

Intra-specific agonistic interactions between hypogeous anthophorids in Kibale National Park

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Abstract

Several aggregations of an anthophorid bee of unknown identity in Kibale National Park were observed engaging in agonistic interactions. To determine the nature of this interaction, a number of bees were trapped, marked and released, and subsequent interactions were observed. Ownership of the hole did not affect the outcome of the interaction, but being in the hole significantly improved chances of winning. The fights won by the dragging bee were significantly shorter than those won by the bee occupying the hole, while duration was also positively correlated with time of day. These interactions may be the result of nest parasitism or poor hole recognition.

INTRODUCTION

Solitary bees (order Hymenoptera) make up the majority of bee species (Stone, 1995). In typical hypogeous (ground nesting) bees, the female digs the nest and provisions the offspring, with no parental care by the male (Stone, 1995; Paxton, 2005). Females provide brood cells with a mixture of pollen and nectar, which the larvae feed on during development (Muller *et al.*, 2006). The distribution of receptive females defines the mating system, either resource based or non-resource based (Paxton, 2005). In either case territorial behaviour can exist (Paxton, 2005). If resources are moderately concentrated and relatively patchy, an individual can increase its fitness by defending an area where resources are found (Price, 1984). Territorial behaviour should be selected for whenever the benefits of keeping a territory are greater than the costs of doing so (Krebs & Davies, 1993). A territorial contest can be illustrated in this respect with the following equation: " $V_A / K_A < V_B / K_B$ ", where V is the value of the resource and K the rate at which contestants accumulate costs during the contest, A and B being the contestants (Krebs & Davies, 1993). In the example above, A is expected to give up the contest when it reaches the stage illustrated by the equation.

On the dirt roads entering Makerere University Biological Field Station (MUBFS), Kibale National Park, there are several aggregations of ground nesting bees. Aspects of their ecology are described in this paper's sister paper, by Macleod & Pluch. It was observed that pairs of bees were involved in what seemed to be agonistic interactions at the mouth of the holes in the ground, which could last for several minutes. The nature of this interaction was unclear. We were informed that little work

had been carried out on such ground nesting bees, and as such this interaction could represent new territory for scientific research. Of particular interest were the characteristics of the interaction:

- Is it an intra or intersexual interaction?
- Is it a result of some kind of territorial behaviour?
- What affects the outcome of the interaction?
- What affects the duration of the interaction?
- What is in the holes, so what resource are the bees competing for?

METHOD

Study site

All work was carried out between 8/7/2011 and 13/7/2011, at MUBFS, Kibale National Park, Uganda.

Identification

An identification key was followed from *Hymenoptera of the world: An identification guide to families*, to determine the family of our study organism (Goulet & Huber, 1993).

Sexing

Ten bees were fixed in ethyl acetate, pinned and dissected to determine their sex; three of these bees were engaged in interactions when caught, four were caught flying and three were caught when exiting a hole. All interacting bees were sexed when possible. Sex was determined by examining the rear of the abdomen, if a sting was present the bee was assumed to be female. Since males lack pollen-collecting structures (Goulet & Huber, 1993), the presence of pollen in the scopae of an interacting bee indicated that the individual was a female.

Marking

Two sites were selected for complete trapping. These were sites C1 and C2. These were chosen as they were the smallest (27 and 17 holes respectively), so it was more feasible to attempt to trap these sites in their entirety than either of the larger sites.

Cylindrical traps were handmade from mosquito net and a roll of transparent OHP sheeting, approximately 10 mm in diameter and 50 mm tall. These were fixed over the holes early in the morning (the last at approximately 09:30) on 10/7/2011. Preliminary investigations revealed that no

bees fly from their holes before this time (MacLeod & Pluch, unpublished data), so it was assumed that any bee residing in a hole would be prevented from leaving by the trap.

Once a bee attempted to leave its hole, it was caught and transferred to a collection tube, and placed in a refrigerator. The site ID, hole number, and time caught were recorded. This bee was termed the hole's owner.

From 11:30 the bees were removed from the fridge, in the order they were caught. Markings were applied using enamel paint on the thorax and abdomen. Paints and inks were among the first material used to mark insects, and due to their low cost and ease of re-sampling are still commonly used (Hagler & Jackson, 2001). The markings on the thorax indicated the site: green for C1 and orange for C2. Trial studies had marked bees from site A with red and bees from site B with yellow. For individual identification, combinations of one, two or three colours (red, yellow and green) were placed on the abdomen. The bees were placed in a collection tube with a net lid to dry, and were then returned to the fridge.

All bees were released at 14:00 the same day, next to the hole they were caught from. The bees were allowed to warm up before release, to minimise predation due to sluggish behaviour.

Interactions

Interactions were observed from 8/7/2011 to 12/7/2011. All interactions at sites A and B were between unmarked bees, while from 10/7/2011 to 12/7/2011 interactions at sites C1 & C2 had the possibility of involving marked bees. The site was scanned, and when an interaction was detected it was observed until it ended. The end of an interaction was determined to be when the bee dragging the other one out of the hole (the "dragger") gave up and flew off, or it successfully removed the bee in the hole (the "hole bee") and replaced it in the hole. The site ID, hole number, duration, end time, presence and absence of pollen for each individual, markings (if present) and outcome was recorded for each interaction.

Mapping

Four sites were found, sites A and B being on the roads entering the field station, while sites C1 and C2 were on the road connecting the field station and the lower camp. The GPS reading of the sites was recorded. For each site all the bee holes present were identified, and each hole was marked with a flat-top pin with the hole number written in permanent pen on the pinhead. The flat top pins were resistant to weather and vehicle damage, and avoided disrupting the immediate environment around

the hole. The boundaries of each site were identified, and scale maps hand drawn of each. Sites A, B and C2 were drawn at a 1:25 scale, while site C1 was drawn at a 1:10 scale. The direction of north was also recorded on each map.

Site A was mapped on 9/7/2011, site B on 8/7/2011 and sites C1 and C2 on 10/7/2011. If any new holes were subsequently detected they were marked, numbered and added to the map.

Area (m^2) of each site was measured by constructing a rectangle, with the outermost holes defining the boundaries. The density of the site was found by dividing the total number of holes by the area to give the number of holes per m^2 . The density experienced by an individual hole was determined by counting the number of holes within 0.56 m, giving the number of holes within a circle with an area of 1 m^2 .

Hole function

To investigate hole function, molten candle wax was poured down hole 15 in site A and hole 6 in site B. This was performed between 07:30 and 08:30, 12/7/2011. These were chosen as they were on the periphery of a main aggregation. This allowed minimum disruption while excavating.

Excavation of hole 15 at site A and hole 6 at site B was begun between 17:30 and 18.30, 12/7/2011. Due to the length of time required to excavate the hole, only these two could be completed. Water was used to soften the soil prior to digging. Excavation of both holes was completed by 10:30, 13/7/2011. Pieces of the wax model were cleaned in water, dried and attached together in the correct order using pins and additional hot wax.

Statistical analysis

Analysis of results was performed in the statistics programme MINITAB. Where possible parametric tests were carried out, otherwise binary and nonparametric tests were used.

RESULTS

Identification

The pinned specimens of the study organism were identified as belonging to the family Anthophoridae. It was assumed that all other studied individuals were of the same species as the pinned specimens.

Sexing

All the bees that were sexed were found to be female. No morphological differences were observed between bees interacting or involved in any other behaviour in and around the hole. This suggests all bees observed in interactions and around the holes were female.

Interactions

In total, 111 interactions were observed. The “hole bee” won 88.3% percent of the interactions, which is significantly more than that predicted by a random distribution (50%) (Binomial test; $N = 111$; Sample $P = 0.126$; $p < 0.001$).

Only four out of 40 interactions (10%) in site C1 where both participant bees were identified did not involve the hole owner. This is significantly different from the expected percentage of interactions involving any particular bee in a random distribution (7.4%) (Binomial test: $N = 40$; Sample $P = 0.900$; $p < 0.001$).

When comparing the influence of being the “hole bee” or the “dragger”, and whether the bee was the owner of the hole or not, it was found that ownership of the hole had no influence over the outcome of the fight, but being the “hole bee” or the “dragger” did have a significant effect (Binary logistic regression: $G = 81.971$, $df = 2$, $p < 0.001$; Ownership of hole: $Z = 0.49$, $p = 0.624$; “Hole bee” or “dragger”: $Z = -5.23$, $p < 0.001$).

It was found that there was no significant relationship between duration of the interaction and the outcome (Figure 1) (Binary logistic regression; $N=111$; $Z = 1.22$; $p = 0.221$). However, all but one of the interactions in which the “dragger” won were relatively short (< 270 seconds) whilst there was one extreme outlier (2,670 seconds). A non-parametric test was used, to take account of the single extreme value. This showed that interactions are significantly shorter when the “dragger” wins (Mann-Whitney U-test: $W = 437.5$, $p = 0.0021$).

In 23 out of 36 interactions (63.9 %) where the hole owner was involved, the hole owner acted as the “dragger”. This did not prove to be different from a random distribution (50%) (Binomial test: $N = 36$; sample $P = 0.639$; $p = 0.132$).

There was no significant difference between the duration of fights where the owner of the hole was the “dragger”, and when the owner was not involved in the fight (so was neither the “hole bee” nor the “dragger”) (Mann-Whitney U-test, $W = 349.0$, $p = 0.9738$).

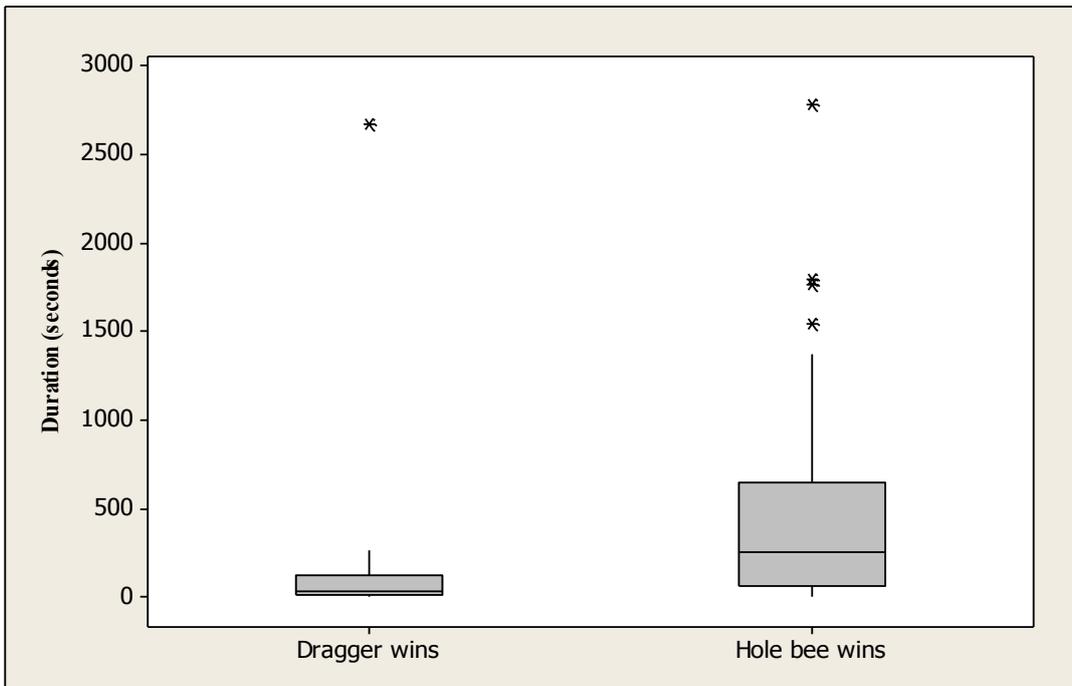


Figure 1. Outcome of interaction against duration of interaction

Duration was significantly positively correlated with time of day (Figure 2, regression analysis; $T = 2.71$; $p = 0.008$).

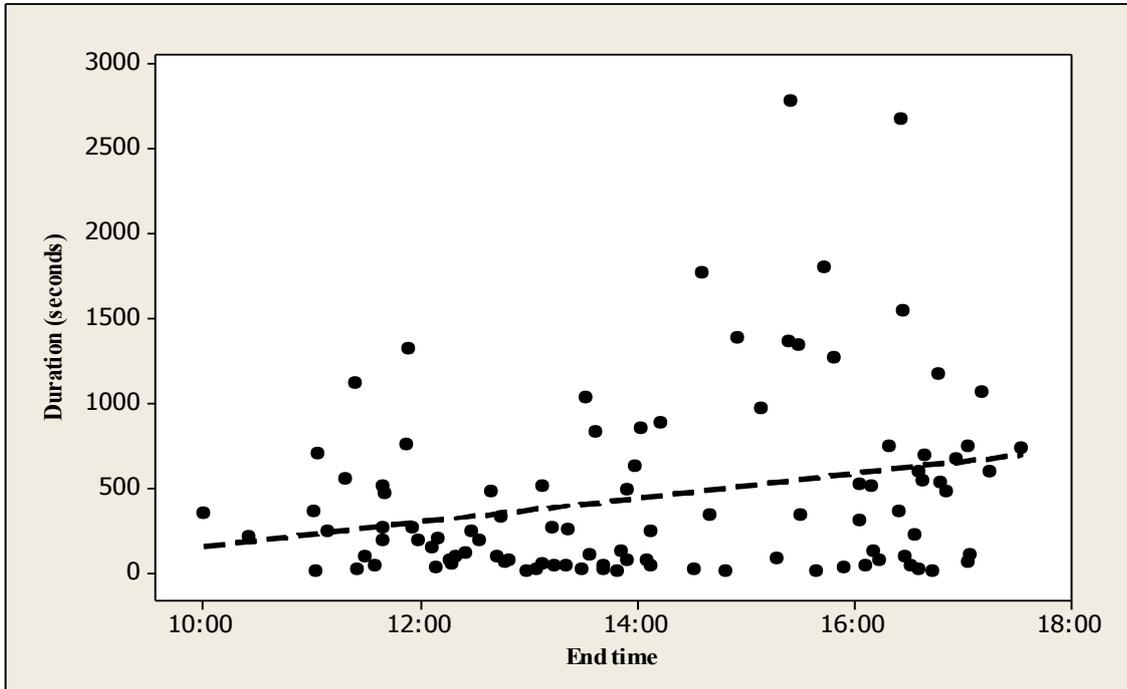


Figure 2. Duration of interaction (seconds) against end time (24 hour clock). Dotted line is regression fit ($y = -563 + 1718x$)

Effect of density on interactions

Local density of holes that hosted interactions was significantly higher than the average density of the site where the interaction occurred (chi-squared goodness-of-fit test: $\chi^2 = 898.390$; $df = 39$; $p < 0.001$).

Mapping

Table 1. Results from mapping and marking of study sites

Site	Area (m ²)	Holes	Density (Holes/m ²)	Co-ordinates
A	15.0	137	9.1	N: 00°33'45.3" E: 030°21'21.1"
B	10.7	45	4.2	N: 00°33'40.1" E: 030°21'22.1"
C1	9.0	27	3.0	N: 00°33'45.0" E: 030°21'21.0"
C2	11.0	17	1.6	N: 00°33'47.7" E: 030°21'21.5"

Trapping

Out of 94 traps, 26 caught zero bees, 65 caught one bee and only three caught two bees.

DISCUSSION

Members of the Anthophoridae are frequently observed to nest in open, firm ground, like that of the study site (Skaife, 1979; Roubik, 1989). Further identification was not possible, but several specimens have been kept and will be sent to the appropriate specialists in an attempt to further identify this species.

All interactions appeared to be between females. Either no morphological differences that are detectable with the naked eye exist between the sexes, or no males were observed interacting. Intrasexual competition between females in the absence of males is likely to be due to resource competition. It is common among insects that females defend a territory where food for the progeny is stored (Price, 1984). This suggests the hole is, or contains, a valuable resource.

The “hole bees” were much more successful in interactions than “draggers”. This suggests that being in the hole gives a great advantage when competing for possession of the hole. This could influence other aspects of behaviour, such as foraging time. Length of foraging trips can be reduced when risk of nest parasitism is higher (Neff, 2008).

Ninety percent of interactions between bees, whose original hole was known, involved the original owner of the hole. This result suggests that the hole acts as a territory which the bees are aware of and defend, otherwise there would not be so many interactions involving the owner. However, the duration of fights when the owner was the “dragger” was not different from the duration of fights when the owner was not involved. This suggests a bee will not fight harder for a nest that it was assumed it owned. Either the assumption of ownership was incorrect, or it is not cost efficient to engage in a prolonged contest over a hole when there are other holes that could be usurped or a new hole could be dug. It was also observed that interactions were shorter when the “dragger” won. In 50% of the cases when the identity of the winning “dragger” was known, these draggers were the actual owner of the hole in which the interaction took place. This may suggest that the value of the hole for the “hole bee” (known not to be the owner of the hole) was not high enough to invest energy in a long interaction. However, due to the low sample size (only four interactions were observed when the winner was a “dragger” of known identity) further analysis of this pattern is not possible at the moment and more research is required.

The function of the hole does not appear to be for rearing larvae in, as no cells or chambers were discovered on excavation of two holes in different sites. Instead, the holes were a series of downwards tunnels, with a thicker lateral portion at approx. 5 cm intervals. Both holes finished in a small pollen store. Although pollen is typically used by bees to provision offspring, it can also be used to produce toxins that are involved in repelling ants from nests (Roubik, 1989). This occurs in variety of social and solitary bees (Roubik, 1989). Ants were observed investigating the entrance to bee holes, but not typically entering, so these bees may be employing a chemical defence. A bee that guards using chemicals will need to forage for pollen, or have a supply in the nest which may explain the pollen store in a nest without any offspring (Roubik, 1989). Investigations at different times of year should reveal if and/or when the tunnel is used for rearing a brood. Our results in this aspect must be taken carefully, because, due to time limitations, we were not able to examine more than two holes.

Duration of interaction was positively correlated with time of day. This suggests that the interacting bees valued the hole more highly near the end of the day, as they were more willing to invest energy in interactions. A possible explanation for this is that possession of a hole increases survival chances during night-time. Hole bees have a large advantage, hence our results fit the asymmetric war of attrition, which states that individuals invest more energy in the fight when the value of the resource is larger (Krebs & Davies, 1993).

Distribution of hole owners in an interaction as either the “hole bee” or as the “dragger” was not different from a 50/50 distribution. This suggests that the interaction is not consistently a result of bees attempting to parasitise unguarded nests, as otherwise the hole owner would be the “dragger” significantly more often. Instead, it may be that when the bees return from foraging trips they may mistake one hole for another. If the study species typically nests in aggregations at lower densities than those observed at our study site, then the nest recognition system may not be particularly precise. This could lead to confusion over hole ownership, resulting in the interaction that was observed. Observational evidence suggests the bees investigate several holes before settling in one (MacLeod & Pluch, unpublished data). However, when presented alone this does not necessarily suggest confusion is occurring.

For each site, holes hosting interactions experienced a higher local density than the average for that site. This is not surprising, seeing that the likelihood of confusing the hole entrance with another is naturally increased at higher densities. Additionally, the possibility of having a nest parasitised by a conspecific is increased the more densely populated an aggregation is.

To experimentally differentiate between the causes, a test where nest recognition is examined should be carried out. A simple method would be to add landmarks near the nest, and allow the bees to habituate to them. If moving the landmarks to other holes affected the rate of interactions, it is likely that inaccurate nest recognition has an influence.

Presumably, an alternative strategy of nest parasitism would be frequency dependant, as it would be easier to steal a hole when many others are building them, but harder if more individuals are attempting to parasitise rather than construct their own hole (Krebs & Davies, 1993). The nature of this relationship could be explored by identifying individual strategies, and monitoring reproductive success depending on strategy choice in the rest of the population, or experimentally altering the availability of holes. A similar study has been carried out on another hymenopteran, the digger wasp (*Sphex ichneumoneus*) by Brockman *et al.* (1979). As the nature of the interaction in our study system appears to be comparable, an equivalent assessment of strategy choice may be appropriate.

The lack of males observed is puzzling. There are a few possible explanations for this. First, if the females mate soon after they have emerged, then males will only be present when the females are young (Skaife, 1979; Paxton, 2005). If the hatching of females is seasonal, then our study may simply have taken place outside the mating season.

Secondly, there may be a “male-killing” bacterium vastly altering the sex ratio. In a wide range of parasitic wasps, *Wolbachia* can induce parthenogenesis, which would make males obsolete (H. Roy, pers. comms.). Prevalence of male killing in populations can reach 95%, which would mean the observation of a male in our short study period would have been unlikely (H. Roy, pers. comms.). In one experiment, removal of male odonates caused the females to start exhibiting territorial behaviour (Jacobs, 1955). While this is typically rare in odonates, a similar system could be occurring in this bee species, where rarity of the opposite sex causes territorial behaviour in females (Suhonen *et al.*, 2008). Male-killing has been reported in the Hymenoptera, but whether it appears in this species requires further investigation (Majerus, 2003).

Thirdly, the distribution of males may not have crossed into our study site. For oligolectic species, where the distribution of females is predictable, males will wait on the flowers for mating opportunities, while in polylectic species the females are more widely distributed, so the males use flyways or landmarks to find females (Paxton, 2005). If the nest density is high, and female emergence is synchronised, males will search nest sites for mating opportunities, but as the nest density often varies across a species’ range describing the density as aggregated or dispersed may not be appropriate (Paxton, 2005). As we do not know of the range of this species, we are unable to make any assumptions on typical nest density. Although the pollen carried by the females has been identified to family level and matched to a particular plant, no instances of the bees from the study site visiting any flowers was observed (MacLeod & Pluch, unpublished data). The pollen did appear to be of consistent structure and colour, suggesting a single plant species was being visited. Once identification of the location of the flowers that the females are visiting has been achieved, males may be located.

The number of bees caught from each trap indicates that each hole only hosts a single bee. This suggests this species is solitary, but choice of site for hole construction may be communal, due to patchiness of suitable habitats (Skaife, 1979). Other explanations for aggregations of a single sex include: primitive sociality; increased temperature (and associated benefits) due to clustering, and improved vigilance for anti-predator defence (Starr & Velez, 2009).

The traps that were used were effective for catching bees that had emerged from the hole. However, it cannot be ruled out that the traps may have interfered with natural behaviour, perhaps causing the bees to remain in the hole. Subsequently, we would not have captured them, and this would affect the estimate of number of bees per hole and any population estimates from the trapping. Additionally, if the bees were communally nesting to protect from predators or nest parasitism, only

one bee may leave the hole at any one time. As such our method would still only detect one bee per hole, as the trapped bee was removed and not returned until it was marked.

CONCLUSION

Our study still leaves us with many questions, but helps us understand a little better the nature of the agonistic interactions observed between ground nesting anthophorids in Kibale National Park. Inter-sexual interactions can be discarded, seeing that all sexed individuals were found to be females. Instead, the interactions seem to be the result of competition between females for the possession of a hole. This may be due to either attempts to parasitise a hole, or deficient nest recognition.

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