

Lethal combat over limited resources: testing the importance of competitors and kin

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Although most animals employ strategies to avoid costly escalation of conflict, the limitation of critical resources may lead to extreme contests and fatal fighting. Evolutionary theories predict that the occurrence and intensity of fights can be explained by resource value and the density and relatedness of competitors. However, the interaction between these factors and their relative importance often remains unclear; moreover, few systems allow all variables to be experimentally investigated, making tests of these theoretical predictions rare. Here, we use the parasitoid wasp *Melittobia* to test the importance of all these factors. In contrast to predictions, variation in contested resource value (female mates) and the relatedness of competitors do not influence levels of aggression. However, as predicted, fight intensity increased with competitor density and was not influenced by the greater cost of fighting at high density. Our results suggest that in the absence of kin recognition, indirectly altruistic behavior (spite) is unlikely to evolve, and in such circumstances, the scale of competition will strongly influence the amount of kin discrimination in the form of level of aggression as observed in *Melittobia* species. *Key words*: fatal fighting, kin discrimination, *Melittobia*, relatedness, resource competition, spite. [*Behav Ecol*]

When individuals compete for resources, their interactions span the entire spectrum of behavior from cooperative resolution to escalated conflict (Maynard-Smith and Price 1973). Escalated and violent interactions are rare and only predicted under conditions where the benefit of winning far outweighs the potential cost of conflict (Maynard-Smith and Price 1973; Enquist and Leimar 1990). When competition occurs over a finite resource of extremely high value, fights can escalate and competing individuals risk death in violent contests (Enquist and Leimar 1987, 1990). While competition over mates does not always lead to conflict, many known examples of lethal conflict result from competition between males over potential mates or access to mating opportunities with females (Enquist and Leimar 1987, 1990), such as in fig wasps and *Cardiocondyla* ants (Hamilton 1979; Murray 1987; Anderson et al. 2003). As mating is directly related to male fitness, access to female mates is extremely important to males. Consequently, when females are limited in time, space, or both, then extreme competition and fatal fighting can evolve (Maynard-Smith and Price 1973; Hamilton 1979; Murray 1987; Enquist and Leimar 1990; Reinhold 2003).

Evolutionary theory predicts that the occurrence and intensity of fights will vary with resource value (Enquist and Leimar 1987, 1990), the number of competitors (Murray and Gerrard 1984, 1985; Murray 1987, 1989), and their relatedness (Hamilton 1979; Reinhold 2003). First, although mates are always a valuable resource, theory suggests that what matters for the evolution of extreme conflict is the value of a current resource relative to its likely future value (Enquist and Leimar 1990). When competitors are likely to have many mating opportunities in the future, each current mating constitutes a small fraction of their potential lifetime reproductive success

(LRS). Therefore, there is relatively little to be gained by fighting for access to mates as the potential costs of doing so are high (Hamilton 1979; Enquist and Leimar 1990). In contrast, if future mating opportunities are unlikely (e.g., due to available mates becoming scarce over time), then each mating represents a considerably larger proportion of lifetime reproduction, and so, the potential benefits of winning can exceed the costs of fatal fighting (Hamilton 1979; Murray 1987; Enquist and Leimar 1990; Cook et al. 1999). Second, variation in competitor density is predicted to have several different effects on the intensity and frequency of fatal fighting. As the number of competitors increases, so does their encounter rate, which results in a higher frequency of fighting, but also decreases the payoff from winning each fight as a higher number of opponents must be defeated (Murray 1987). When these effects are considered simultaneously in a model, a domed relationship is predicted in which fight intensity is highest at intermediate competitor density, a pattern partially supported by observational data from fig wasps (Murray 1987; Figure 1a). Third, when kin can be discriminated from nonkin, theoretical models based on fig wasp's fighting behavior predict that fight intensity will decrease between competitors that are more closely related: Due to the indirectly altruistic benefits of preferentially harming nonrelatives to increase the fitness of relatives, male competitors are expected to selectively fight unrelated competitors only, resulting in a relatively lower overall fight intensity (Hamilton 1979; Reinhold 2003). Recently, it has also been argued that if this model is extended and both the number of competitors and their relatedness are considered simultaneously, fight intensity is instead predicted to decrease with competitor density for a given level of relatedness (Reinhold 2003; Figure 1b). However, as model systems suitable for testing all these hypotheses are scarce, there have been few experimental tests of these alternative theoretical predictions (West et al. 2001; Reinhold 2003; Moore et al. 2008).

Here, we present a series of experiments to directly test how the perceived value of the contested resource and variation in

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Received 6 March 2010; revised 30 August 2010; accepted 7 September 2010.

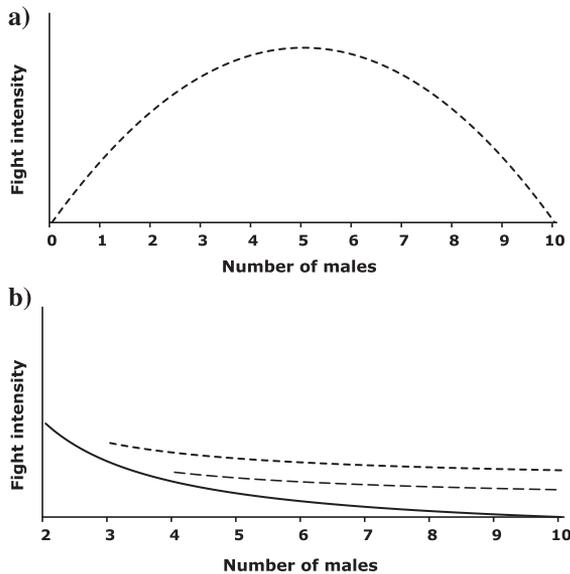


Figure 1

Theoretical predictions for the relationship between number of competitors and fight intensity; a) increasing encounter rate is counteracted by increased cost of fighting as the number of competitors increases (Murray, 1987); b) fight intensity varies with competitor number and the relatedness of competitors, where competitors are either closely related (1 foundress female; solid line), a mixture of related and unrelated males (2 foundresses; dotted line) giving intermediate average relatedness, or have low average relatedness (3 foundresses; dashed line) (Reinhold, 2003). In both cases, the y axis corresponds to increasing fight intensity.

the density and relatedness of competitors influence the intensity and frequency of fatal fights. We use the parasitoid wasp *Melittobia* because male *Melittobia* are restricted in their spatial and temporal opportunities to gain mates (Hamilton 1979; Matthews et al. 2009). Consequently, as their entire LRS is at stake when males compete, they engage in extremely violent fatal fighting (see MATERIALS AND METHODS). In the first experiment, we manipulate males perception of resource value—mating opportunities—by allowing some males to mate prior to fighting, whereas some remain unmated. This manipulation creates a difference in the ratio between the current and potential future value of the resource for males from the 2 treatments, where future reproductive opportunities are perceived to be a greater component of potential lifetime reproduction for a virgin male than a previously mated male. The manipulation could affect resource value in a number of ways: We expect future access to mates to be of higher value to a virgin male—more affected by the lack of current mating opportunities—than a mated male and predict that virgin males will fight more often, more intensely, or both; however, it is also possible that mated males will use previous matings as a cue for the availability of mates and fight more intensely than virgin males over a mating resource perceived to be large. Crucially, in either case, we expect to see a difference in fighting behavior between the 2 treatments (see MATERIALS AND METHODS). Second, we manipulate the density of competitors by creating arenas with different numbers of males and measure fight intensity. We expect the frequency of fights to increase with group size and fight intensity to either be greatest at intermediate density (Murray 1987; Figure 1a) or to decrease with increasing density (Reinhold 2003; Figure 1b) depending on whether the cost of fighting or benefit of winning is more influential. Third, we vary competitor density and relatedness between competitors simultaneously by creating arenas in

which males compete with different numbers of related males—all equally related to a given male—or a mixture of related and unrelated males—such that a given male is relatively more related to some competitors compared with others (see MATERIALS AND METHODS [Experiment 3: relatedness and competitor density]). If individuals are able to recognize kin, then we predict lower overall fight intensity in the highly related groups (Reinhold 2003; Figure 1b), unless any benefit of behaving less violently toward closer relatives is canceled by the potential for competition between relatives (West et al. 2002). If, however, *Melittobia* do not recognize kin (or kin selection benefits are negligible), we expect that the frequency and intensity of fights will simply be determined by competitor density (Reinhold 2003). In all experiments, we collect data to quantify fight intensity at a number of levels by considering the pattern of mortality, the incidence and severity of injuries, and several measures of aggressive behavior.

MATERIALS AND METHODS

Background biology and general methods

Melittobia acasta and *M. australica* (Hymenoptera: Eulophidae) are gregarious ectoparasitoid wasps with similar natural history, host range, sex ratio patterns, and fighting behavior to other *Melittobia* species (Van den Assem et al. 1980; Gonzalez, Abe, et al. 2004; Gonzalez, Genaro, et al. 2004; Matthews et al. 2009 and see Abe et al. 2003, 2005; Innocent et al. 2007; Reece et al. 2007 for further details of their natural history). *Melittobia* species parasitize a wide range of hosts, particularly other species of Hymenoptera (Balfour Browne 1922; Freeman and Parnell 1973; Freeman 1977; Van den Assem et al. 1980; Dahms 1984; Cooperband and Vinson 2000; Gonzalez, Genaro, et al. 2004; Matthews et al. 2009). Sexual dimorphism is pronounced: Males are blind, flightless, and remain on their natal host to compete locally for mates, whereas females have fully functioning eyes and wings and may disperse after mating (Buckell 1928; Dahms 1984; Gonzalez, Genaro, et al. 2004; Matthews et al. 2009). Male mandibles are highly modified weapons used in violent lethal combat prior to female eclosion: Males sever competitor's limbs and decapitate opponents in fights to the death (Balfour Browne 1922; Buckell 1928; Dahms 1984; Abe et al. 2003, 2005; Hartley and Matthews 2003; Innocent et al. 2007; Reece et al. 2007; see also Hamilton 1979). Any male remaining alive when the female's eclose gains the opportunity to mate. *Melittobia* produce extremely female-biased offspring sex ratios (85–95% female; Abe et al. 2003, 2005; Cooperband et al. 2003; Gonzalez, Genaro, et al. 2004; Innocent et al. 2007), so the proportion of male offspring eclosing from a host is low; however, the precise number of males varies with both the number of females laying eggs on the host, oviposition period, and the host species. For example, the number of males per host in culture ranges from 0–1 (1 foundress, 24-h oviposition) to 15–17 (50 foundresses, 48-h oviposition) on *Caliphora vomitae* pupae (Innocent et al. 2007) and from 0–7 (1 foundress, 72 h) to as many as 80 (15 foundresses, 144 h) males on *Bombus terrestris* pupae (Innocent et al. 2010). Male emergence time varies: For instance, when reared at 30 °C with a 16:8 h light:dark photoperiod, development time is in the region of 9–14 days for males in comparison with 14–21 days for females; under these conditions, the average lifespan of isolated virgin males is approximately 7 days; however, male lifespan is strongly influenced by the degree of fighting (Innocent et al. 2007 and Innocent TM, West SA and Reece SE, unpublished data). The degree of relatedness between male competitors is determined by the number and relatedness of female foundresses (Innocent et al. 2010). Given the potential for multiple

sequentially ovipositing foundresses on a single host (Schmieder 1933; Freeman and Ittayeipe 1976, 1982, 1993; Van den Assem et al. 1982; Dahms 1984; Cooperband et al. 2003; Matthews et al. 2009), male emergence may vary through time; as a result of staggered emergence (rather than learned differences), males differ in fighting ability (e.g., with variation in age; Abe et al. 2007; Innocent et al. 2007) and experience variation in the local or temporal availability of females.

Although the biology of *M. acasta* and *M. australica* is widely reported to be similar (e.g., Matthews et al. 2009), in laboratory culture, the patterns of development can vary (*M. acasta*: Innocent et al. 2007; *M. australica*: Abe et al. 2003, 2005). We utilize the differences between our stock cultures of *M. acasta* and *M. australica* to match the logistical requirements of our experiments. *M. acasta* has more synchronous development in culture, enabling the production of large numbers of age-matched males, and consequently was the most suitable species for use in experiment 1, whereas a larger bank of unrelated stock lines was available to us for *M. australica*, a critical prerequisite for experiments 2 and 3. Crucially, previous experiments using the same stock cultures of these species have found similar patterns of aggressive behavior and similar sex ratio patterns (Abe et al. 2003, 2005; Innocent et al. 2007). Thus, by using these species, with a high degree of similarity in relation to the expression of the traits we focus upon in our experiments—patterns of sex allocation and fighting behavior—we are able to better test complementary aspects of our hypotheses. Moreover, the shared natural history of *M. acasta* and *M. australica* suggests that these traits are shaped by similar evolutionary pressures. We cultured *M. acasta* stock on *C. vomitae* pupae at 30 °C (see Reece et al. 2007; Innocent et al. 2007). *M. australica* lines were collected throughout Japan (by Jun Abe, 1999–2000; see Abe et al. 2003, 2005), with lines originating from wasps collected in different regions at different times. Since collection, each line has been cultured separately and within replicates, and no 2 lines from the same region were used. We reared all experimental *M. australica* lines on *B. terrestris* pupae (Koppert, Berkel en Rodenrijs, The Netherlands) allocated evenly by mass across groups, incubating them at 30 °C with a 16:8 h light:dark photoperiod. The general protocols—common to both species—are given below.

To produce males, we collected virgin female pupae from the stock culture: As sex determination in *Melittobia* is haplodiploid, virgins lay unfertilized eggs, which produce exclusively male offspring (Cook 1993). We placed groups of 60 virgin females with hosts for oviposition (as above) and incubated them at 30 °C. To minimize variation in age of male offspring, we gave virgin females hosts synchronously and limited oviposition to an 8-h period. We collected male pupae from hosts approximately 8 days after oviposition and isolated each male individually in a hollow gelatin capsule of similar dimension to host pupae (volume = 0.21 ml) to prevent any aggressive male–male interactions prior to the experiment. We checked males daily, grouped them by eclosion date, and used males from the same 24-h emergence period within experimental replicates. By testing male fighting behavior in the absence of females, we mirrored the natural history of *Melittobia* species, where the majority of fighting occurs before female emergence. Given the variation in male emergence times, few males would be fighting in the presence of females; however, this has the potentially confounding effect on male behavior and the investment of energy by males in mating rather than fighting, which we controlled for by ensuring that mating was not possible during experiments. We collected data for measures of fight intensity based on behavior, injury, and mortality using 2 types of fighting arena. We used holes punched in sheet metal (5-mm diameter and 3-mm thick) encased by glass cover slips as arenas for data collection on

focal males (experiment 1). These were cleaned between replicates to avoid any potential influence of chemical signals from previous contests. To collect group-level data (experiments 2 and 3), we used gelatin capsules as arenas (as above).

Experiment 1: resource value

We tested whether virgin males compete more intensively over mating opportunities than previously mated males. We placed each male from the mated treatment with 5 virgin females (from stock synchronized with male emergence) for 2 h at 30 °C. Males are able to mate many females as evidenced by the extremely female-biased sex ratios and large clutch sizes produced by single foundress females (e.g., Innocent et al. 2007). Previous experiments have shown that females produce an average clutch size of 100–200 offspring of which an average of 4 are males; with variation in male survival due to fatal fighting, mating rate is likely to vary, but on average, a single male is likely to be able to mate with 25–50 females (Innocent et al. 2010). Mating with 5 females therefore represents less than the maximum potential male mating rate but constitutes a significant proportion of the average mating success. We restricted male mating time to avoid any negative effects of multiple mating such as significant investment of resources that could confound reduced fight intensity in response to a decrease in perceived resource value, and individual males were given different females so that no effects of sperm competition could occur. We simultaneously placed the remaining virgin males at 30 °C for 2 h and isolated individually in gelatin capsules. Subsequently, we paired males in 3 combinations, with 20 replicates of each: mated male + mated male (MM), virgin male + virgin male (VV), and mated male + virgin male (MV). We expected that virgin males would fight more fiercely relative to those who had already achieved some matings, given that future mating opportunities represented their entire LRS. However, it is possible that matings themselves are used as a cue for mate availability by male *Melittobia*, and if so, mated males may fight more intensely as they perceive the total available resource to be of greater value; in either case, the critical factor is that we expect to see a difference between the treatments in fighting behavior. We painted each male's abdomen for identification; color was assigned randomly across pairs and combinations. We observed each male separately for 5 min, recording the number of movements between sectors of an arena to estimate individual baseline activity level. Next, we paired males in a new arena and observed them for 30 min during which we recorded the time interactions began and finished and the identity of the male initiating and retreating from each bout. We also recorded key aggressive behaviors defined in preliminary studies: 1) “boxing,” where a male hits their opponent using limbs only; 2) “locking” of opponent, where a male grabs hold of his opponent around the body, preventing the movement of the opponents limbs; and c) “biting,” where a male attempts to attack using his mandibles. We defined interactions as >5 s of physical contact between males and considered them antagonistic if we observed any of these aggressive behaviors. We scored the relative size of each pair of males by eye—recording pairs as the same size or noting the identity of the larger male—known to correlate with weapon size and fighting success (Innocent et al. 2007; Reece et al. 2007). After observation, we incubated each pair in a gelatin capsule (as above, suitable for incubation and similar in dimension to host pupae) at 30 °C; we recorded the outcome of each contest after 24 h (win/lose or draw), the identity of male(s) remaining alive, and any injuries to either male visible by eye with thorough examination under a microscope.

Experiment 2: competitor density

We tested Murray's (1987) prediction that fight intensity is influenced by the number of competitors and is greatest at intermediate male densities. We set up 6–12 replicates for each of 5 competitor densities: 2, 5, 10, 15, and 25 male *M. acasta* (49 replicates in total). We placed groups of age-matched males into capsule arenas (see above) and incubated them at 30 °C for 24 h. We recorded the number of males dead at 2, 4, 6, 8, and 24 h (Olympus SZX10 microscope) to estimate the time of the first death and proportion of males dead at 24 h. We froze all arenas at 24 h and scored injuries visible with a microscope for each male according to a scale of 0–7 (e.g., loss of an antennae scored 0.5 points, whereas loss of head scored 7 points) adapted from Murray (Murray and Gerrard 1984, 1985; Murray 1985, 1987, 1989, 1990). We then calculated mean injury per wasp (lifetime extent of injury [LEI]), the proportion of males injured, and the proportion with severe injury (total score >7) for each arena.

Experiment 3: relatedness and competitor density

We varied relatedness between male *M. australica* using 2 treatments: “related”—all males came from the same line and “mixed relatedness”—males came from 3 different lines (in a combination drawn from 6 available lines), meaning that males were relatively more related to males of the same line and relatively less related to males from any of the unrelated lines. If the effect of localized competition with relatives cancels the benefits of kin-selected altruism, “all relatives” and “all nonrelatives” are comparable scenarios because there is no difference in the average relatedness between competitors. For example, in fig wasps, where males competes locally for mates, if all competitors are brothers—resulting from a single foundress female—then there is no reason to spare one brother at the cost of fighting another, and so, males fight all competitors indiscriminately (West et al. 2001). However, we might still expect indirectly altruistic behavior—in the form of choosing to fight nonrelatives over relatives—in mixed relatedness groups, where relative relatedness can differ: Compared with the average relatedness of the competing population, a focal male would be positively related to brothers and negatively related to nonsibs. Under these circumstances, fighting unrelated male competitors is beneficial to males' siblings because it reduces the number of competitors they face, and thus, it is considered indirectly altruistic or spiteful behavior.

We cross-factored relatedness treatments with a competitor density treatment using 2 group sizes, 3 or 6 males. Overall, we therefore had 4 possible treatment combinations, representing scenarios where either 1 foundress female (“related” treatment) or 3 foundress females (“mixed relatedness”) produced a total of either 3 or 6 male offspring as follows: (a) 3 males from the same line; (b) 3 males, 1 from each of 3 different lines; (c) 6 males from the same line; and (d) 6 males, 2 each from 3 different lines—thus keeping the ratio of relatedness the same between group sizes of 3 and 6 for the mixed relatedness treatment. Each line contributed equally to both related and mixed relatedness treatment groups, ensuring that line differences did not confound treatment effects (and see Statistical methods). Preliminary data showed that different male lines demonstrated similar patterns of aggressive behavior, such as fighting any male opponent without an initial assessment phase (Innocent TM, West SA and Reece SE, unpublished data). This experimental design allowed us to examine the importance of relative relatedness by testing for differences in the level of aggression within groups where males were related to all their competitors (combinations [a]

and [c], above), none of their competitors (b), or were relatively more related to some competitors than others (d).

We placed males in gelatin capsule arenas, which we mounted and observed with a microscope (as experiment 2) for 30 min. We recorded the number of fights and the number of males engaged in fighting at 1-min intervals through this period. Males were not marked individually as this is difficult for the large number of males required here and because we were interested in average levels of aggression of groups. Following observation, we incubated arenas at 30 °C, recording the number of males dead at 90 and 180 min and 24 h in order to estimate the time of first death and calculate the proportion of males dead at 24 h. We froze all arenas at 24 h, scored injuries for each individual, and then calculated LEI, the proportion of males injured, and the proportion with high levels of injury per arena (as above). We collected data from 9 replicates for each of treatments (b) and (d) (those with 3 different lines represented) and then 27 replicates for each of (a) and (c) (i.e., a single-line replicate for each line represented in a mixed-line replicate).

Statistical methods

Where necessary, data were transformed (using square-root, log, or arcsine square-root transformation) to normalize the error variances. We used linear models to test for the effect of group size on the time of first male death, the proportion of males dead at 24 h, and the proportion of males injured in experiment 2 and all behavioral measures of fight intensity from experiment 1. For experiment 2, we also tested for a quadratic relationship between each factor and group size. We used generalized linear models (GLIMs) to analyze fight resolution and occurrence of injury data (experiment 1), assuming a binomial error distribution and using a logit link function for maximum power. Model simplification was based on analysis of deviance comparing changes in deviance between models to the chi-squared distribution. We tested for overdispersion of data by calculating the heterogeneity factor (HF), where $HF < 4$ data was scaled and significance tested using the *F* distribution to correct for overdispersion (Crawley 2007). We included resource value treatment, focal size (scored as same size, larger, or smaller than competitor), difference in activity level, and 2-way interactions between treatment and other variables in the model as possible explanatory variables. To examine the effect of both group size and relatedness between competitors on mortality, injury, and behavior in experiment 3, we used linear mixed-effect models to account for repeated measures on multiple different individuals from the same lines and thus avoid pseudoreplication. We used the measures of fight intensity as response variables for each model, including group size and relatedness in the model as fixed effects; and fitted line identity as a random effect to control for any differences between lines. Interactions are presented only where significant at the level of $P < 0.01$ —using more stringent criteria for significance as recommended when testing interactions (Crawley 2007). All analyses were carried out in R (R version 2.3.1, Copyright 2006, The R Foundation for Statistical Computing).

RESULTS

Experiment 1: resource value

In contrast to predictions of Enquist and Leimar (1990), we found that variation in resource value (whether or not males had previously mated with females) did not significantly influence fight intensity as measured by mortality, injury, or

behavior. The likelihood of at least one male dying within the first 24 h was not significantly influenced by resource value (male mating status: $F_{2,55} = 0.60$, $P = 0.55$), size difference ($F_{1,57} = 0.47$, $P = 0.49$), or individual activity level ($F_{1,54} = 0.26$, $P = 0.61$). We scored all visible injuries and did not find any significant correlation with male mating status ($F_{2,55} = 1.95$, $P = 0.15$), size difference ($F_{1,55} = 1.6$, $P = 0.20$), or activity level ($F_{1,55} = 0.10$, $P = 0.76$). Similarly, there were no significant correlations between the mean number of fights per minute and male mating status ($F_{2,55} = 1.75$, $P = 0.18$; Figure 2), size difference ($F_{2,56} = 3.38$, $P = 0.07$), or difference in individual activity between males ($F_{1,53} = 0.01$, $P = 0.91$). We also found the same qualitative pattern with 2 other measures of aggressive behavior, the total number of fights, and the proportion of the observation period individuals spent fighting ($P > 0.25$ in all cases).

Experiment 2: competitor density

The proportion of males dying within 24 h was positively correlated with group size ($F_{1,43} = 14.74$, $P = 0.0004$; Figure 3a), and the first male death was significantly earlier in larger groups ($F_{1,43} = 48.32$, $P < 0.0001$). There was no significant quadratic relationship with group size in either case ($P > 0.1$). We found no significant effect of increasing group size on the proportion of males injured (linear: $F_{1,43} = 1.17$, $P = 0.29$, quadratic: $F_{1,42} = 0.26$, $P = 0.61$; Figure 3b). Similarly, there was no significant effect of group size on the proportion of males with high injury score or mean injury per wasp ($P > 0.35$); there were no quadratic relationships ($P > 0.35$ in all cases).

Experiment 3: relatedness and competitor density

The proportion of males dead at 24 h increased significantly with increasing group size ($F_{1,58} = 6.56$, $P = 0.01$; Figure 4a) but not with variation in male relatedness ($F_{1,11} = 1.12$, $P = 0.27$; Figure 4a; Table 1). Similarly, the time of first death was significantly earlier in larger groups ($F_{1,58} = 12.23$, $P < 0.0001$) but was unaffected by relatedness ($F_{1,11} = 2.39$, $P = 0.13$). The proportion of males injured did not vary significantly with increasing group size ($F_{1,58} = 0.53$, $P = 0.47$) or relatedness within groups ($F_{1,11} = 2.71$, $P = 0.13$; Figure 4b; Table 1). We found a similar pattern for the proportion of males with severe injury and the LEI ($P > 0.1$). The mean proportion of males fighting increased with group size ($F_{1,58} = 11.34$, $P = 0.001$; Figure 4c) but did not vary with relatedness ($F_{1,11} = 1.21$, $P = 0.27$; Figure 4c; Table 1). Similarly, the mean number of fights per minute increased with

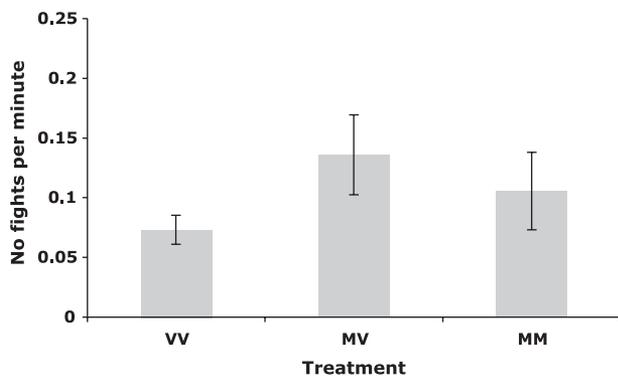


Figure 2

Mean fighting rate between male pairs within 3 treatment combinations: both virgins (VV), both mated (MM), and a mated versus a virgin male (MV). Error bars indicate standard errors.

group size ($F_{1,58} = 38.90$, $P = 0.0001$) but did not vary with relatedness ($F_{1,11} = 1.87$, $P = 0.18$).

DISCUSSION

We experimentally tested theoretical predictions for how resource value, competitor density, and relatedness between rivals will influence the frequency and intensity of fatal fighting (Figure 1). We found that 1) males do not adjust their level of aggression in response to perceived variation in the contested resource value according to whether or not they had previously mated (Figure 2), 2) the intensity of fighting increased linearly with increasing competitor density (Figure 3), 3) levels of aggression did not vary with the relatedness between interacting individuals (Figure 4). Overall, our results suggest that male *Melittobia* exhibit a relatively fixed behavioral strategy, do not exhibit kin discrimination with respect to fighting behavior, and when given the opportunity are likely to engage in potentially lethal combat.

Theory suggests that the more valuable a contested resource, the more likely competitors are to risk costly escalated conflict to obtain it, as documented for a range of animals, from red deer to fig wasps (Hamilton 1979; Enquist and Leimar 1987, 1990; Cook et al. 1999). Here, we find no evidence that the intensity of fighting between male *Melittobia* varies according to whether the contested resource has the same or different value for the competitors (Figure 2). One possible explanation is that, given the short lifespan of males and limited opportunity to gain mates, the best strategy may be to fight whenever another male is encountered without making any assessment. Alternatively, the finite number of potential matings along with any impacts of senescence on fighting ability—in the extreme, a terminal investment—may lead males, particularly mated males, to fight vigorously against all competitors. Another possibility is that the value of past resources has no impact on the ratio of current to future resource value (Dawkins and Carlisle 1976) and that fighting over potential LRS is always favored in these species. In this case, we may also have failed to detect an influence of resource value that could be found using an alternative manipulation, such as varying the number of females present at the time of fighting, whereas patterns of sperm precedence could alter the potential benefits of fighting.

The intensity of fighting is predicted to show either a domed (Murray 1987) or negative (Reinhold 2003) relationship with competitor density. Increased competitor density leads to a higher number of interactions between males, and hence the possibility for more violent conflicts, but this can be negated at high density if this also leads to an increased cost due to fighting more competitors (Murray 1987). We found that a greater number of competing males led to a monotonic increase in fight intensity across a biologically relevant range of densities (Figure 3). If encounter rate does increase with the number of competitors (Murray 1987; Reece et al. 2007), our results suggest that males do not modify their fighting behavior in response to the increasing costs of fighting more opponents (Murray and Gerrard 1984; Murray 1987, 1989). Ideally, an alternative method for testing fighting behavior in the natural competitive environment of a host would allow these hypotheses to be tested in context. An additional factor to consider is that these species differ in male aggression; however, this seems unlikely to be the case, given the degree of similarity in natural history and, in particular, the considerable evidence for comparable patterns of sex allocation and fighting behavior—the key traits we focus on—in *Melittobia* species (reviewed in Matthews et al. 2009). Furthermore, these results confirm previous observations of fighting behavior that conflict limitation through opponent assessment does not occur in *Melittobia* (Reece et al. 2007).

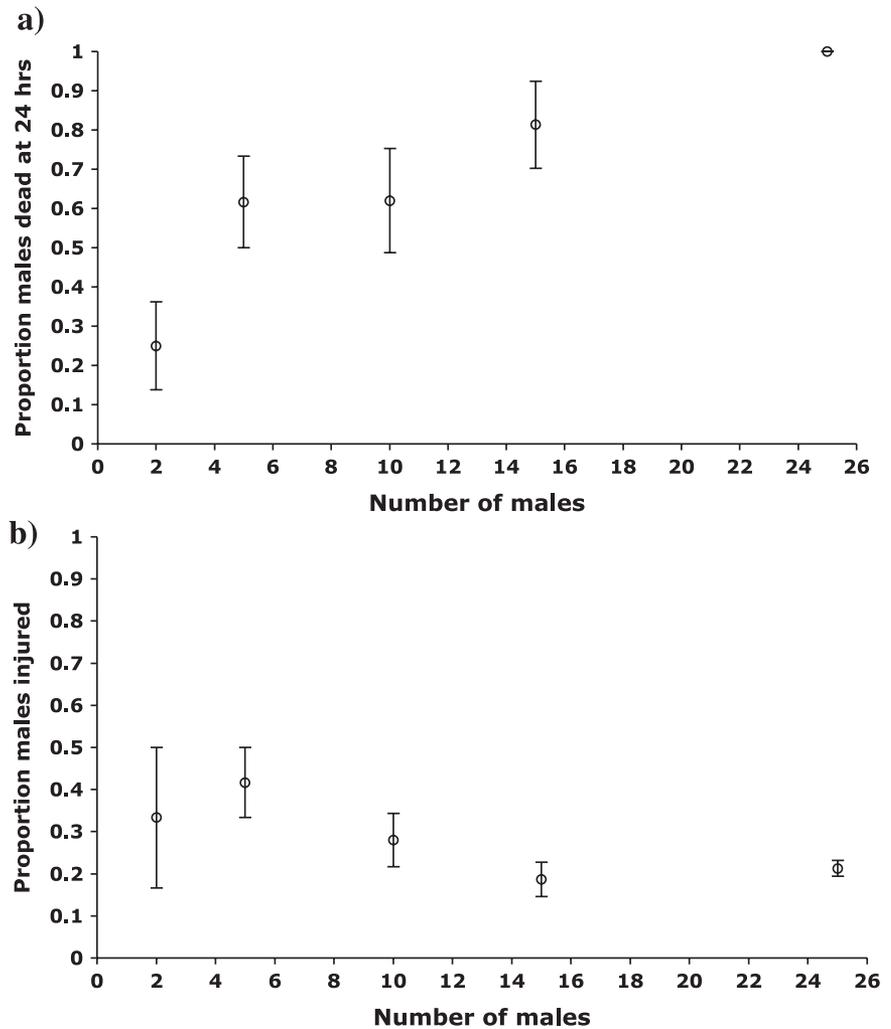


Figure 3

The influence of competitor density on mortality and injury measures within groups for a range of group sizes; (a) the pattern of mortality, shown as the proportion of males within a group dead at 24 h and (b) the pattern of injury, shown as the proportion of males injured within 24 h. Error bars show mean values \pm standard errors.

Theory predicts that if individuals are able to discriminate relatives from nonrelatives (kin discrimination), then competition should be less aggressive between relatives (Hamilton 1979; Reinhold 2003). Specifically, individuals should be more violent to nonrelatives because any harm caused would 1) not lead to an indirect fitness cost and 2) potentially benefit relatives, who would experience reduced competition with the harmed individual. As fighting can be costly, it can therefore be favored as a selfish or spiteful (indirectly altruistic) behavior (Gardner and West 2004; Gardner et al. 2007; West and Gardner 2010). We allowed male *Melittobia* to interact with both relatives and nonrelatives in their arenas and found no evidence that they adjust their fighting behavior in response to relatedness (Figure 4, see also Abe et al. 2003). It is possible that treatments resulting in a wider range of degrees of relative relatedness might detect an influence on fighting behavior. In contrast to our findings, Giron et al. (2004) tested similar hypotheses in the polyembryonic wasp *Copidosoma floridanum* and found that the level of aggression exhibited by soldier larvae decreased as relatedness to potential competitors increased but was unaffected by the severity of resource competition. However, theory predicts that it is hard to maintain variability in genetic cues of relatedness (Rousset and Roze 2007), a problem that appears to be sidestepped in *C. floridanum* by using genes whose variability is maintained for host resistance (Giron and Strand 2004). Our results suggest that *Melittobia* are unable to discriminate kin, which is consistent with data from other non-social insects, where kin discrimination is rarely found (Fellowes

1998; Reece et al. 2004; Shuker et al. 2004; although also see Marris, et al. 1996; Lalonde 2005; Lize et al. 2006). While this may be because relatedness shows little variation (Herre 1987), theory predicts that kin discrimination based on genotype will be rare because common alleles will be recognized more often, and hence, kin discrimination would eliminate the genetic diversity that it requires in order to operate (Crozier 1986; Rousset and Roze 2007). More generally, our data support observational studies showing that local competition within fig fruits means that any kin-selected benefit from reducing conflict with relatives is negated by the increased competition with other relatives (West et al. 2001). Thus, one possible explanation for our results is that the benefits of indirect altruism are canceled by the local scale of competition in *Melittobia*. Put simply, there is no benefit in being less aggressive toward a brother if any benefit they obtain comes at a cost to another brother (West et al. 2002).

How does fighting in *Melittobia* compare with other species in which extreme contests over limited resources are found? A common feature of species in which males engage in lethal combat is that potential mates are aggregated both spatially and temporally for a short time only (Hamilton 1979; Enquist and Leimar 1990). For instance, some wingless male fig wasps engage in lethal combat within fig fruit for access to locally emerging females, and wingless male *Cardiocondyla* ants will kill rivals within the nest during competition for mates (Hamilton 1979; Murray and Gerrard 1984, 1985; Murray 1987, 1989, 1990; Cook et al. 1997, 1999; Bean and Cook

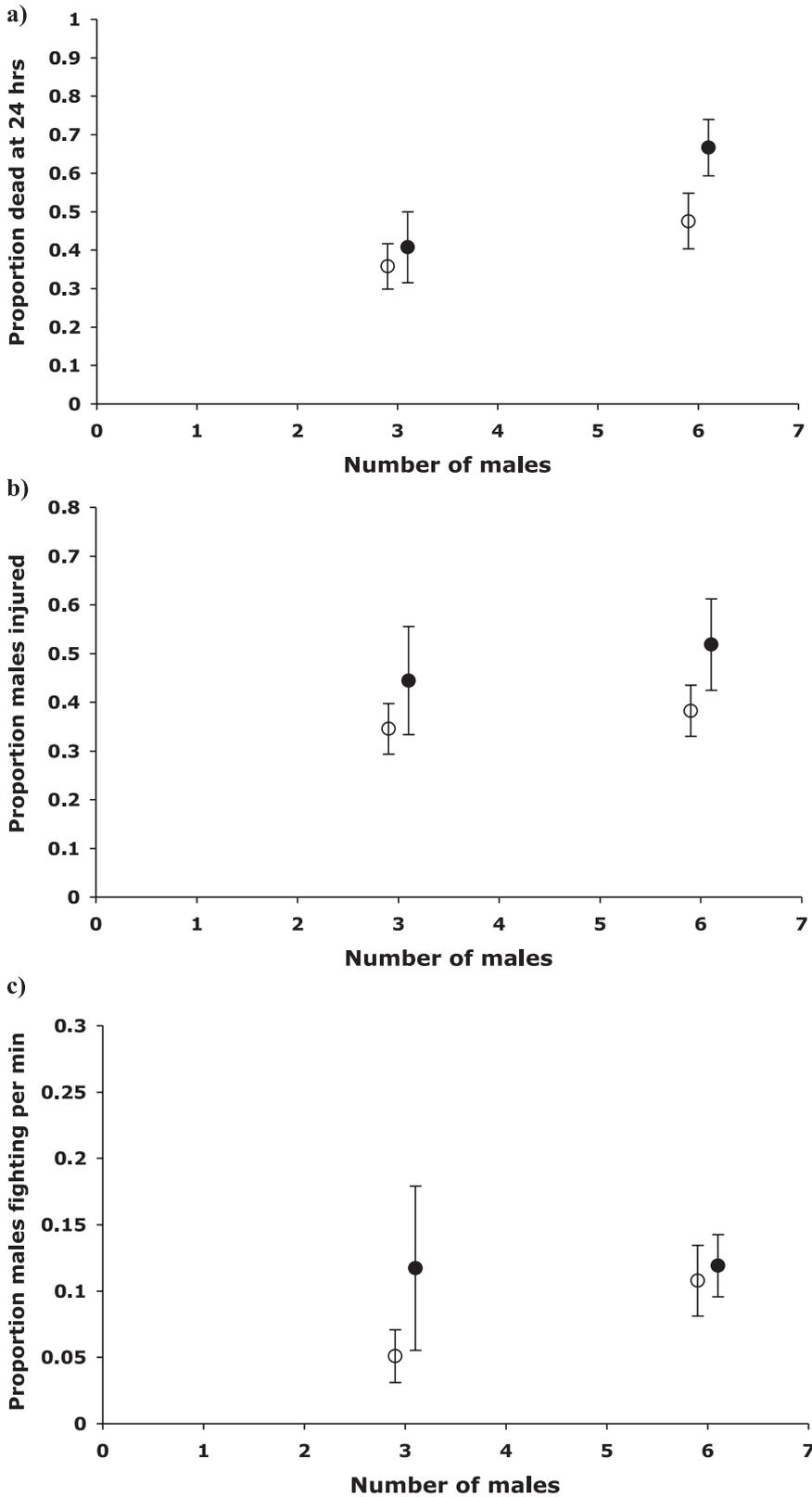


Figure 4

The influence of group size and relatedness on fight intensity (y axis: intensity increases from 0 to 1) in *M. australica* as measured by (a) mortality, shown as proportion of males dead at 24 h; (b) proportion of males injured; and (c) proportion of males fighting per minute for related (open circles) and mixed relatedness (closed circles) groups of 3 or 6 male competitors. Error bars indicate mean value \pm standard errors and x axis staggered for clarity.

2001; Anderson et al. 2003; Greeff et al. 2003; Cook and Bean 2006; Moore et al. 2008; Nelson and Greeff 2009). The highly localized competition in these species could theoretically favor the evolution of indirectly altruistic behavior, where an

individual can fight nonrelatives to reduce competition faced by relatives and gain indirect fitness benefits. However, if the lack of kin discrimination observed in *Melittobia* species is found more generally, this suggests that the indirect benefits

Table 1
Mean values and 95% confidence intervals for the effect of relatedness treatment on measures of fight intensity in experiment 3

	Proportion males dead at 24 h	Proportion males injured	Proportion males fighting per minute
(a) 3 males, related	0.36 ± 0.1	0.35 ± 0.1	0.05 ± 0.04
(b) 3 males, mixed	0.41 ± 0.2	0.44 ± 0.2	0.12 ± 0.1
(c) 6 males, related	0.48 ± 0.1	0.38 ± 0.1	0.11 ± 0.05
(d) 6 males, mixed	0.67 ± 0.1	0.52 ± 0.2	0.12 ± 0.04

of harming nonrelatives have little impact on the pattern of fatal fighting over highly valuable limited resources.

FUNDING

Natural Environment Research Council, the Royal Society, the Leverhulme Trust, and the Wellcome Trust (WT082234MA)

Thanks to N. Colegrave and M. Robinson for statistical advice and useful discussion, J. Abe for comments and discussion, and J. Nunes for assistance in the laboratory.

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