RHP; Maynard Smith, 1974; Parker, 1974), and the value they place on the resource ('Subjective Resource Value', SRV; Maynard Smith & Parker, 1976; Hammerstein, 1981; Enquist & Leimar, 1987). Individuals with higher RHP (e.g., bigger contestants) typically win the resource (Maynard Smith, 1982; Elias et al., 2008; Arnott & Elwood, 2009), and individuals with higher SRV (e.g., with greater egg load or lower food intake) are expected to be more

motivated to fight and have a higher probability of winning contests (Stokkebo & Hardy, 2000; Dissanayake et al., 2009). Moreover, these factors have been shown to influence aggressiveness, the most aggressive contestants being generally more likely to win conflicts (Goubault et al., 2007; Mohamad et al., 2010; Mathiron et al., 2018).

Aggressiveness during conflict can also vary with the degree of genetic relatedness between competitors (West et al., 2002). Kin selection theory postulates that a trait that does not provide a direct fitness benefit (or to some extent, a negative effect) to the individual who expresses it, can be selected for if it improves the fitness of the individuals carrying the same genes as the protagonist (i.e., indirect fitness; Hamilton, 1964). In the context of conflicts, it would be advantageous for an individual to be less aggressive towards genetically related conspecifics (Hamilton & May, 1977; Griffin & West, 2002; Lizé et al., 2012) because this would maximize their inclusive fitness (West et al., 2001). However, avoiding conflicts between relatives implies kin recognition, i.e. the identification (neurophysiological process), and discrimination (the expression of different behaviours) of genetically related individuals. Two types of recognition exist: (i) recognition based on the perception of genetically encoded phenotypic indices (Gadagkar, 1985) and (ii) familiarity, which refers to the recognition of individuals regardless of their genetic relatedness (Lizé et al., 2012). For instance, two individuals can be considered familiar if they meet often, or if they come from the same environment. The ability to recognize and discriminate genetically related individuals has been observed in various animal taxa, such as nematodes (Kapranas et al., 2016), anuran amphibians (Blaustein & Waldman, 1992) and mammals (Heth et al., 1998). In social insects, for example, kin recognition and avoidance of competition between relatives have been demonstrated in many species, like in sweat bees *Lasioglossum zephyrum*, honey bees *Apis mellifera* (West-Eberhard, 1989), and in the socially polymorphic ant, *Pheidole pallidula* (Fournier et al., 2016). In non-social insects, on the other hand, this phenomenon seems rarer (for reviews, see

Fellowes, 1998 and Griffin & West, 2002) or even absent, like in the parasitoid wasps *Aphidius matricariae* (Bourdais & Hance, 2009) and *Cotesia glomerata* (Ruf et al., 2010). Theoretical models also predict that kin discrimination may not evolve in populations with a low dispersion of individuals due to the high risk of competition for resource between relatives (West et al., 2002).

In hymenopteran parasitoids (i.e. organisms that feed and develop at the expense of a host, resulting in its death), females may compete to feed and lay their eggs on (i.e. ectoparasitoid) or in (i.e. endoparasitoid) a host. Because offspring survival directly depends on the mother's ability to acquire and defend the host (Petersen & Hardy, 1996), agonistic interactions may occur between females that may, in some cases, be fatal, as described in some *Melittobia* species (Godfray, 1994; Hartley & Matthews, 2003; Innocent et al., 2011). In this context, four main factors acting on RHP and/or SRV appear to influence conflict resolution: (1) relative size and age of the opponents, (2) order of arrival on the resource (resident or intruder), (3) egg load, and (4) previous experience. Bigger and older females usually win contests (Petersen & Hardy, 1996; Humphries et al., 2006; Tsai et al., 2014), as do residents (Maynard Smith & Parker, 1976; Enquist & Leimar, 1987), even if they are smaller than intruders (Bentley et al., 2009). In addition, individuals with a greater egg load are generally advantaged during combat; these females value the resource more because a greater egg load would allow them to oviposit on the host sooner (Stokkebo & Hardy, 2000; Mohamad et al., 2010; but see Goubault et al., 2007). Finally, females that have experienced low quality hosts or low host densities are usually more aggressive and win more often in the presence of high quality hosts (Mohamad et al., 2010; Mathiron et al., 2018). Although the ability to treat kin differentially may influence the dynamics of contests between parasitoids competing for hosts, this has been rarely tested empirically. To our knowledge, only two studies measured the effect of kin recognition on competition avoidance and aggression in hymenopteran parasitoids: (1)

in the polyembryonic wasp (i.e. clonal multiplication of eggs within the host) *Copidosoma floridanum* (Giron et al., 2004) and (2) in the gregarious (i.e. several larvae develop on the same host) parasitoid *Goniozus legneri* (Lizé et al., 2012). In the latter study, Lizé et al. (2012) showed that adult females recognized their relatives through phenotypic cues and familiarity (development on the same host), adjusting their aggressiveness accordingly. Familiarity would thus act as a proxy for kinship. However, no study has measured the effect of genetic relatedness on aggression and conflict resolution in a solitary parasitoid species.

Here, we explored the effect of kin recognition on aggressiveness and resolution of conflict in females of the solitary ectoparasitoid wasp, *Eupelmus vuilleti* (Hymenoptera: Eupelmidae). Females parasitize and feed upon larvae and pupae of the cowpea seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae), which infest seeds of *Vigna unguiculata* (Fabaceae). *Eupelmus vuilleti* is a solitary parasitoid, which implies that only one individual develops per host (supernumerary larvae are eliminated by larval competition; Fisher, 1961). When several females are simultaneously present on a patch, they tend to protect the host by directing agonistic behaviours towards conspecific competitors (Mohamad et al., 2010; Mathiron et al., 2018). In sub-Saharan Africa, farmers traditionally stored cowpea seeds in granaries, sometimes resulting in populations of both hosts and parasitoids reaching high densities. Since seeds often contain several hosts, several individuals of *E. vuilleti* can emerge from the same seed, leading females to experience intense intraspecific competition with both kin and non-kin conspecifics.

We therefore tested whether wasps are able to adjust their aggressiveness during contests for host access depending on their level of genetic relatedness with their competitor. Because reducing the costs associated with conflicts between kin may be beneficial in terms of inclusive fitness, we expected a decrease in aggressiveness and conflict escalation between related females. Moreover, if kin recognition is effective in *E. vuilleti* females, this recognition

might be based on phenotypic matching, familiarity, or both. Females may use cues coming from genetically encoded phenotypic traits and/or from a common environment. For example, the seed containing hosts on which wasps developed may itself act as a way to identify kin. Using a full factorial design, we made genetically related or unrelated juveniles develop on hosts within the same seed (familiar) or different seeds (unfamiliar). We then investigated the effects of (1) genetic relatedness among adult females (genetically related vs. unrelated), (2) the seed containing the hosts on which females developed (same vs. different seeds, i.e. familiar vs. unfamiliar), (3) the absolute difference in SRV (number of ready-to-lay eggs) of individuals, and (4) the interaction between these variables on aggressiveness, contest escalation and resolution. As contests are predicted to be clearly settled and less aggressive when contestants show a large difference in SRV (Maynard Smith & Parker, 1976), we expected contests in which the absolute difference in egg load between contestants was large to be less aggressive and often won by the most gravid female. If females recognize kin and avoid fighting against them, this effect of SRV asymmetry should be stronger between unrelated/unfamiliar females.

## MATERIAL AND METHODS

### *Laboratory breeding*

We tested individuals of *E. vuilleti* that were collected on cowpea seeds from crops cultivated in Togo in 2007. Wasps were brought to the laboratory (Institut de Recherche sur la Biologie de l'Insecte, Université de Tours, France) and reared on juvenile stages of *C. maculatus*, as described by Jaloux et al. (2004). We performed all experimental procedures in a climate room (temperature = 30 °C and photoperiod = 12:12 h light: dark).

### *Genotypes*



To study the influence of genetic relatedness between females on competition for host access, we bred four genetically inbred strains. Working on individuals coming from more than two genotypes minimized the risk of bias due to contest behaviours specific to those genotypes. To do so, we separately introduced four mating pairs from the same breeding in Petri dishes (diameter: 8.5 cm, height: 2.7 cm) with cowpea seeds infested by *C. maculatus* and a piece of cotton soaked in water. We isolated females at emergence so that they were virgin and we placed them in the presence of a male. We left couples in contact for one to two days so that females could mate and parasitize hosts. We then removed the wasps to allow offspring development (generation 1, G1). At the emergence of G1 offspring (about 15 days after hatching; Jaloux et al., 2004), we mated one son and one daughter from each original mating pair to produce the second generation (G2). We repeated this operation until the 17<sup>th</sup> generation (G17). Theoretical models estimate that inbred lineages become fixed from G20 in diploid species (Hamilton, 2009). As *E. vuilleti* is a haplodiploid species, fixation process is likely to be more rapid; we therefore assumed that we had generated four different, highly inbred genotypes (referred as genotypes ‘A’, ‘B’, ‘C’ and ‘D’). We then individually placed G17 females in Petri dishes with a brother male and supplied them with infested cowpea seeds for 7 days, in order to obtain G18 daughters that were (1) familiar (same seed) and related (same genotype), (2) familiar (same seed) and unrelated (different genotypes), (3) unfamiliar (different seeds) and related (same genotype) and (4) unfamiliar (different seeds) and unrelated (different genotypes). To obtain familiar – unrelated females (i.e., coming from the same seed but genetically different), we cut seeds into halves, placed each half in different Petri dishes and allowed G17 mothers from different genotypes to separately parasitize hosts contained in each half of the seeds (Fig. 1). The difficulty in obtaining wasps that emerge synchronously from each half seeds explains the relatively small sample size of contests performed between these unrelated–familiar females (see the ‘experimental procedure’ section).

## *Females preparation for contests*

At the emergence of G18 individuals, we isolated wasps to prevent them from having any competition experience and introduced them separately in Petri dishes supplied with a piece of cotton soaked in water (Fig. 1). During the 3 days preceding behavioural observations, we provided females with one *C. maculatus* pupa, which was replaced daily. To do so, we dissected cowpea seeds to collect pupae. We then individually placed them in standard transparent gelatine capsule (length: 2 cm, diameter: 0.6 cm). These artificial seeds allow for better observation of laid eggs, without altering females' oviposition behaviour (Gauthier & Monge, 1999; Jaloux et al., 2004). Moreover, as *E. vuilleti* is a synovigenic species (i.e. they possess a few mature eggs at emergence and egg maturation continues throughout adult life), we added a male to the females' petri dishes for 24h to stimulate oogenesis (Terrasse & Rojas-Rousse, 1986). In addition, to facilitate individual identification during contests, we marked them on the dorsal part of their thorax with a dot of bright yellow or bright red acrylic paint. On the day of behavioural observations, we removed the hosts and deprived females of hosts for at least 2h before the start of the contest so that they were more motivated to oviposit. We then observed contests between females fighting for a 4<sup>th</sup> instar larva of *C. maculatus*.

## *Experimental procedure*

At the start of the observation, we simultaneously introduced two parasitoid females into an arena consisting of a plastic block made of three chambers linked by a narrow channel (adapted from Petersen & Hardy, 1996). We placed females in the central chamber already containing a 4<sup>th</sup> instar larva of *C. maculatus* previously placed in a capsule. Mathiron et al. (2018) showed that females with oviposition pre-experience on pupae were more motivated to gain access to a 4<sup>th</sup> instar larva. Contestants were of the same age (3 days old), marked with

different colours and visually matched for size (*a posteriori* analysis confirmed that contestants did not differ significantly in body mass; Wilcoxon signed-ranks test:  $T = 2463$ ,  $N = 92$ ,  $P = 0.14$ ). We set up four different types of contests (Fig. 1), with different combinations of genotypes: (1) females emerged from the same seed that were from the same genotype (i.e. familiar – related,  $N = 19$ ; genotype combinations: ‘AA’,  $N = 7$ ; ‘BB’,  $N = 5$ ; ‘CC’,  $N = 2$ ; ‘DD’,  $N = 5$ ), (2) females emerged from the same seed that were from different genotypes (i.e. familiar – unrelated,  $N = 9$ ; genotype combinations: ‘AD’,  $N = 1$ ; ‘BC’,  $N = 2$ ; ‘BD’,  $N = 1$ ; ‘CD’,  $N = 5$ ), (3) females emerged from different seeds that were from the same genotype (i.e. unfamiliar – related,  $N = 25$ ; genotype combinations: ‘AA’,  $N = 9$ ; ‘BB’,  $N = 5$ ; ‘CC’,  $N = 7$ ; ‘DD’,  $N = 4$ ) and (4) females emerged from different seeds that were from different genotypes (i.e. unrelated – unfamiliar,  $N = 39$ ; genotype combinations: ‘AB’,  $N = 6$ ; ‘AC’,  $N = 5$ ; ‘AD’,  $N = 8$ ; ‘BC’,  $N = 6$ ; ‘BD’,  $N = 7$ ; ‘CD’,  $N = 7$ ). Wasps from the same genotype were full sisters.

During all tests, we recorded oviposition and aggressive behaviours displayed by each female. When wasps detect a conspecific (identified by females raising their antennae in the direction of their opponent), they frequently stop their behavioural oviposition sequence (Mohamad et al., 2010). Females can either display defensive behaviour, kicking the opponent with their legs without taking their ovipositor out of the capsule, or they can exhibit a full attack, in which case one parasitoid usually hits her opponent with her head, chases her away from the capsule, or even mounts her. This hitting, chasing, or mounting can lead the loser to quit the central chamber of the arena. We therefore considered only these behaviours, collectively termed as ‘attack’, in the rest of the study. We conducted behavioural observations during 1h or stopped the contests when one female left the central chamber for at least 2 min. We also stopped contests when neither of the females touched the host nor displayed any aggressive interactions for at least 2min. We considered a contest as resolved when a female chased her

opponent out of the central chamber, or when only one female displayed oviposition behaviour (i.e. the winning female) during the experiment time.

At the end of experiments, we immediately froze the wasps at -20°C. We then weighed them using an electronic balance (Ohaus Discovery® model, accuracy: 0.01mg) and we counted the number of mature eggs in their abdomen (i.e. egg load) by dissecting them. Finally, we noted for each contest the number of eggs laid on the host by the winning females to determine their initial egg load (i.e. at the start of the contest).

### *Statistical analysis*

We analysed our data with the software R (R Development Core Team, 2016; version 3.5.0), using  $\alpha = 0.05$ . Instead of transforming data to fit standard assumptions, we chose to perform parametric analyses in which the assumed distribution of residuals was matched to the data (Wilson & Hardy, 2002; Briffa et al., 2013).

We first defined contest aggressiveness as a binary response: 0 = no aggressive behaviour occurred during a replicate and 1 = aggressive behaviours occurred ( $N = 92$  contests). We then performed a generalized linear model (GLM) with a binomial error distribution (link function = 'logit') to investigate the influence of absolute difference in initial egg load between contestants, genetic relatedness and familiarity among females on the probability that aggressive behaviours would be exhibited during contest. The model included the three main effects and two-way interactions between variables. We used the same procedure to explore the influence of absolute difference in initial egg load between contestants, genetic relatedness and familiarity among females on the probability of contest resolution. We thus defined contest resolution as a binary response: 0 = the contest was unresolved and 1 = there was a clear winner ( $N = 92$  contests).

We finally examine the influence of absolute difference in initial egg load between contestants, genetic relatedness and familiarity among females on the total number of attacks

displayed by both females during contest. To account for overdispersion, we ran a GLM assuming a quasi-Poisson distribution of residuals (link function = 'log'), considering only aggressive contests (i.e. contests where at least one attack was observed;  $N = 76$ ). The model included main effects and two-way interactions between variables. We also included in the model the logarithm of contest duration as an offset term to account for differences in contest duration.

For all analyses, we started with the maximal model and removed non-significant interactions to test the significance of main effects. Likelihood-ratio chi-square and F-statistics were calculated using the 'Anova' function (package *car*), which performs type-II analysis of variance for GLM models, i.e. without *a priori* of the sequence order of explanatory variables (Fox & Weisberg, 2019). Where we found significant interactions between factors, we ran Dunn's post-hoc multiple comparison tests to establish which modalities significantly differed from each other (Dunn, 1961).

### *Ethical Note*

We used 372 individuals in this study: 186 females for contests, and 186 males for female insemination. No animal ethics approval was required for this study. Nevertheless, we handled all individuals with care and we kept handling time to an absolute minimum. Acrylic paint used for marking does not cause injury to wasps. Subjects submitted to dissection were chilled in a freezer prior to decapitation.

## **RESULTS**

### *Effect of genotype combination*

We first verified that the genotype combination of the contestants did not affect the probability of contests being aggressive (GLM with binomial distribution of residuals:  $G_9 = 12.72$ ,  $P = 0.18$ ), the probability of contest being resolved (GLM with binomial distribution of residuals:  $G_9 = 9.91$ ,  $P = 0.36$ ), or the number of attacks during contest (GLM with quasi-Poisson distribution of residuals:  $F_{9,66} = 1.90$ ,  $P = 0.07$ ). However, this could reflect a lack of statistical power due to small sample sizes.

### *Escalation and resolution of conflicts*

The probability of contests being aggressive was significantly influenced by the interaction between genetic relatedness and familiarity of females (GLM with binomial distribution of residuals: estimates  $\pm$  S.E. =  $-17.29 \pm 1312.5$ ,  $G_1 = 5.54$ ,  $P = 0.02$ , Fig. 2a). Contest escalation was indeed less likely when contestants were familiar and related than when they were familiar and unrelated (post-hoc Dunn's multiple comparison test:  $P = 0.02$ , Fig. 2a), but no difference was observed between unfamiliar-related and unfamiliar-unrelated females (post-hoc Dunn's multiple comparison test:  $P = 0.27$ ; Fig. 2a). Moreover, no difference was observed between familiar-related and unfamiliar-related females (post-hoc Dunn's multiple comparison test:  $P = 0.05$ ; Fig. 2a), and between unfamiliar-related and unfamiliar-unrelated females (post-hoc Dunn's multiple comparison test:  $P = 0.10$ ; Fig. 2a). The probability of contest being aggressive did not significantly vary with the absolute difference in female egg load (GLM with binomial distribution of residuals: estimates  $\pm$  S.E. =  $-0.115 \pm 0.184$ ,  $G_1 = 0.38$ ,  $P = 0.54$ ). All other interactions were also non-significant.

The probability of a contest being clearly resolved varied significantly with familiarity (GLM with binomial distribution of residuals: estimates  $\pm$  S.E. =  $2.216 \pm 0.802$ ,  $G_1 = 11.32$ ,  $P < 0.001$ ). Contests between familiar females had a greater chance of showing a clear winner than contests between unfamiliar females (Fig. 2b). However, we found no effect of genetic

relatedness (GLM with binomial distribution of residuals: estimates  $\pm$  S.E. =  $-0.332 \pm 0.507$ ,  $G_1 = 0.430$ ,  $P = 0.51$ ), the absolute difference in female egg load (GLM with binomial distribution of residuals: estimates  $\pm$  S.E. =  $-0.201 \pm 0.158$ ,  $G_1 = 1.634$ ,  $P = 0.2$ ), or any 2-way interactions between variables.

### *Number of attacks during conflicts*

The combined total number of attacks displayed by the two wasps during a contest was influenced by the absolute difference in female egg load, with contests being more aggressive when this difference was larger (GLM with quasi-Poisson distribution of residuals: estimates  $\pm$  S.E. =  $0.229 \pm 0.041$ ,  $F_{1,72} = 28.01$ ,  $P < 0.001$ ; Fig. 3). This total number of attacks did not vary with female genetic relatedness (GLM with quasi-Poisson distribution of residuals: estimates  $\pm$  S.E. =  $-0.059 \pm 0.148$ ,  $F_{1,72} = 0.1593$ ,  $P = 0.69$ ), familiarity (GLM with quasi-Poisson distribution of residuals: estimates  $\pm$  S.E. =  $-0.323 \pm 0.170$ ,  $F_{1,72} = 3.795$ ,  $P = 0.06$ ) or any 2-way interactions between these variables.

## **DISCUSSION**

The main goals of our study were: 1) to investigate whether *E. vuilleti* females adjust their aggressive behaviour during contests for host access according to the genetic relatedness with their competitor, and if so, 2) to explore on which components (phenotypic matching and/or familiarity) kin recognition is based. We observed that both genetic relatedness and familiarity influence the probability of conflict being aggressive: contest escalation was the least likely when contestants were both familiar and related. Moreover, familiarity affected conflict resolution, contests being more likely to be resolved when females were familiar. We thus highlighted kin discrimination in *E. vuilleti*, a solitary parasitoid with low dispersion ability

(Cortesero et al., 1997). This evidence is all the more important that, in species with limited dispersion, the high risk of competition between kin may weaken the evolution of kin discrimination (West et al., 2001, 2002) and behaviours favouring relatives may not be selected for, as observed in fig wasps (West et al., 2001).

Our results first indicate that *E. vuilleti* individuals were able to recognize both genetically related females and familiar females, and then decide whether to engage in an aggressive contest or not. Recognition of relatives in *E. vuilleti* agrees with what was found in the polyembryonic wasp genus *Copidosoma*, in which attack rates by the larval soldier caste was inversely correlated with competitor relatedness (Giron et al., 2004). Moreover, in the gregarious parasitoid wasp, *G. legneri*, contests between adult females over hosts were less aggressive when competitors were more closely related and when females had developed on the same host (i.e., familiar, Lizé et al., 2012). However, several other studies of parasitoid hymenopterans have reported that kin recognition did not affect fights between males (West et al., 2001; Innocent et al., 2011), mate choice (Bourdais & Hance, 2009; Ruf et al., 2010), or sex allocation behaviour (Reece et al., 2004). Recognition of genetically encoded phenotypic cues can operate through two mechanisms: (1) allelic recognition (greenbeard effect), i.e., recognition of an allele that underlies the expression of a phenotypic trait (Gardner & West, 2010) and (2) phenotypic matching, which is based on overall phenotypic similarity between related individuals (Gadagkar, 1985; Mateo, 2004). The first mechanism leads to the recognition of similarity at the locus responsible for expression of the phenotypic trait (Gardner & West, 2010), while the second mechanism leads to relatedness recognition at the genome scale if there is a strong correlation between both genetic and phenotypic similarity (Mateo, 2004; Gardner & West, 2010). Because allelic recognition may involve cooperation between unrelated individuals expressing the trait encoded by a given allele, fixation in a population is expected to be rare owing to invasion by cheaters (Gardner & West, 2010). As such, phenotypic



matching is more plausible in the current case. Phenotype matching, in which the genotype of individuals is expressed through their phenotype, contrasts to familiarity in which phenotypic cues may derive from genotypes and/or environment (Lizé et al., 2012). Even if phenotypic matching is generally referred to as a different mechanism from familiarity, phenotypic matching may thus be seen as an extension of familiarity (Mateo, 2004). Here, because the probability of a conflict being aggressive was influenced by the interaction between genetic relatedness and familiarity among females, this suggests that phenotypic matching mechanism may be an extension of familiarity in *E. vuilleti* females. In accordance with the study of Lizé et al. (2012) in *G. legneri*, our experiments highlighted that these two components of kin recognition affect contests for hosts in a parasitoid wasp. More strikingly, we showed for the first time that both components can interact to affect intraspecific competition for resources in a solitary insect species.

While we also expected the total number of aggressive behaviours displayed during contests to be influenced by both kin recognition components, only the difference in competitors' egg load had a significant effect: fights were less aggressive when the opponents had a similar number of mature eggs. This unexpected result seems in contradiction with theory models predicting that symmetry between individuals leads to more escalated contests of longer duration (Parker, 1974; Maynard Smith & Parker, 1976). Here, one explanation is that the total number of attacks during a contest may have been driven by the behaviour of the contestant with the highest egg load. As egg load is known to influence competitors' subjective value of the resource (Enquist & Leimar, 1987; Stokkebo & Hardy, 2000), the more gravid females (i.e. with more mature eggs in their ovaries) are expected to be more motivated to fight for winning access to the host. Opponent females can match their aggressiveness, resulting in an increase in the total number of aggressive behaviours during conflicts. Additional analyses seem to support this hypothesis: the total number of attacks displayed by the two wasps during contests

significantly increased with the egg load of the most gravid contestant (GLM with quasi-Poisson distribution of residuals: estimates  $\pm$  S.E. =  $0.11 \pm 0.04$ ,  $F_{1,74} = 6.20$ ,  $P < 0.02$ ) and the number of attacks displayed by both females are positively correlated (GLM with quasi-Poisson distribution of residuals: estimates  $\pm$  S.E. =  $0.04 \pm 0.01$ ,  $F_{1,74} = 36.27$ ,  $P < 0.001$ ). Furthermore, our results add to the evidence that egg load is the major factor driving variation in aggressive behaviours during contests for hosts in *E. vuilleti* (Mohamad et al., 2010, 2012). The underlying mechanism is still unknown, but the involvement of hormones has been suggested (Stokkebo & Hardy, 2000; Goubault & Decuignière, 2012). In particular, juvenile hormone is known to be involved in egg maturation in many insect species (reviewed by Nijhout, 1994) and associated with aggressiveness in different hymenopteran species such as paper wasps (Tibbetts & Huang, 2010) and honeybees (Pearce et al., 2001). Ecdysteroids also play a major role in the regulation of oogenesis in *E. vuilleti* females (Bodin et al., 2009), and may affect female aggressive behaviours. Nevertheless, additional work is required to investigate to what extent hormones may affect female aggressiveness during contest over host.

In contrast with Lizé et al. (2012) who found no effect of familiarity or genetic relatedness on the probability of contests being resolved in *G. legneri*, we showed that conflicts between familiar females were more likely to result in a clear winner and loser. In parasitoid hymenopterans, offspring survival, hence females' direct fitness, depends directly on their ability to acquire, parasitize and defend hosts (Petersen & Hardy, 1996). However, exploiting a host, and in particular drilling through the seed to access it, has been shown to be highly energetically costly in this species (Boisseau et al., 2017). Quitting a host, to the benefit of another female, is thus a waste of potential fitness return, but may save energy. Nevertheless, if both contestants are relatives, the increase in inclusive fitness may counterbalance the cost of surrendering the host to a conspecific female, as predicted by kin selection theory (Hamilton, 1964). Behavioural mechanisms that allow higher probabilities of contest resolution between

familiar females in *E. vuilleti* would therefore be adaptive, as long as emerging from hosts that developed in the same seed is a good predictor of genetic relatedness in this species. In *E. vuilleti*, mothers often monopolize a cowpea seed infested by several juvenile *C. maculatus* (Mohamad et al., 2012), and wasps that emerge from the same seed are therefore highly likely to be sisters.

Altogether, we observed that *E. vuilleti* females variably rely on genetic relatedness and familiarity cues depending on the contest stage: they first rely on both types of cues when deciding whether to escalate the conflict or not. Then, during escalated contests, aggressiveness varied only with SRV asymmetry between contestants. Finally, contest resolution was based on familiarity cues only. If this may be surprising at first glance, the type of information used by animals for decision-making is classically known to change over contests (see Arnott & Elwood, 2009 for a review of ‘assessment’ models). For example, Elias et al. (2008) showed that males of the jumping spider, *Phidippus clarus*, use multimodal signals during aggressive interactions: the duration of pre-contact phases was based on differences in vibration behaviour between males, while motivation to escalate towards contact phases was mainly based on body size. Moreover, in the mangrove rivulus fish, *Kryptolebias marmoratus*, males use different assessment strategies during conflicts. Fish usually adopt ‘mutual assessment’ to decide whether to escalate the contest, and then change to a ‘self-assessment’ to whether persist the escalation (Hsu et al., 2008). Overall, our results demonstrated that wasps are able to use different cues at each phase of the conflict to make their behavioural decisions.

The mechanisms underlying the identification of genetic relatedness and familiarity remain to be explored in *E. vuilleti*. However, *E. vuilleti* females are known to adjust their behaviour based on the detection of conspecific cuticular hydrocarbons (CHC; Darrouzet et al., 2010). Because the wasps, in our study, discriminated competitors of the same genotype and those from the same seed, they may have detected genetically encoded or environmentally

influenced differences in the CHC profile of their opponent. Studies in parasitoid hymenoptera suggest that CHC may be characteristic of related individuals (Höller et al., 1991; Howard, 2001). Several studies have also measured biochemical convergence between the surface lipids of host plants and cuticular compounds of insect herbivores and their predators or parasitoids (reviewed in Blomquist & Bagnères, 2010). Kühbandner et al. (2012) found, in the pteromalid wasp *Lariophagus distinguendus*, that shifting host during only one generation led to distinguishable CHC profiles among females. Moreover, since familiar *E. vuilleti* females came from the same seed on which the hosts fed, it is possible that females developing on hosts that exploit the same resource might express a similar biochemical profile that allows for recognition of familiar individuals (Ode et al., 1995; Singer, 1998). For example, von Beeren et al. (2011) showed that ant parasites *Malayatelura ponerophila* acquire cuticular hydrocarbons directly from their host *Leptogenys distinguenda*. However, *E. vuilleti* females may have simply acquired seed-specific compounds by friction when emerging out of the seed. Physical contacts can also generate variation in the chemical cuticular hydrocarbon profile: when stroking their abdomens on the nest surface of their host, both parasite species *Sulcopolistes sulcifer* and *Polistes semenowi* acquire host hydrocarbons from the comb (Turillazzi et al., 1990; Zacchi, 1995). Moreover, in social wasps, a hydrocarbon layer similar to that of resident wasps covers nest paper, so that physical contact between parasite cuticle and nest hydrocarbons may favour the transfer of odours between parasites and comb (Lorenzi et al., 1996; Singer, 1998). We call future studies to test these hypotheses and identify the cues involved in the different components of kin recognition.

## Conclusion

By investigating the effects of both genetic relatedness and common developmental environment on contest behaviour and resolution in *E. vuilleti*, we were able to demonstrate

that two kin recognition components (phenotypic matching and familiarity) mediate the occurrence of escalated conflicts and contest resolution in a parasitoid wasp. We showed for the first time that both components can interact to affect intraspecific competition in a solitary insect species. Additionally, in species with limited dispersion like *E. vuilleti* (Cortesero et al., 1997), high competition risk for resources between kin may weaken the evolution of reducing aggressive behaviours toward related conspecifics (West et al., 2001, 2002). Our study therefore shows that kin discrimination during intraspecific competition can also evolve in a species with limited dispersion.

## References

- Abrams, P. (1981). Competition in an Indo-Pacific hermit crab community. *Oecologia*, 51, 240–249.  
<https://doi.org/10.1007/BF00540608>
- Arnott, G., & Elwood, R. W. (2008). Information gathering and decision making about resource value in animal contests. *Animal Behaviour*, 76(3), 529–542.  
<http://doi.org/10.1016/j.anbehav.2008.04.019>
- Barton, R. A. (1993). Sociospatial mechanisms of feeding competition in female olive baboons, *Papio anubis*. *Animal Behaviour*, 46(4), 791–802.
- Bentley, T., Hull, T. T., Hardy I. C. W., & Goubault, M. (2009). The elusive paradox: owner-intruder roles, strategies, and outcomes in parasitoid contests. *Behavioral Ecology*, 20(2), 296–304.
- Blaustein, A. R., & Waldman, B. (1992). Kin recognition in anuran amphibians. *Animal Behaviour*, 44, 207–221. [https://doi.org/10.1016/0003-3472\(92\)90027-7](https://doi.org/10.1016/0003-3472(92)90027-7)
- Blomquist, G. J., & Bagnères, A. G. (2010). Insect hydrocarbons: biology, biochemistry, and chemical ecology. Cambridge University Press. <https://doi.org/10.1017/CBO9780511711909>
- Bodin, A., Jaloux, B., Delbecq, J.-P., Vannier, F., Monge, J.-P., & Mondy, N. (2009). Reproduction in a variable environment: How does *Eupelmus vuilleti*, a parasitoid wasp, adjust oogenesis to

host availability? *Journal of Insect Physiology*, 55(7), 643–648.

<http://doi.org/10.1016/j.jinsphys.2009.04.003>

Boisseau, R. P., Woods, H. A., & Goubault, M. (2017). The metabolic costs of fighting and host exploitation in a seed-drilling parasitic wasp. *Journal of Experimental Biology*, 220, 3955–3966. <https://doi.org/10.1242/jeb.160887>

Bourdais, D., & Hance, T. (2009). Lack of behavioural evidence for kin avoidance in mate choice in a hymenopteran parasitoid (Hymenoptera: Braconidae). *Behavioural Processes*, 81(1), 92–94. <https://doi.org/10.1016/j.beproc.2009.02.015>

Branch, G. M. (1975). Mechanisms Reducing Intraspecific Competition in *Patella* Spp.: Migration, Differentiation and Territorial Behaviour. *Journal of Animal Ecology*, 44(2), 575–600.

Brick, O., & Jakobsson, S. (2002). Individual variation in risk taking: the effect of a predatory threat on fighting behavior in *Nannacara anomala*. *Behavioral Ecology*, 13(4), 439–442. <https://doi.org/10.1093/beheco/13.4.439>

Briffa, M., Hardy, I. C. W., Gammell, M. P., Jennings, D. J., Clarke, D. D., & Goubault, M. (2013). Analysis of animal contest data. In I. C. W. Hardy & M. Briffa (Eds.), *Animal Contests* (pp. 47–85). Cambridge University Press.

Chase, I. D. (1974). Models of hierarchy formation in animals. *Behavioural Science*, 19, 374–382.

Cortesero, A. M., Monge, J. P., & Huignard, J. (1997). Dispersal and parasitizing abilities of *Eupelmus vuilleti* (Hymenoptera: Eupelmidae) within a column of cowpea seeds. *Environmental Entomology*, 26(5), 1025–1030.

Darrouzet, E., Lebreton, S., Gouix, N., Wipf, A., & Bagnères, A. G. (2010). Parasitoids modify their oviposition behaviour according to the sexual origin of conspecific cuticular hydrocarbon traces. *Journal of Chemical Ecology*, 36(10), 1092–1100. <https://doi.org/10.1007/s10886-010-9845-8>

Davies, N. B. (1978). Territorial defense in the Speckled Wood Butterfly (*Pararge aegeria*): the resident always wins. *Animal Behaviour*, 26(1), 138–147. [https://doi.org/10.1016/0003-3472\(78\)90013-1](https://doi.org/10.1016/0003-3472(78)90013-1)

493 Díaz-Uriarte, R. (1999). Anti-predator behaviour changes following an aggressive encounter in the  
 494 lizard *Tropidurus hispidus*. *Proceedings of the Royal Society B: Biological Sciences*, 266, 2457–  
 495 2464. <https://doi.org/10.1098/rspb.1999.0946>  
 496 Dissanayake, A., Galloway, T. S., & Jones, M. B. (2009). Physiological condition and intraspecific  
 497 agonistic behaviour in *Carcinus maenas* (Crustacea: Decapoda). *Journal of Experimental Marine*  
 498 *Biology and Ecology*, 375(1–2), 57–63. <https://doi.org/10.1016/j.jembe.2009.05.007>  
 499 Dunn, O. J. (1961). Multiple comparisons among means. *Journal of the American Statistical*  
 500 *Association*, 56(293), 52–64.  
 501 Elias, D. O., Kasumovic, M. M., Punzalan, D., Andrade, M. C. B., & Mason, A. C. (2008).  
 502 Assessment during aggressive contests between male jumping spiders. *Animal Behaviour*, 76(3),  
 503 901–910. <http://doi.org/10.1016/j.anbehav.2008.01.032>  
 504 Enquist, M., & Leimar, O. (1987). Evolution of fighting behaviour: The effect of variation in resource  
 505 value. *Journal of Theoretical Biology*, 127(2), 187–205. [https://doi.org/10.1016/S0022-](https://doi.org/10.1016/S0022-5193(87)80130-3)  
 506 5193(87)80130-3  
 507 Fellowes, M. D. E. (1998). Do non-social insects get the (kin) recognition they deserve? *Ecological*  
 508 *Entomology*, 23(2), 223–227. <https://doi.org/10.1046/j.1365-2311.1998.00128.x>  
 509 Festa-Bianchet, M., Apollonio, M., Mari, F., Rasola, G., 1990. Aggression among lekking male fallow  
 510 deer (*Dama dama*): territory effects and relationship with copulatory success. *Ethology*, 85, 236–  
 511 246.  
 512 Fisher, R. C. (1961). A study in insect multiparasitism. I. Host selection and oviposition. *Journal of*  
 513 *Experimental Biology*, 38(3), 267–275.  
 514 Fletcher, D. J. C., and Michener, C. D. (1987). Kin recognition in Humans. New York.  
 515 Fournier, D., De Biseau, J. C., De Laet, S., Lenoir, A., Passera, L., & Aron, S. (2016). Social structure  
 516 and genetic distance mediate nestmate recognition and aggressiveness in the facultative  
 517 polygynous ant *Pheidole pallidula*. *PLoS ONE*, 11(5).  
 518 <https://doi.org/10.1371/journal.pone.0156440>  
 519 Fox, J., & Weisberg, S. (2019). An R companion to applied regression, third edition, Sage.

520 Gadagkar, R. (1985). Kin recognition in social insects and other animals-A review of recent findings  
 521 and a consideration of their relevance for the theory of kin selection. *Proceedings of the Indian*  
 522 *Academy of Science: Animal Science*, 94(6), 587–621. <https://doi.org/10.1007/bf03191863>

523 Gardner, A., & West, S. A. (2010). Greenbeards. *Evolution*, 64(1), 25–38.  
 524 <https://doi.org/10.1111/j.1558-5646.2009.00842.x>

525 Gauthier, N., & Monge, J. P. (1999). Could the egg itself be the source of the oviposition deterrent  
 526 marker in the ectoparasitoid *Dinarmus basalis*? *Journal of Insect Physiology*, 45(4), 393–400.  
 527 [https://doi.org/10.1016/S0022-1910\(98\)00138-3](https://doi.org/10.1016/S0022-1910(98)00138-3)

528 Giron, D., Dunn, D. D., Hardy, I. C. W., & Strand, M. R. (2004). Aggression by polyembryonic wasp  
 529 soldiers correlates with kinship but not resource competition. *Nature*, 430, 676–679.  
 530 <https://doi.org/10.1038/nature02631.1>

531 Goubault, M., & Decuignière, M. (2012). Previous experience and contest outcome: winner effects  
 532 persist in absence of evident loser effects in a parasitoid wasp. *The American Naturalist*, 180(3),  
 533 364–371. <https://doi.org/10.1086/667192>

534 Griffin, A., & West, S. (2002). Kin selection: fact and fiction. *Trends in Ecology & Evolution*, 17(1),  
 535 15–21.

536 Gustafsson, L. (1986). Inter- and intraspecific competition for nest holes in a population of the Collard  
 537 flycatcher *Ficedula albicollis*. *Ibis*, 130, 11–16.

538 Hack, M. A. (1997). Assessment strategies in the contests of male crickets, *Acheta domesticus* (L.).  
 539 *Animal Behaviour*, 53, 733–747. <https://doi.org/10.1006/anbe.1996.0310>

540 Hamilton, M. B. (2009). Population Genetics. *Wiley-Blackwell*.  
 541 <https://www.ncbi.nlm.nih.gov/pubmed/21290736>

542 Hamilton, W. D., & May, R. M. (1977). Dispersal in stable habitats. *Nature*, 269, 578–581.  
 543 <https://doi.org/10.1038/269578a0>

544 Hammerstein, P. (1981). The role of asymmetries in animal contests. *Animal Behaviour*, 29(1), 193–  
 545 205. [https://doi.org/10.1016/S0003-3472\(81\)80166-2](https://doi.org/10.1016/S0003-3472(81)80166-2)



- Hardy, I. C. W., Griffiths, N. T., & Godfray, H. C. J. (1992). Clutch size in a parasitoid wasp: a manipulation experiment. *Journal of Animal Ecology*, 61, 121–129.  
<https://doi.org/10.1098/ROYAL/>
- Hardy, I. C. W., & Briffa, M. (2013). *Animal contests*. Cambridge University Press.
- Hartley, C. S., & Matthews, R. W. (2003). The effect of body size on male- male combat in the parasitoid wasp *Melittobia digitata* Dahms (Hymenoptera : Eulophidae). *Journal of Hymenoptera Research*, 12, 272–277. Retrieved from <http://biostor.org/reference/311>
- Heth, G., Todrank, J., & Johnston, R. E. (1998). Kin recognition in golden hamsters: Evidence for phenotype matching. *Animal Behaviour*, 56(2), 409–417. <https://doi.org/10.1006/anbe.1998.0747>
- Höller, C., Williams, H. J., & Vinson, S. B. (1991). Evidence for a two-component external marking pheromone system in an aphid hyperparasitoid. *Journal of Chemical Ecology*, 17(6), 1021–1035.  
<https://doi.org/10.1007/BF01402931>
- Howard, R. W. (2001). Cuticular hydrocarbons of adult *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) and two larval hosts, angoumois grain moth (Lepidoptera: Gelechiidae) and cowpea weevil (Coleoptera: Bruchidae). *Annals of the Entomological Society of America*, 94(1), 152–158. [https://doi.org/10.1603/0013-8746\(2001\)094\[0152:CHOAPC\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2001)094[0152:CHOAPC]2.0.CO;2)
- Hrdy, S. B. (1974). Male-male competition and infanticide among the langurs (*Presbytis entellus*) of Abu, Rajasthan. *Folia Primatologica*, 22(1), 19–58. <https://doi.org/10.1159/000155616>
- Hsu, Y., Lee, S. P., Chen, M. H., Yang, S. Y., & Cheng, K. C. (2008). Switching assessment strategy during a contest: fighting in killifish *Kryptolebias marmoratus*. *Animal Behaviour*, 75(5), 1641–1649. <http://doi.org/10.1016/j.anbehav.2007.10.017>
- Innocent, T. M., West, S. A., Sanderson, J. L., Hyrkkänen, N., & Reece, S. E. (2011). Lethal combat over limited resources: Testing the importance of competitors and kin. *Behavioral Ecology*, 22(5), 923–931. <https://doi.org/10.1093/beheco/arq209>
- Jakobsson, S., Brick, O., & Kullberg, C. (1995). Escalated fighting behaviour incurs increased predation risk. *Animal Behaviour*, 49(1), 235–239. [https://doi.org/10.1016/0003-3472\(95\)80172-3](https://doi.org/10.1016/0003-3472(95)80172-3)

573 Jaloux, B., Sanon, A., Huignard, J., & Monge, J. P. (2004). Interspecific relationships between the  
574 solitary ectoparasitoid, *Eupelmus vuillei* (Crw.) (Eupelmidae), and its sympatric species,  
575 *Dinarmus basalis* (Rond.) (Pteromalidae), in the presence of their host, *Callosobruchus*  
576 *maculatus* pic (Coleoptera: Bruchidae). *Journal of Insect Behaviour*, 17(6), 793–808.  
577 <https://doi.org/10.1023/B:JOIR.0000048989.36181.4d>

578 Kapranas, A., Maher, A. M. D., & Griffin, C. T. (2016). Higher relatedness mitigates mortality in a  
579 nematode with lethal male fighting. *Journal of Evolutionary Biology*, 29(2), 344–351.  
580 <https://doi.org/10.1111/jeb.12786>Kühbandner, S., Hacker, K., Niedermayer, S., Steidle, J. L. M.,  
581 & Ruther, J. (2012). Composition of cuticular lipids in the pteromalid wasp *Lariophagus*  
582 *distinguendus* is host dependent. *Bulletin of Entomological Research*, 102(5), 610–617.  
583 <http://doi.org/10.1017/S000748531200017X>Le Boeuf, B. J. (1974). Male-male competition and  
584 reproductive success in elephant seals. *American Zoologist*, 176(July), 163–176.

585 Lizé, A., Khidr, S. K., & Hardy, I. C. W. (2012). Two components of kin recognition influence  
586 parasitoid aggression in resource competition. *Animal Behaviour*, 83(3), 793–799.  
587 <https://doi.org/10.1016/j.anbehav.2012.01.001>

588 Lorenzi, M.C., L., Bagnères, A-G., & Clément, J.L. (1996). The role of cuticular hydrocarbons in  
589 social insects: is it the same in paper wasps? In S. Turillazzi & M. West-Eberhard (Eds.), *Natural*  
590 *History and Evolution of Paper Wasps* (pp. 178–189). Oxford, UK: Oxford Univ. Press.

591 Mateo, J. M. (2004). Recognition systems and biological organization: The perception component of  
592 social recognition. *Annales Zoologici Fennici*, 41(6), 843–858.

593 Mathiron, A. G. E., Pottier, P., & Goubault, M. (2018). Let the most motivated win : resource value  
594 components affect contest outcome in a parasitoid wasp. *Behavioral Ecology*, 29(5), 1088–1095.  
595 <https://doi.org/10.1093/beheco/ary084>

596 Maynard Smith, J., & Parker, G. A. (1976). The logic of asymmetric animal contests. *Animal*  
597 *Behaviour*, 24(1), 159–175.

598 Maynard Smith, J. (1974). The theory of games and the evolution of animal conflicts. *Journal of*  
599 *Theoretical Biology*, 47(1), 209–221. [https://doi.org/10.1016/0022-5193\(74\)90110-6](https://doi.org/10.1016/0022-5193(74)90110-6)

600 Maynard Smith, J., & Price, G. R. (1973). The logic of animal conflict. *Nature*, 246(5427), 15–18.  
601 <https://doi.org/10.1038/246015a0>

602 Maynard Smith, J. (1988). Evolution and the Theory of Games. In *Did Darwin Get It Right?* (pp. 202–  
603 215). Boston, MA: Springer US. [https://doi.org/10.1007/978-1-4684-7862-4\\_22](https://doi.org/10.1007/978-1-4684-7862-4_22)

604 Mohamad, R., Monge, J. P., & Goubault, M. (2010). Can subjective resource value affect  
605 aggressiveness and contest outcome in parasitoid wasps? *Animal Behaviour*, 80(4), 629–636.  
606 <https://doi.org/10.1016/j.anbehav.2010.06.022>

607 Mohamad, R., Monge, J. P., & Goubault, M. (2012). Wait or fight? Ownership asymmetry affects  
608 contest behaviours in a parasitoid wasp. *Behavioral Ecology*, 23(6), 1330–1337.  
609 <https://doi.org/10.1093/beheco/ars125>

610 Nijhout, H. F. (1994). *Insect Hormones*. Princeton: Princeton University Press.

611 Ode, P. J., Antolin, M. F., & Strand, M. R. (1995). Brood-mate avoidance in the parasitic wasp *Bracon*  
612 *hebetor* Say. *Animal Behaviour*, 49(5), 1239–1248. <https://doi.org/10.1006/anbe.1995.0156>

613 O'Neill, D. J., & Cobb, J. S. (1979). Some factors influencing the outcome of shelter competition in  
614 lobsters (*Homarus americanus*). *Marine Behaviour and Physiology*, 6, 33–45.  
615 <https://doi.org/10.1080/10236247909378551>

616 Palombit, R.A., (1993). Lethal territorial aggression in a white-handed gibbon. *American Journal of*  
617 *Primatology*. 31(4), 311–318. <https://doi.org/10.1002/ajp.1350310407>

618 Parker, G. A. (1974). Assessment strategy and the evaluation of fighting behaviour. *Journal of*  
619 *Theoretical Biology*, 47, 223–243.

620 Pearce, A. N., Huang, Z. Y., and Breed, M. D. (2001). Juvenile hormone and aggression in honey  
621 bees.” *Journal of Insect Physiology*, 47(11), 1243–1247.

622 Petersen, G., & Hardy, I. C. W. (1996). The importance of being larger: parasitoid intruder – owner  
623 contests and their implications for clutch size. *Animal Behaviour*, 51, 1363–1373.  
624 <https://doi.org/10.1006/anbe.1996.0139>

625 Poole, J. H. (1989). Mate guarding, reproductive success and female choice in African elephants.  
626 *Animal Behaviour*, 37, 842–849. [https://doi.org/10.1016/0003-3472\(89\)90068-7](https://doi.org/10.1016/0003-3472(89)90068-7)

627 Reece, S. E., Shuker, D. M., Pen, I., Duncan, A. B., Choudhary, A., Batchelor, C. M., & West, S. A.  
 628 (2004). Kin discrimination and sex ratios in a parasitoid wasp. *Journal of Evolutionary Biology*,  
 629 17(1), 208–216. <https://doi.org/10.1046/j.1420-9101.2003.00640.x>  
 630 Riechert, S. E. (1998). Game theory and animal contests. In L. A. Dugatkin & H. K. Reeve (Eds.),  
 631 *Game theory and animal behaviour* (pp. 64–93). Oxford University Press.  
 632 Rowland, W. J. (1989). The effects of body size, aggression and nuptial coloration on competition for  
 633 territories in male threespine sticklebacks, *Gasterosteus aculeatus*. *Animal Behaviour*, 37, 282–  
 634 289. [https://doi.org/10.1016/0003-3472\(89\)90117-6](https://doi.org/10.1016/0003-3472(89)90117-6)  
 635 R Development Core Team. (2016). R: A language and environment for statistical computing. Vienna,  
 636 Austria: R Foundation for Statistical Computing.  
 637 Ruf, D., Mazzi, D., & Dorn, S. (2010). No kin discrimination in female mate choice of a parasitoid  
 638 with complementary sex determination. *Behavioral Ecology*, 21(6), 1301–1307.  
 639 <https://doi.org/10.1093/beheco/arq148>  
 640 Schwagmeyer, P. L., & Woontner, S. J. (1985). Mating competition in an asocial ground-squirrel,  
 641 *Spermophilus tridecemlineatus*. *Behavioral Ecology and Sociobiology*, 17(3), 291–296.  
 642 <https://doi.org/10.1007/BF00300149>  
 643 Segoli, M., Keasar, T., Harari, A. R., & Bouskila, A. (2009). Limited kin discrimination abilities  
 644 mediate tolerance toward relatives in polyembryonic parasitoid wasps. *Behavioral Ecology*,  
 645 20(6), 1262–1267. <https://doi.org/10.1093/beheco/arp125>  
 646 Simmons, L. W. (1986). Intermale competition and mating success in the field cricket, *Gryllus*  
 647 *bimaculatus* (Degeer). *Animal Behaviour*, 34, 567–579. [https://doi.org/10.1016/S0003-](https://doi.org/10.1016/S0003-3472(86)80126-9)  
 648 3472(86)80126-9  
 649 Singer, T. L. (1998). Roles of hydrocarbons in the recognition systems of insects. *American Zoologist*,  
 650 38(July), 394–405.  
 651 Stokkebo, S., & Hardy, I. C. W. (2000). The importance of being gravid: egg load and contest  
 652 outcome in a parasitoid wasp. *Animal Behaviour*, 59(6), 1111–1118.  
 653 <https://doi.org/10.1006/anbe.2000.1407>

654 Terrasse, P. C., & Rojas-Rousse, D. (1986). Distribution de la ponte et évitement du superparasitisme  
 655 chez l'hyménoptère solitaire *Bruchocida vuilleti* Cwf. (Hym., Eupelmidae), parasite des stades  
 656 larvaires de son hôte, *Callosobruchus maculatus* F. (Col., Bruchidae). *Journal of Applied*  
 657 *Entomology*, 101, 243–256. <https://doi.org/10.1111/j.1439-0418.1986.tb00855.x>  
 658 Tibbetts, E. A., & Huang, Z. Y. (2010.) The challenge hypothesis in an insect: juvenile hormone  
 659 increases during reproductive conflict following queen loss in *Polistes* wasps. *The American*  
 660 *Naturalist*, 176(2), 123–130.  
 661 Turillazzi, S., Cervo, R., & Cavallari, I. (1990). Invasion of the Nest of *Polistes dominulus* by the  
 662 Social Parasite *Sulcopolistes sulcifer* (Hymenoptera, Vespidae). *Ethology*, 84, 47–59.  
 663 Vogel, E. R. (2005). Rank differences in energy intake rates in white-faced capuchin monkeys, *Cebus*  
 664 *capucinus*: The effects of contest competition. *Behavioral Ecology and Sociobiology*, 58(4),  
 665 333–344. <https://doi.org/10.1007/s00265-005-0960-4>  
 666 von Beeren, C., Schulz, S., Hashim, R., & Witte, V. (2011). Acquisition of chemical recognition cues  
 667 facilitates integration into ant societies. *BMC Ecology*, 11. [https://doi.org/10.1186/1472-6785-11-](https://doi.org/10.1186/1472-6785-11-30)  
 668 30  
 669 Waldman, B. (1988). The Ecology of kin recognition. *Annual Review of Ecology and Systematics*,  
 670 19(1), 543–571. <https://doi.org/10.1146/annurev.es.19.110188.002551>  
 671 West, S. A., Murray, M. G., Machado, C. A., Griffin, A. S., & Herre, E. A. (2001). Testing Hamilton's  
 672 rule with competition between relatives. *Nature*, 409, 510–513.  
 673 West, S. A., Pen, I., Griffin, A. S. (2002). Cooperation and competition between relatives. *Science*,  
 674 296, 72–75.  
 675 West-Eberhard, M. J. (1979). Sexual selection, social competition, and speciation. *Proceedings of the*  
 676 *American Philosophical Society*, 123(4), 222–234. <https://doi.org/10.1086/413215>  
 677 West-Eberhard, M. J. (1989). Kin recognition in animals. *Evolution*, 43(3), 703–705.  
 678 <https://doi.org/10.1080/0810902032000050000>  
 679 Wilson, K., & Hardy I. C. W. (2002). Statistical analysis of sex ratios: an introduction. In *Sex ratios:*  
 680 *concepts and research methods. Part 2. Statistical analysis of sex ratio data* (pp. 46–92), edited  
 681 by I. C. W. Hardy. New York: Cambridge University Press.

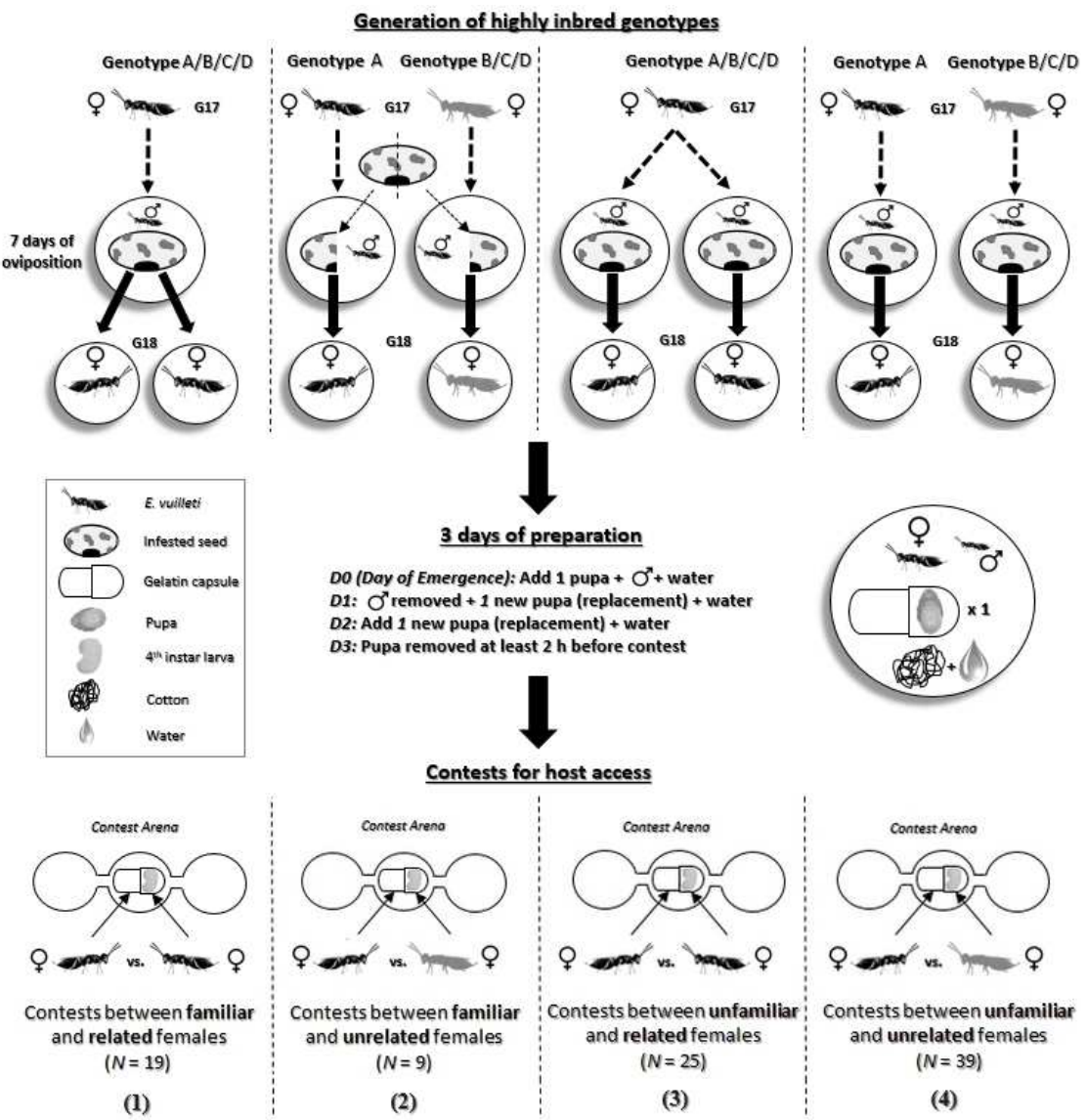
682 Zacchi, F., Cervo, R., & Turillazzi, S. (1996). How *Polistes semenowi*, obligate social parasite,  
683 invades the nest of its host, *Polistes dominulus* (Hymenoptera, Vespidae). *Insect Social Life*, 1,  
684 125–130.

## Figure captions

**Figure 1.** Experimental design that allowed us to investigate the role of genetic relatedness and familiarity on female aggressiveness and conflict resolution over host access. Parasitoid females from different genotypes ('A', 'B', 'C' and 'D') mated and then parasitized infested cowpea seeds for 7 days, in order to obtain adult wasps that have emerged from the same seed or not, and that were genetically related or not. After 3 days of preparation, we observed contests between females that were (1) familiar-related, (2) familiar-unrelated, (3) unfamiliar-related and (4) unfamiliar-unrelated, fighting for accessing to a host.

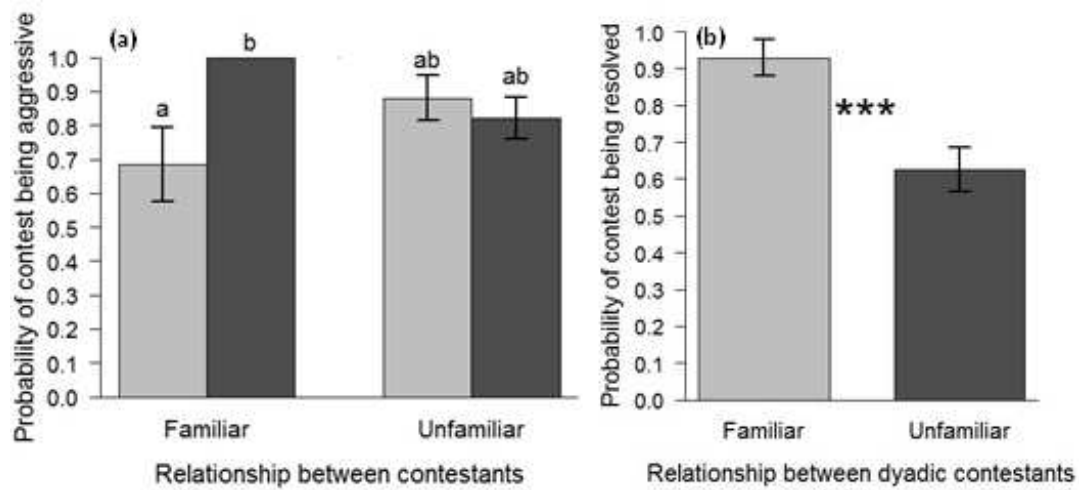
**Figure 2.** (a) Probability ( $\pm$ S.E.M.) of a contest being aggressive when familiar contestants were genetically related (light bars,  $N = 19$ ) or unrelated (dark bars,  $N = 9$ ), or when unfamiliar contestants were genetically related (light bars,  $N = 25$ ) or unrelated (dark bars,  $N = 39$ ). Different letters indicate significant differences based on post-hoc Dunn's multiple comparison test. (b) Probability ( $\pm$ S.E.) of a contest being resolved when contestants were familiar (light bars,  $N = 28$ ) or unfamiliar (dark bars,  $N = 64$ ). \*\*\*:  $P < 0.001$ .

**Figure 3.** Combined total of number of aggressive behaviours observed during contest according to the absolute difference in initial egg load between females of a same dyad. \*\*\*:  $P < 0.001$ . Statistical analyses accounted for differences in contest duration, and showed that effect of the absolute difference in female egg load was still significant when removing the contest during which we observed 100 attacks (GLM with quasi-Poisson distribution of residuals: estimates  $\pm$  S.E. =  $0.175 \pm 0.049$ ,  $F_{1,71} = 4.87$ ,  $P < 0.01$ ).

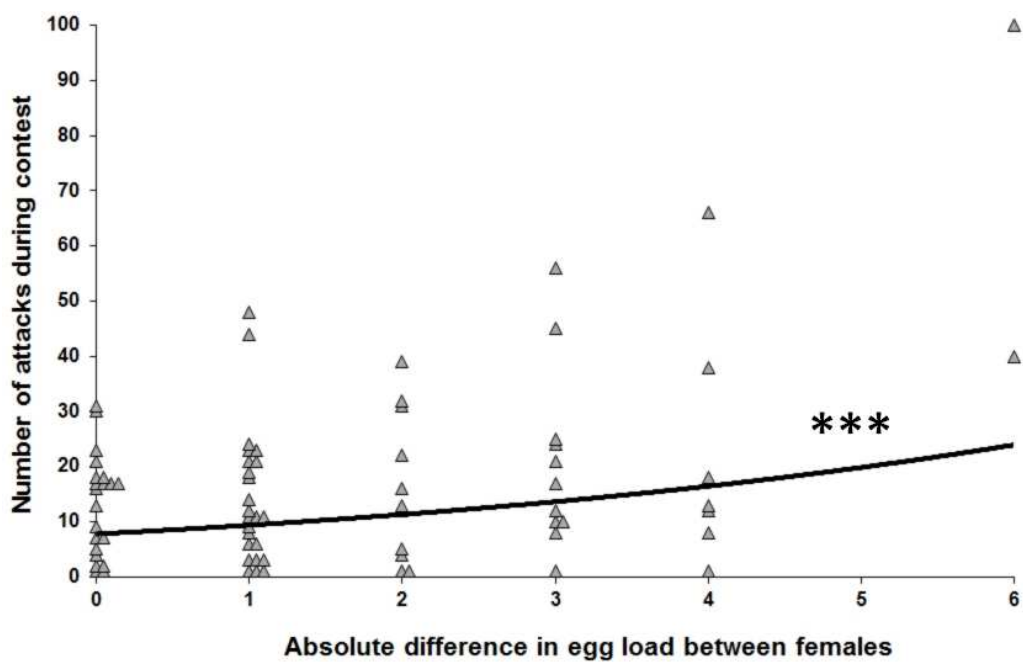


**Figure 1.**





**Figure 2.**



**Figure 3.**