



Cumulative effects of body size and social experience on aggressive behaviour in a subsocial bee

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Abstract

Dominance hierarchies represent some of nature's most rudimentary social structures, and aggression is key to their establishment in many animal species. Previous studies have focused on the relative influences of prior experience and physiological traits of individuals in determining social rank through aggression. Here we examine the behavioural potential for dominance hierarchy formation in the subsocial small carpenter bee, *Ceratina calcarata*. Both physiological traits and social experience were found to play partial roles in predicting future interactive behaviour in this species. Our results suggest that individual size is associated with dominance in initial encounters, while prior experience plays a larger role in predicting dominance in subsequent encounters. Social systems in the early stages of social evolution may well have followed these same predictive factors and these factors are key targets for future studies of social evolution and the behavioural origins of dominance hierarchies.

Keywords

aggression, sociality, social experience, size.

1. Introduction

Aggression is one of the most common types of behaviour between conspecifics, and is exhibited across numerous taxa (Huntingford, 1976). However, many social species rely on cooperation to function (Wilson, 1971; West et al., 2007), requiring a trade-off between aggression and cooperation. Aggression can often be used to establish dominance through the formation of hierarchies, and subsequent aggressive behaviour maintains this order (Kim & Zuk, 2000; Daws et al., 2002; Wong & Balshine, 2011; Bang

& Gadagkar, 2016). Dominance manifests as aggression in many species (Syme, 1974), with more aggressive individuals tending to achieve the higher social rank (Amsalem & Hefetz, 2010). Aggressive behaviour has been repeatedly linked to sociality (Wcislo, 1997; Cameron & Jost, 1998; Arneson & Wcislo, 2003). As the organisation of a social system becomes more complex and cooperation increases, aggression within a social unit decreases (Sumana & Gadagkar, 2001; Arneson & Wcislo, 2003; West et al., 2007). Thus, varying levels of aggression in social species help indicate evolutionary transitions in social organisation. Studying the influences of aggression on social behaviour can further explain the evolution of complex social systems.

Solitary living is the simplest life history strategy of a species, with conspecific interactions limited to mating (Michener, 1974). Accordingly, solitary life represents an evolutionary antecedent to all forms of social organisation. The simplest form of social behaviour is subsociality, in which a mother stays with and provides extended care for offspring (Michener, 1974). The most derived form of social organisation, eusociality, is defined by generational overlap, a dominant reproductive caste, and non-reproductive subordinates, which care for the offspring of the dominant reproductive (Michener, 1974). One of the more significant aspects of eusociality is the reproductive hierarchy of its caste system, which is evolutionarily preceded by dominance hierarchies (West-Eberhard, 1967; Gadagkar, 1980), and which may be enforced by aggression in more primitively eusocial groups (Platt et al., 2004). Even largely solitary species may possess the behavioural precursors necessary to form social dominance hierarchies when forced to interact (Arneson & Wcislo, 2003). This suggests that latent differences in behavioural tendencies among solitary individuals (Garamszegi & Herczeg, 2012; Jandt et al., 2013) can provide insights into the earliest stages of social group formation. Hymenopterans (bees, wasps and ants), in particular, exemplify a diversity of social structures, from solitary to a range of social interactions (Wilson, 1971), making these a useful target for study.

Individuals' physiological traits, such as size, are known to play a significant role in determining dominance (Tokarz, 1985; Rowland, 1989). This is typically gauged by consistency of contest outcomes in repeated encounters, and by predictive correlations between traits and winning, in studies ranging from anemones (Brace et al., 1979) to ponies (Rutberg & Greenberg, 1989). Both size and age are frequent considerations in studies of repeated

interaction outcomes in social insects (Hughes & Strassmann, 1988; Higashi et al., 1994; Heinze & Oberstadt, 1999), spiders (Kasumavic et al., 2009), and vertebrates alike (Kim & Zuk, 2000). Reproductive status has also proven a major contributor to dominance in social insects (Chandrashekara & Gadagkar, 1991; Arneson & Wcislo, 1993; Wcislo, 1997; Cameron & Jost, 1998; Rehan & Richards, 2013).

In agonistic interactions, the outcome of a contest can largely predict future behaviour for each individual (Sneddon et al., 1997; Earley & Dugatkin, 2006; Hsu et al., 2006; Hiadlovská et al., 2015). More dominant individuals, who win in contests, will be more likely to escalate future interactions, while more subordinate individuals, who lose these contests, will have decreased willingness to engage in future encounters. The lasting impressions of victory and defeat dictate future decisions, the basis of what is called the social cue hypothesis (Rutte et al., 2006). The behavioural outcomes that result from repeated dominant and subordinate interactions rapidly become dominance hierarchies (Hogendoorn & Velthuis, 1999; Kim & Zuk, 2000; Daws et al., 2002; van Doorn, 2008; Fewell et al., 2009; Amsalem & Hefetz, 2010; Amsalem et al., 2013). However, previous studies in hymenopterans have not examined the effects of such social experience in naturally occurring simple societies (i.e., subsocial groups). This indicates that although there are specific physiological traits affecting dominance in many species, the effect of social experience at the most basic level of sociality remains unknown.

Cumulative effects of both social experience and physiological traits have also been observed in multiple taxa (Earley & Dugatkin, 2006; Tanner et al., 2011). Physiological traits and social environment are co-predictors of dominance in many systems, with greater effects resulting from experience (Chase et al., 2002) or individual characteristics (Kasumavic et al., 2009), and often with both contributing equally (Schuett, 1997; Earley & Dugatkin, 2006; Seebacher & Wilson, 2007; Tanner et al., 2011). In social insects, this is evident in several species whose reproductive dominance is controlled by a combination of aggressive behaviour, size, and age (reviewed in Hogendoorn & Velthuis, 1999). Winning and losing effects are only recently being explored in a broader array of social life histories (Bang & Gadagkar, 2016), but not with corresponding consideration for physiological trait effects against experience. By also comparing the physiologies of individuals, we can further assess the contributing influences of physiological traits and social experience on dominance.

Direct observation of individuals within a nest is often impractical or impossible, but the use of artificial arenas offers a means to replicate nest conditions (Bell & Hawkins, 1974; Brothers & Michener, 1974; Breed et al., 1978). For hymenopterans, this type of observation can be performed via the circle tube assay, wherein two individuals are introduced into a clear plastic tube, the ends are joined, and the pair is allowed to interact (Breed et al., 1978). These one-on-one interactions also mirror those expected inside the colony, and can even allow for characterisation of many species' whole social hierarchies without any need for intranidal observations (Packer, 2006). Circle tube observation has shown that solitary individuals tend to be much more aggressive than social individuals (Richards & Packer, 2010). Similarly, eusocial workers that are very aggressive toward non-nestmates are instead cooperative with nestmates (Packer et al., 2003). The versatility, specificity, and comparability of circle tube assays allow researchers to identify behaviours that are indicative of evolutionary changes in social organisation.

Within Hymenoptera, the bees represent a broad social diversity (Kocher & Paxton, 2014). Subsocial species exhibit the simplest form of social organization with prolonged maternal care and mother-offspring interaction, while eusocial colonies are complex societies characterized by overlapping generations, cooperative brood care and reproductive division of labour (Michener, 1974). The bee subfamily Xylocopinae (Hymenoptera: Apidae) exhibits not only a wide range of social forms, but also contains a number of incipiently social species (Rehan et al., 2012), providing meaningful comparisons across the full social spectrum including contrasts with both simple and complex societies (Rehan & Toth, 2015). In particular, the small carpenter bee *Ceratina calcarata* offers important insights. Previous forced association study on the species has found that it is subsocial, but capable of nestmate recognition (Rehan & Richards, 2013), a characteristic more typical of eusocial organisms (Flores-Prado et al., 2008; Boesi & Polidori, 2011). While studies like these offer insights into certain pre-conditions for more advanced sociality, few studies have focused on behaviours in the context of social experience (Arneson & Wcislo, 2003). Likewise, observation of physiological traits, such as ovarian development, have found that reproductively active *C. calcarata* mothers are highly aggressive, pre-reproductive females are less aggressive, and daughters and post-reproductive females are largely non-aggressive (Rehan & Richards, 2013). Thus, *C. calcarata* expresses a behavioural repertoire similar to that of a primitive dominance

hierarchy (Breed et al., 1978; Wcislo, 1997), making this subsocial species well-suited for investigation into physiological traits and experience effects as pre-conditions for more complex social organisation.

Here we examined the behavioural potential for dominance hierarchy formation in a subsocial bee using circle tube assays. The aims of this study were threefold: first, to determine whether the physiological traits of size, age or reproductive development have an effect on dominance behaviour in *C. calcarata*; second, to determine whether social experience contributes to this behaviour; and third, to determine if there is a cumulative effect between social and physiological factors. If physiological traits predict dominance, individual traits should significantly predict behaviour outcomes regardless of prior experience. Conversely, if experience is predictive of dominance hierarchies, then behaviours of repeated interactions should match those of initial encounters regardless of physiological differences between paired individuals. If the effect is cumulative, both experience and physiological traits should measurably contribute to dominance after repeated interactions.

2. Methods

2.1. Nesting biology of *Ceratina*

Ceratina calcarata females create new nests each spring by excavating the pith of dead plant stems (Rehan & Richards, 2010). Solitary foundresses forage for pollen and nectar to create individual pollen balls upon which they lay their eggs. After mass provisioning and oviposition, females cap the brood cell and the process is repeated in a linear fashion, filling the nest. Females are considered to be in the ‘active brood’ stage when pollen masses, eggs or small larvae are present in the nest, indicating that clutches are incomplete (Daly, 1966; Rehan & Richards, 2010). It is during this active brood stage in early summer that females are the most aggressive towards unrelated conspecifics in order to prevent nest usurpation (Rehan & Richards, 2013), making this the ideal time period for studies of dominance behaviour. Mothers continue nest maintenance and guarding until autumn. In autumn, both male and female adult offspring eclose and remain in the nest for overwintering until dispersal and mating occurs the following spring (Rehan & Richards, 2010).

2.2. Bee collection

Active brood nests were collected prior to 8 am between 11 June and 10 July 2014 from dead, broken stems of staghorn sumac, *Rhus typhina*. Nests were collected across Strafford County, NH, USA (43°08'N, 70°55'W), and chilled at 4°C until processing. The nests were then split longitudinally to extract adult females (one per nest), which were kept on ice in microfuge tubes until initiation of the behaviour trials. All behavioural trials were conducted no more than two hours after nest processing to minimize stress-induced behavioural changes in the bees (Pabalan et al., 2000). Pairs were randomly chosen and individuals were uniquely colour coded with a Sharpie® brand paint marker on the top of the thorax in such a manner as not to affect movement (Arneson & Wcislo, 2003; Rehan & Richards, 2013).

2.3. Behaviour trials

Ceratina calcarata individuals were simultaneously placed in opposite ends of a clean, unused polyethylene tube with an internal diameter of 4 mm and a length of 30 cm, twice the average *C. calcarata* head width and 40 times the average body length, respectively. These dimensions allowed for the possibility of both mutual passage and forced blockage (Packer, 2005), while simultaneous introduction eliminated established territorial effects (Wcislo, 1997). The tube's ends were joined and trial timing ($t = 20$ min) began when either individual became active. Behavioural interactions were recorded every time the bees were within one body length of each other (Kukuk, 1992; Packer, 2005). Encounter behaviours were classified as either aggressive, avoidant, following, or tolerant (Table 1). Depending on the species, following can be considered representative of any one of the other three behavioural categories, so these interactions were classified separately as per Packer (2006). Mutually performed tolerance behaviours were recorded as single events for both individuals, whereas all other behaviours were recorded as separate events per individual (Table 1). Behaviours were recorded in terms of latency to first instance and frequency of each event. To assess pair-wise differences, behaviours were also quantified as a difference in frequency between the two bees in a pair. Bees were randomly assigned as 'Bee 1' or 'Bee 2', and frequency of each behaviour was subtracted (Bee 1 – Bee 2) so that relationships between behaviour differences could be assessed.

After the 20-min trial, individuals were removed from the circle tubes and placed back on ice in microfuge tubes. Following a 20-min recovery

Table 1.
Ethogram of *C. calcarata* circle tube behaviours.

Category	Behaviour	Description
Aggression	Biting	Mandibles of one bee grab hold of body part of another.
	C-posture	Curling body into a C-shape with mandibles and stinger pointed at other bee.
	Nudging	One bee applying force to other with its head (Arneson & Wcislo, 2003); also called ‘pushing’ (Boesi & Polidori, 2011) and ‘lunging’/‘headbutting’ (Packer et al., 2003).
Avoidance	Back	Backing away from other bee without turning.
	Reverse	Making a 180° turn and moving away from other bee.
Following	Follow	Moving toward other bee while it moves away.
Tolerance	Pass	Both bees arrange themselves to fit past one another in tube.
	Antennate	In frontal encounter, stopping and touching one another with antennae.
	Head-head touch	In frontal encounter, stopping with faces in contact with one another.

From Rehan & Richards (2013).

period, individuals were then paired with a different partner and the trial was repeated as before. Upon completion of their second trial, all bees were flash frozen in liquid nitrogen and stored at -80°C for future dissection.

2.4. *Physiological measurements*

Immediately before the behaviour trials, head width and wing wear of individuals were measured using a Nikon SMZ800 dissecting scope with mounted Unitron 15854 LED light. Head width, measured as the maximum distance across the compound eyes, is an accurate predictor of body mass in this species (Rehan & Richards, 2010). Wing wear, damage on the wing margin ranked on a scale from zero to five (unmarred to heavily damaged) serves as a proxy for age and foraging activity (Mueller & Wolf-Mueller, 1993). Metasomas of frozen bees were thawed in 70% ethanol for dissection. Reproductive development was quantified as the sum of the three largest terminal oocyte lengths in each individual (Rehan et al., 2009). The ovaries enlarge as eggs develop throughout the active brood stage, and then are re-sorbed after the breeding season, making ovary development a useful metric for reproductive developmental stage. These measurements allowed assessment of the effects of physiological traits on contest outcomes.

2.5. Statistical analyses

Calculations were made in R version 3.1.1 (R Core Team, 2014). Shapiro–Wilk tests were used to test for normal distributions. Ovarian development was normally distributed, so parametric pair-wise comparisons for this variable were made with a Student’s *t*-test, and group wise comparisons made with an ANOVA. Correlations to ovarian development were made with a Pearson product-moment correlation. Head width, wing wear and all behaviour frequency and latency data were not normally distributed, so non-parametric pair-wise comparisons for these variables were made with the Mann–Whitney *U*-test and Wilcoxon signed-rank test. Group-wise comparisons were made with a Kruskal–Wallis one-way ANOVA followed by a Tukey’s honest significance test for any significant differences found. All subsequent linear correlations were calculated using a Spearman’s rank correlation. Logistic regressions were used to calculate the effect of each physiological and behavioural metric on trial outcome.

3. Results

3.1. Behaviour trials

A total of 54 individuals were randomly paired for each of two trials (27 pairs per trial). Total frequency of interactions for combined first and second trials was 23.63 ± 18.70 encounters (mean \pm SD). Individuals’ overall interaction frequency did not vary significantly from the first trial to the second (Wilcoxon signed rank test: $V = 651.5$, $p = 0.74$). Average latency to first interaction in both trials was 2.46 ± 4.96 min, with no significant difference between first and second trials ($V = 736$, $p = 0.96$). Separately, aggression, following, and tolerance behaviour frequencies were not significantly different between trials, while frequency of avoidance behaviour was significantly lower in the second trial (4.15 ± 3.96 encounters) versus the first (5.54 ± 6.26 encounters; $V = 736$, $p = 0.03$). Latency for each of the four behavioural categories did not significantly differ between trials. Tolerance was the most frequently observed interaction in the first trial (13.3 ± 21.2 encounters; Kruskal–Wallis: $\chi_3^2 = 30.5$, $p < 0.0001$) as well as in the second trial (15.6 ± 15.7 encounters; $\chi_3^2 = 49.9$, $p < 0.001$). Latency to avoidance was shortest of all behaviour categories in both the first trial (5.22 ± 7.28 min; $\chi_3^2 = 28.1$, $p < 0.0001$) and second trial ($5.16 \pm$

7.51 min; $\chi_3^2 = 30.9$, $p < 0.0001$). There was no significant difference between trials for latency to aggression, following, or tolerance.

3.2. *Pair-wise behavioural comparisons*

There were no trials that lacked aggressive interactions. There was a significant negative linear correlation in the differences between paired individuals for aggression and avoidance frequencies ($r_s = -0.36$, $N = 54$, $p < 0.01$; Figure 1), and a significant positive linear correlation in pair-wise behaviour differences for aggression and following ($r_s = 0.57$, $N = 54$, $p < 0.0001$; Figure 1). There was a positive but non-significant trend between frequency differences in aggression and tolerance ($r_s = 0.25$, $N = 54$, $p = 0.07$; Figure 1). Based on the negative correlation between aggression and avoidance in all trials, we classified the more aggressive individual in each dyad as the ‘winner’ and the more avoidant individual as the ‘loser’ as per a simplified version of accepted dominance indices and terminology from similar studies (Bang et al., 2010; Manfredini et al., 2013). Given these binary outcome possibilities, logistic regressions were used to assess behaviours and physiological metrics as predictors of trial outcome.

By these definitions, winners in the first trial were individuals with significantly higher aggressive frequency (5.03 ± 4.76) than losers (1.55 ± 2.38 ; Mann–Whitney U -test: $W = 192$, $p < 0.001$), while losers were those with significantly higher avoidance frequency (7.83 ± 7.42) than winners (3.52 ± 4.09 ; $W = 552$, $p = 0.04$; Figure 2). Likewise, second trial winners were those with significantly higher frequency of aggressive behaviour (4.00 ± 3.02) than losers (1.11 ± 1.55 ; $W = 134.5$, $p < 0.0001$), and losers were those with significantly higher avoidance behaviour frequency (4.96 ± 3.99) than winners (3.25 ± 3.68 ; $W = 517$, $p = 0.04$; Figure 2).

Experience effect was measured by treating first trial behaviour frequency as a predictor of second trial outcome in a logistic regression. Each additional aggressive behaviour in the first trial increased the odds of winning in the second trial 1.43 times ($\log(\text{odds}) = 0.359$, $p < 0.001$), and each avoidant behaviour decreased the odds of winning 0.912 times ($\log(\text{odds}) = -0.0922$, $p < 0.05$). First trial following frequency also increased the odds of winning in the second trial by 1.35 times ($\log(\text{odds}) = 0.304$, $p < 0.01$).

3.3. *Physiological correlations*

Average female head width was 1.95 ± 0.16 mm, and average wing wear score was 2.07 ± 1.43 . Wing wear was not significantly correlated with

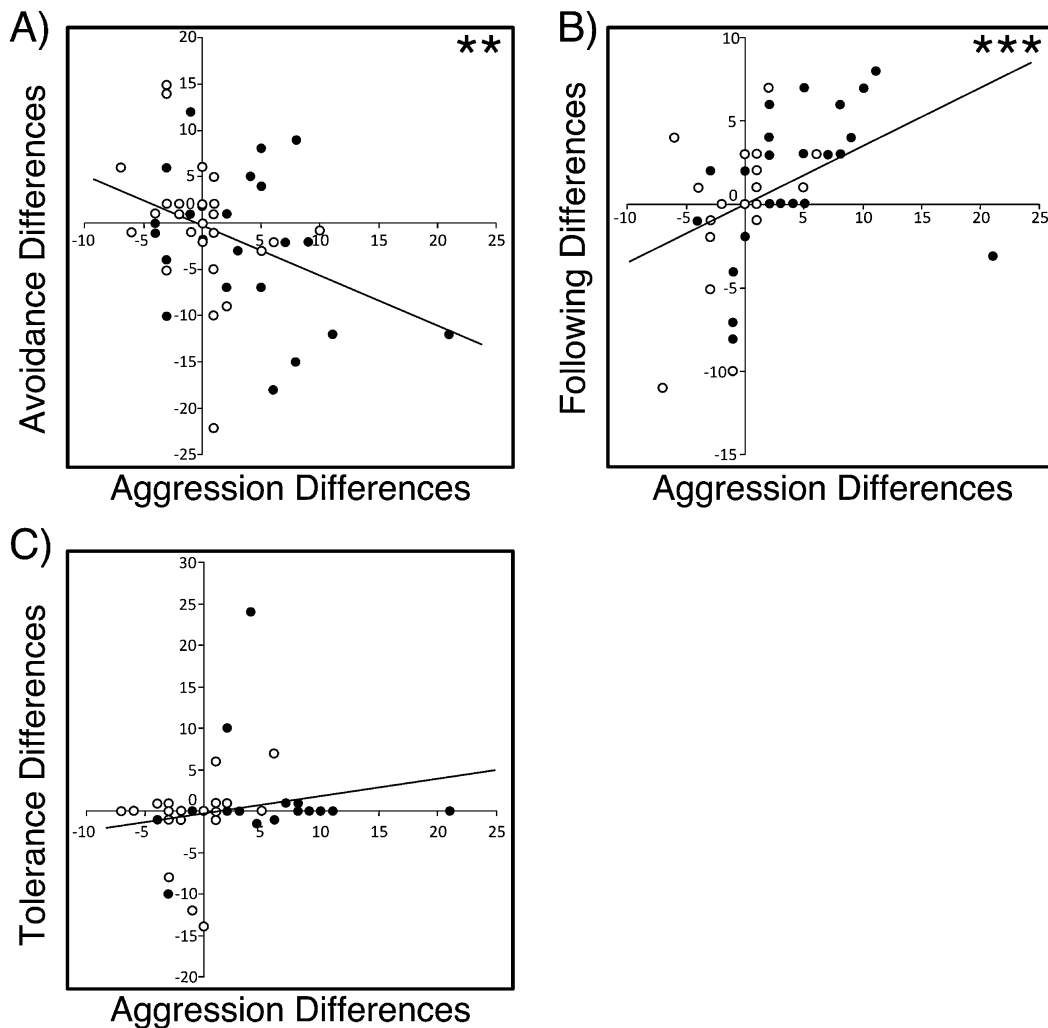


Figure 1. Pair-wise differences in individual behaviour frequency (randomly assigned, Bee 1 – Bee 2) for each trial ($N = 27$ pairs of individuals per trial). Trial 1 is represented by filled dots and Trial 2 is represented by unfilled dots. Frequency is plotted by behaviour type and correlated by (A) aggression vs. avoidance, (B) aggression vs. following, and (C) aggression vs. tolerance. Spearman's rank coefficient for significant correlations: (A) $r_s = -0.36$ and (B) $r_s = 0.57$. Two asterisks denotes significance at $p < 0.01$, and three asterisks denotes significance at $p < 0.001$. The negative correlation between aggression and avoidance serves as a proof-of-concept for 'winning' and 'losing' terminology. Note that some points may overlap.

either head width (Spearman's rank correlation: $r_s = 0.10$, $N = 46$, $p = 0.474$) or ovarian development (Pearson's product moment correlation: $r_p = 0.02$, $N = 54$, $p = 0.43$). There was a significant positive linear correlation between head width and ovarian development ($r_p = 0.61$, $N = 49$, $p < 0.0001$), so ovarian data were subsequently normalised as the ovarian

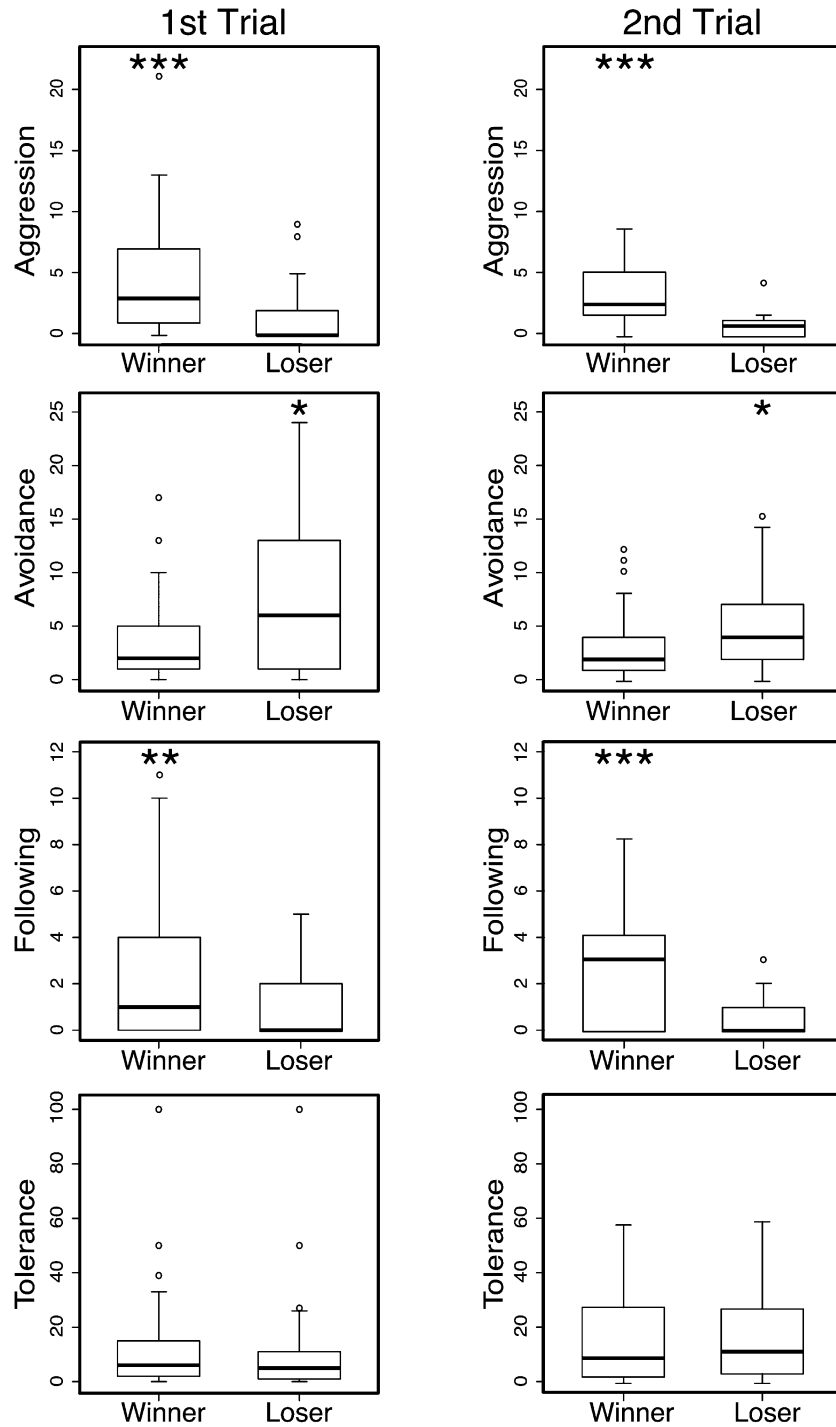


Figure 2. Behavioural comparisons demonstrating differences between designation of ‘winners’ and ‘losers’ in first and second trials for the frequency of aggression, avoidance, following, and tolerance encounters. Boxes represent medians and upper/lower quartiles, and whiskers represent maxima and minima. One asterisk denotes significance at $p < 0.05$, two asterisks $p < 0.01$, and three asterisks $p < 0.001$. $N = 54$ individuals.

development ratio: ovarian sum (mm)/head width (mm). Average ovarian development ratio was 1.35 ± 0.28 . Aggression, avoidance and following frequencies were not significantly correlated with head width, wing wear, or ovarian development ratio in either of the two trials. The frequency of tolerance behaviour also showed no significant correlation with head width or wing wear, but showed a significant positive correlation with ovarian development ratio in the second trial ($r_p = 0.35$, $N = 46$, $p = 0.02$).

Ovarian development and wing wear did not differ significantly between winning and losing females in separated first or second trials. Similarly, logistic regressions of wing wear and ovarian development ratio showed no significant predictor effect on winning in either trial. Head width was significantly greater in winning females (2.0 ± 0.14 mm) than in losing females (1.88 ± 0.16 mm) in the first trial ($W = 161$, $p < 0.01$; Figure 3), but not in the second trial. Moreover, logistic regression of head width in the first trial indicated that this was a significant predictor of winning, with each 0.01 mm increase in head width raising the odds of winning 2.5 times ($p < 0.01$).

4. Discussion

Dominance hierarchies are among the most rudimentary social structures, and aggression has been coopted as the basis of their formation (West-Eberhard, 1967; Gadagkar, 1980). Previous studies on hierarchical structure have focused on the relative influences of prior experience and physiological traits of individuals in determining social rank through aggression (reviewed in Syme, 1974; Hsu et al., 2006). Although these studies span many taxa, they deal almost exclusively with obligately social species, allowing for minimal comparison to more incipient stages of social organisation.

This study found that aggressive behaviour between reproductively active females of the subsocial bee *C. calcarata* was higher in certain individuals than in others, resulting in an aggression/avoidance dichotomy matching the social expectations for the early stages of dominance hierarchy formation (Syme, 1974). Repeated trials demonstrated partial effects of both social experience and the physiological trait of body size on future outcomes, suggesting a cumulative role for these factors in early dominance hierarchy behaviour in this species.

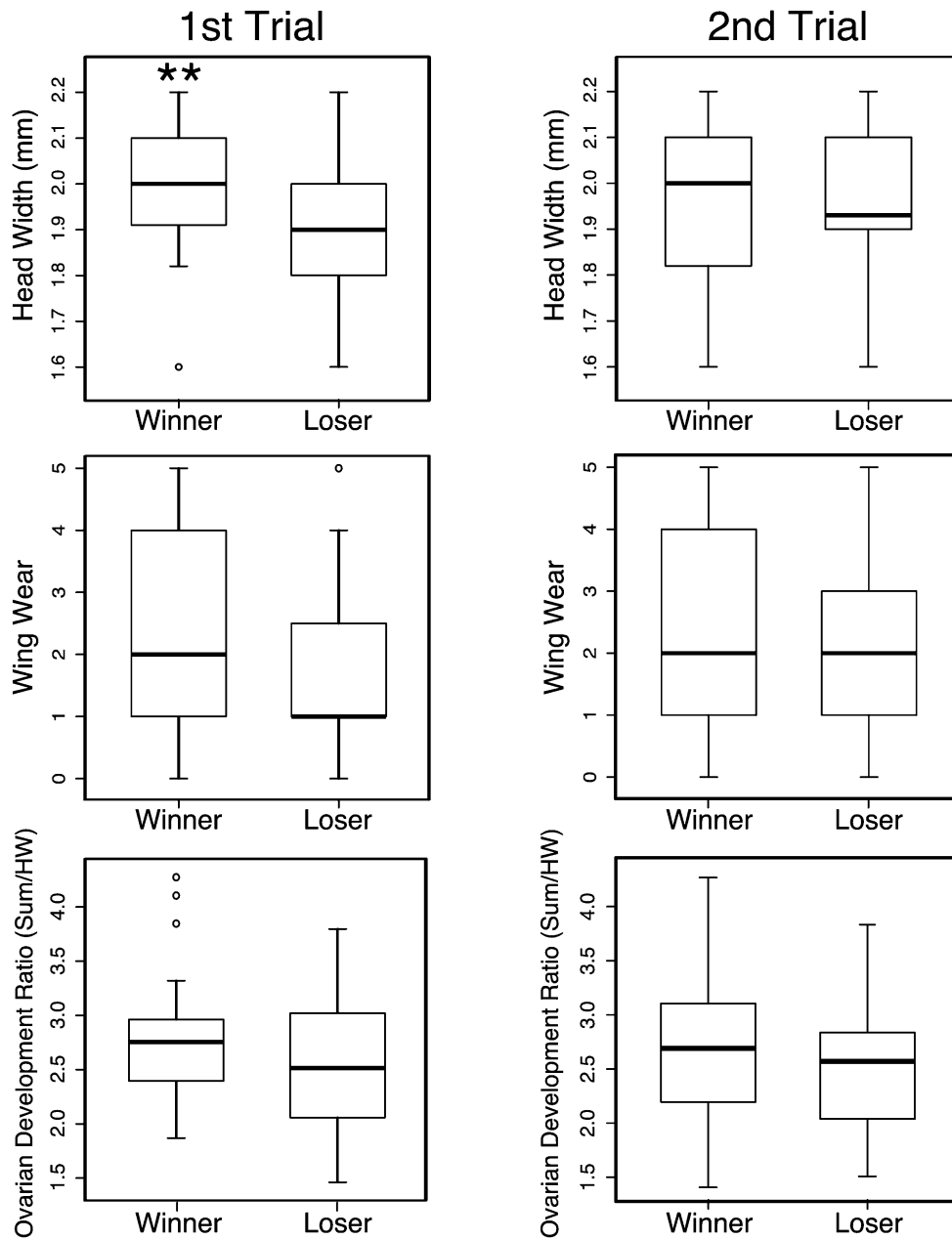


Figure 3. A physiological comparison of winners and losers in first and second trials for head width, wing wear, and ovarian development normalised for body size as the ovarian development ratio: ovarian sum (mm)/head width (mm). Boxes represent medians and upper/lower quartiles, and whiskers represent maxima and minima. Two asterisks denote significance at $p < 0.01$. Note: average head width of all individuals was significantly greater in winners, but members of each pair did not significantly differ in size for either trial. $N = 54$ individuals.

4.1. *Physiological trait effects*

Physiological characteristics of individuals are known predictors of social behaviour (Brace et al., 1978; Rutberg & Greenberg, 1989; Sneddon et al., 1997; Earley & Dugatkin, 2006). When these traits serve as the principle predictors of contest outcomes, results are due to physical differences between individuals independent of experience (Chandrashekar & Gadagkar, 1991; Arneson & Wcislo, 1993; Wcislo, 1997; Cameron & Jost, 1998; Heinze & Oberstadt, 1999; Kasumavic et al., 2009). Given the physiological metrics quantified here for *C. calcarata*, there is evidence that physiological traits are partly predictive of contest outcome.

Winning individuals had greater head width in the first trial. Larger individuals were more likely to win and thus to be dominant (the odds of winning increased 2.5 times for every 0.01 mm larger head width). Body size has been known to contribute, in varying degrees, to behaviour in many other species (Kim & Zuk, 2000; Daws et al., 2002; Kasumavic et al., 2009). Moreover, the reproductive dominants in *Ceratina* colonies made through forced association were larger females, while non-reproductive, foraging behaviour was exhibited in smaller females (Sakagami & Maeta, 1995). Although we found that body size was a very strong predictor of winning in this first trial, it was not significantly predictive in the second.

4.2. *Social experience effects*

Prior experience is another known predictor of future behaviours in many animal species (Hsu et al., 2006; Rutte et al., 2006; Seebacher & Wilson, 2007; Jeanson & Fewell, 2008; Wong & Balshine, 2011; Manfredini et al., 2013; Stevenson & Rillich, 2013), including social insects (van Doorn, 2008; Amsalem & Hefetz, 2010; Amsalem et al., 2013). The negative correlation between aggression and avoidance suggests two distinct behaviour outcomes, whereby those two behaviours are, in their extremes, mutually exclusive (Figure 1). These consistent binary behaviour differences displayed between groups are consistent with behavioural contests involving winners and losers (Whitehead, 2008). Given the winner or loser effect that results from first trial experience (Rutte et al., 2006), winning and losing as an outcome revealed patterns based on individuals with like behaviours for trial outcomes: when the behaviours of the first trial were used as predictors of second trial winning and losing, aggression, avoidance, and following behaviour frequency in the first trial all significantly predicted winning and losing in the second trial.

Based on this expectation of the social cue hypothesis, behavioural responses completely attributable to social experience should manifest as constant or even increased aggression and avoidance in repeated encounters, and tolerance should decrease accordingly (Stevenson et al., 2005; Rutte et al., 2006; Stevenson & Schildberger, 2013). Instead, avoidance in individuals that lost both trials and aggression in individuals that won both trials significantly decreased in the second trial, while all other behaviour types were unchanged between trials. Past studies involving forced association in other *Ceratina* species have resulted in induced dominance hierarchies with the necessary mutual tolerance to achieve cooperation (Sakagami & Maeta, 1984, 1989, 1995). Pre-conditions such as mutual tolerance in forced associations facilitate more complex social evolution (Michener, 1985). The observed decrease in aggression and avoidance and the uniform tolerance between the two trials in *C. calcarata* could thus suggest similar behavioural precursors requisite for more complex sociality.

4.3. *Cumulative effects*

A combined role for social experience and physiological traits is common in nature for determining dominance hierarchies (Berdoy et al., 1995; Earley & Dugatkin, 2006; Seebacher & Wilson, 2007; Tanner et al., 2011), and the most likely explanation for the results of this study. Here we found that size may predict dominance in single interactions, but that prior experience predicts dominance in repeated interactions. Furthermore, this experience effect may suggest a prominent role of memory and learning in social interactions of the species, which is known to be characteristic of more complex social life histories (Dukas & Real, 1991). The short-term results of repeated interactions seen here may foreshadow those of the prolonged exposure seen in a cohabitating social group.

4.4. *Future directions*

The social plasticity observed throughout the subfamily Xylocopinae offers an opportunity to compare dominance behaviour across the full social spectrum of species ranging from solitary to eusocial (Rehan & Toth, 2015). An interesting next step would be to study the relative effects of physiological traits and social experience on dominance behaviour in species at varying stages of social complexity. One potential candidate for this work is *C. australensis*, which is facultatively social (Rehan et al., 2010, 2011, 2014) and

thus an interesting source of comparison for understanding the earliest stages in the evolution of sociality.

This study also revealed that in *C. calcarata*, following is likely a form of aggressive behaviour. Increases in following behaviour were strongly associated with increases in aggression, and therefore with defined winners. Following as an aggressive behaviour has been previously observed in halictid bees (Brothers & Michener, 1974). Further studies characterizing following across the social spectrum from simple to complex colony organization is necessary to understand this behaviour at each level of social complexity and independent origin of sociality.

Another important consideration for future work is the role of cuticular hydrocarbons and reproductive pheromones as these are crucial chemical cues known to elicit behavioural responses associated with dominance in solitary and eusocial species (Howard, 1993). Extensive study on what factors dictate hierarchy formation can therefore provide a deeper understanding of the social environment and physiological pre-conditions required for the evolutionary transition from solitary to eusocial.

This study demonstrates that first size and then experience determine hierarchy structure in a species with very simple sociality, matching similar patterns in a variety of taxa. Size and experience may therefore be key to the formation of basic social structures, and should be strongly considered in future studies of social evolution.

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