Natural History Note

Bees at War: Interspecific Battles and Nest Usurpation in Stingless Bees

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abstract: We provide the first evidence for interspecific warfare in bees, a spectacular natural phenomenon that involves a series of aerial battles and leads to thousands of fatalities from both attacking and defending colonies. Molecular analysis of fights at a hive of the Australian stingless bee *Tetragonula carbonaria* revealed that the attack was launched by a related species, *Tetragonula hockingsi*, which has only recently extended its habitat into southeastern Queensland. Following a succession of attacks by the same *T. hockingsi* colony over a 4-month period, the defending *T. carbonaria* colony was defeated and the hive usurped, with the invading colony installing a new queen. We complemented our direct observations with a 5-year study of more than 260 *Tetragonula* hives and found interspecific hive changes, which were likely to be usurpation events, occurring in 46 hives over this period. We discuss how fighting swarms and hive usurpation fit with theoretical predictions on the evolution of fatal fighting and highlight the many unexplained features of these battles that warrant further study.

Keywords: Tetragonula, Trigona, eusocial, fighting, microsatellite.

Introduction

Fights to the death are rare occurrences in nature, and evolutionary theory proposes that this is because alternative strategies that assess strength and fighting ability (such as displays and assessment) have evolved to avoid this costly behavior (Maynard-Smith and Price 1973; Enquist and Leimar 1990). In species where fighting can escalate to a lethal situation, theory predicts that the risk of death must be outweighed by the benefits of obtaining the resource that is being contested (e.g., food, mates, or nest-

ing sites; Murray 1987; Enquist and Leimar 1990; Shorter and Rueppell 2012).

Fatal fighting is well documented in ants, where intra- and interspecific battles between neighboring colonies can lead to enormous numbers of fatalities from both sides (Batchelor and Briffa 2011) and involve a wide rage of behaviors and outcomes, such as slave making (Hölldobler and Wilson 1990; Foitzik and Herbers 2001; Pohl and Foitzik 2011), raiding of nest provisions (Hölldobler and Lumsden 1980; Zee and Holway 2006; Hölldobler et al. 2011), or fights on territorial boundaries or foraging trails (Hölldobler and Lumsden 1980; Hölldobler 1981; Adams 1994; Whitehouse and Jaffe 1996; van Wilgenburg et al. 2005). In these eusocial insects, the fighting individuals are usually sterile workers, and their self-sacrificing behavior can be explained in evolutionary terms through the indirect fitness benefits of protecting the colony's reproducing adults (Hamilton 1964; Enquist and Leimar 1990; Boomsma and Franks 2006; Shorter and Rueppell 2012). Large-scale battles are most likely when the future of the nest is at stake, since all members of the colony stand to lose their genetic contribution to the next generation if the nest is lost (Scharf et al. 2011).

Although there is less evidence for collective fighting in eusocial bees, intra- and interspecific battles are known to occur in the vicinity of foraging sites, where colonies compete for the possession of food resources (Johnson and Hubbell 1974; Nagamitsu and Inoue 1997; Nieh et al. 2005). Since access to foraging sites can influence survival and reproduction within the nest, especially if food is limiting (Roubik 1982), the inclusive fitness benefits to each worker of securing (or losing) one of these sites provide an explanation for why large numbers of fatalities can occur during these territorial battles (Johnson and Hubbell 1974).

When it comes to attacks on the nests, however, there are no examples in the bee literature that resemble the devastating collective attacks of warring ants. Africanized honeybees can invade and usurp the hives of European honeybees (Schneider et al. 2004), but attacking swarms here are relatively small (possibly reproductive or absconding swarms), and fighting is not extensive (Schneider et al. 2004). In the primitive stingless "robber" bee *Lestrimelitta limao*, fights between workers can occur as a result of a nest-raiding strategy, but adult mortality is slight (Sakagami et al. 1993). Scouts of European honeybees are known to fight with workers from competing colonies while locating nest sites, and this can lead to mobbing and killing of those in the minority (Rangel et al. 2010). Fights to the death between queens of primitively eusocial bee and wasp species occur when an usurping queen invades another's nest (Zobel and Paxton 2007), and queen fighting also occurs in bumblebees in the subgenus *Psithyrus*, which are obligate parasites on *Bombus* bumblebee species (Kreuter et al. 2012).

Only one species of bee is currently known to engage in intercolony battles that involve mass fatalities, and that is the Australian stingless bee *Tetragonula carbonaria*. These battles are a spectacular phenomenon, with swarms from attacking and defending hives colliding midair and fighting bees falling to the ground locked in a death grip from which neither combatant survives (Heard 1996). Previous studies have demonstrated that swarming can be initiated by placing *T. carbonaria* workers from one colony at the hive entrance of another (Gloag et al. 2008). These observations have led to the assumption that fighting swarms are intraspecific battles between neighboring *T. carbonaria* colonies, the ecological or evolutionary explanation for these battles remaining elusive.

One likely explanation for these fights is that they are attempts at hive usurpation (Wagner and Dollin 1982), and in this study we aimed to test this hypothesis by identifying a focal *T. carbonaria* hive that was engaging in fights, using behavioral and molecular analysis to determine whether usurpation was occurring. What we found was surprising: the resident colony was being attacked not only by its own species but also by a related species, *Tetragonula hockingsi*. The hive engaged in two subsequent fights, and then after 5 months in which no fighting occurred, we opened the hive and examined the nest architecture (which differs between the two species) and analyzed brood genetics. To verify that this was not an incidental event, we monitored a population of more than 260 commercial (hobby) *T. carbonaria* hives over a 5-year period, recording the prevalence of *T. hockingsi* usurpations through changes in nest architecture.

Methods

Analysis of Sequential Fights at the Focal Hive

The stingless bees, *Tetragonula carbonaria* and *Tetragonula hockingsi* (Apidae: Meliponini), are native to tropical and subtropical Australia (Dollin et al. 1997). Colonies comprise several thousand individuals and a single queen (Wille 1983; Heard 1999; Gloag et al. 2008), with nests commonly found throughout coastal Queensland and northern New South Wales, in logs and tree cavities, in man-made cavities in urban areas, and by amateur beekeepers in more than 600 commercial hives (Halcroft et al. 2013). Small fighting swarms (skirmishes) are commonly observed by beekeepers and occasionally escalate into far more conspicuous battles in which hundreds of dead bees are found scattered near the hive entrance (video 1; fig. 1*a*). Our study followed the fighting activity of a colony of *T. carbonaria* housed in a man-made nest box at a residential property in Brisbane, Australia (27°29′50.11″S, 152°58′48.97″E). We had already observed the hive engaging in small fights (skirmishes, <50 fighting pairs per day) in May and July of 2008, having collected pairs of fighting bees from the July fight. In August 2008 and October 2008, we observed escalated fights (>50 fighting pairs per day). We tracked the progression of the fight each day and at dusk (when fighting ceased) collected all dead bees from the surrounding ground, which we then swept clean. In order to identify dead bees to colony, we carried out a molecular analysis of bees from the July skirmish (fighting pairs only) and over the duration of the two major fights (August 2–17 and October 13–19), collecting the following bees for analysis.

Fighting pairs. Bees locked together in combat within 3 m of the hive entrance (fig. 1*a*, 1*c*).

Video 1: Still photograph from a video (video 1, available online) showing *Tetragonula* fighting swarms and usurpation.

Figure 1: Behaviors observed during fights: *a*, fighting bees on the ground near the hive; *b*, clusters of drones on the nearby foliage; *c*, fighting pair used for molecular identification; *d*, attacking worker dragging a callow (young adult; *left*) from the hive.

Hive entrance bees.   Bees congregating on the surface of the hive (video 1).

Ejected callows.   This behavior has not been previously reported in stingless bee fighting and relates directly to usurpation. We observed workers dragging callow bees (immature adults with light-brown bodies) from the hive (video 1; fig. 1*d*), releasing these bees a meter or so from the hive entrance. In four instances, we captured both the callow bee and the attacking worker.

Drone clusters.   During fights, clusters of bees were seen congregating on surrounding vegetation at the end of each day (fig. 1*b*). These bees were sexed (by dissection and identification of genitalia) and identified as drones.

Brood larvae.   The collection of brood required splitting of the hive, which severely damages the comb. For this reason, the hive was split only once, 5 months after the October fight, during which time no swarming or fighting was observed.

Species identification.   The two species of bee are difficult to tell apart visually (Dollin et al. 1997), and thus we used molecular methods to identify bee species, as reported by Franck et al. (2004). We collected eight individuals from each of six man-made hives (three *T. carbonaria* and three *T. hockingsi*) that had previously been identified to species based on the architecture of the brood comb. *Tetragonula carbonaria* has a spiralling brood chamber, in which the cells are compact and connected by their walls to adjacent cells at the same height, whereas *T. hockingsi* brood takes on a less organized appearance, being an open lattice composed of clumps of around 10 cells connected by vertical pillars (Brito et al. 2012). As indicated by Franck et al. (2004), the two species were also readily separated by analysis of the microsatellite gene frequencies (fig. 2*a*).

We extracted DNA from 572 bees using DNEasy columns (Qiagen), removing each member of the fighting pair from its opponent and avoiding contaminated body parts (e.g., legs held in the mandibles of the opponent). We genotyped individuals using seven previously published microsatellite loci (Green et al. 2001). Individuals that failed to amplify across more than five of the seven loci were considered to be low-quality extractions and were excluded, leaving 523 bees with individual genotypes scored. The seven microsatellites we used were highly variable (mean number of alleles per locus = 9.3; table A1, available online). This complete genotype data set has been deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.2ng61 (Cunningham et al. 2014). We identified each bee as belonging to one of the two species using the Markov chain Monte Carlo clustering algorithm, implemented in the program STRUCTURE (Pritchard et al. 2000) as detailed in the appendix, available online.

Species Divergence Estimates

Franck et al. (2004) reported that *T. hockingsi* might be two species, one closely related to *T. carbonaria* (the sister species) and a more distant member of the carbonaria group. These authors proposed that this group diverged within Australia a minimum of several thousand years ago but were unable to assess divergence times accurately as their mitochondrial marker (cytochrome b) was heavily contaminated by pseudogenes. In order to clarify the divergence of the three *T. hockingsi* colonies in this study, we sequenced the bar-coding region of the cytochrome oxidase 1 gene (COI; appendix). We have deposited the final sequences in GenBank (accession numbers for *T. hockingsi*: KM112224–KM112237; for *T. carbonaria*: KM112238–KM112246).

Longitudinal Study

Over a 5-year period (2008–2012), we examined the brood comb of approximately 260 stingless bee hives across southeast Queensland in order to identify the resident stingless bee species. All colonies had been originally established from wild *T. carbonaria* and transferred into man-made wooden hives between 1985 and 2007 (i.e., from 1 to 22 years before the beginning of our study). Man-made hives are not fundamentally different from natural hollow logs as nest sites (in fact, the hive design attempts to mimic natural locations in terms of construction material [wood], nest volume [approximately 8 L], and entrance size [13 mm]), and colonies are not kept at densities any higher than natural populations, which are around 10 nests/ha in suburban areas.

We used nest architecture to identify the resident *Tetragonula* species, assessing hives annually when they were opened for honey extraction and hive propagation (Heard and Dollin 2000). Each year, we inspected all hives within the study population (between 253 and 274 colonies; a relatively small number of colonies died, were removed, or were added to our study) from 2008 to 2012 and recorded whether the resident species had changed from the previous year. We compared the proportion of hives within the study population that changed from *T. carbonaria* to *T. hockingsi* with changes from *T. hockingsi* to *T. carbonaria* using a χ^2 test.

Results

July Fight

Fighting pairs in this skirmish (<50 fighting pairs/day, <3-day duration) predominantly involved the resident *Tetragonula carbonaria* and a single *Tetragonula hockingsi* colony (fig. 2*c*). There were a number of *T. carbonaria/T. carbon-*

a. Tests to resolve species (six known hives)

T. carbonaria   *T. hockingsi*

C1   C2   C3   H1   H2   H3

b. Drone Clusters

1   2   3   4   5
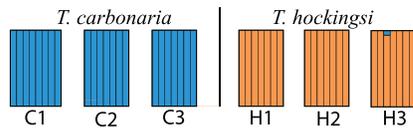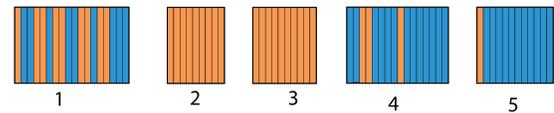
c. July fight
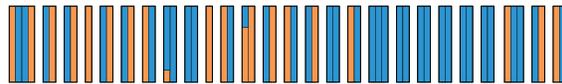
Fighting pairs

carbonaria/carbonaria = 9
carbonaria/hockingsi = 15
hockingsi/hockingsi = 1

d. August fight
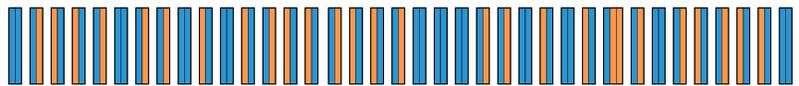
Fighting pairs

carbonaria/carbonaria = 11
carbonaria/hockingsi = 26
hockingsi/hockingsi = 0

Hive Entrance   Callow   D C D C
                                     Callow and
                                     Dragging bee
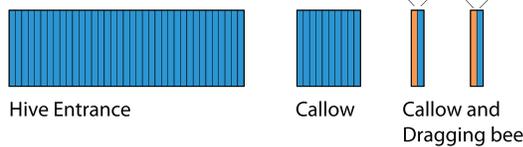
e. October fight
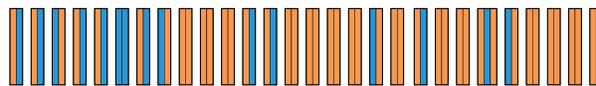
Fighting pairs

carbonaria/carbonaria = 1
carbonaria/hockingsi = 14
hockingsi/hockingsi = 13

Swarm in front of hive

Hive Entrance   Callow

D C D C

Callow and   Queens dragged out
Dragging bee   by attacking bees
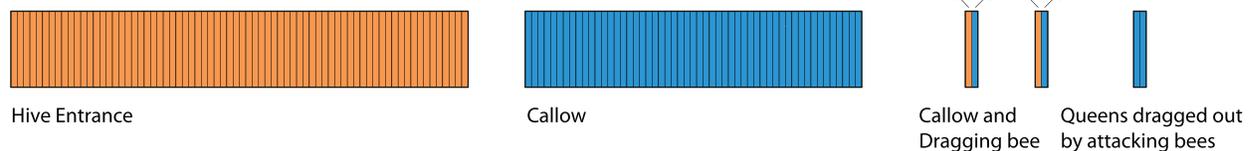
f. March brood (5 months of no fighting)

Brood

Figure 2: Molecular analysis of fighting bees with species analysis performed using STRUCTURE. Each bar represents a stingless bee individual, and the color represents its posterior probability of belonging to each species.

aria pairs recovered during this fight, and analysis of their genotypes indicated that they all came from the defending hive (i.e., from a single queen drone mating).

July Drone Clusters

Subsequent to the July skirmish, clusters of drones congregated on nearby plants (fig. 1*b*). Genetic analyses found

two clusters to be *T. hockingsi* males only, and three were mixed species (fig. 2*b*).

August Fight

This escalated fight began on August 2, 2008, and continued for 15 days (fig. 3). Fighting peaked on day 3 and
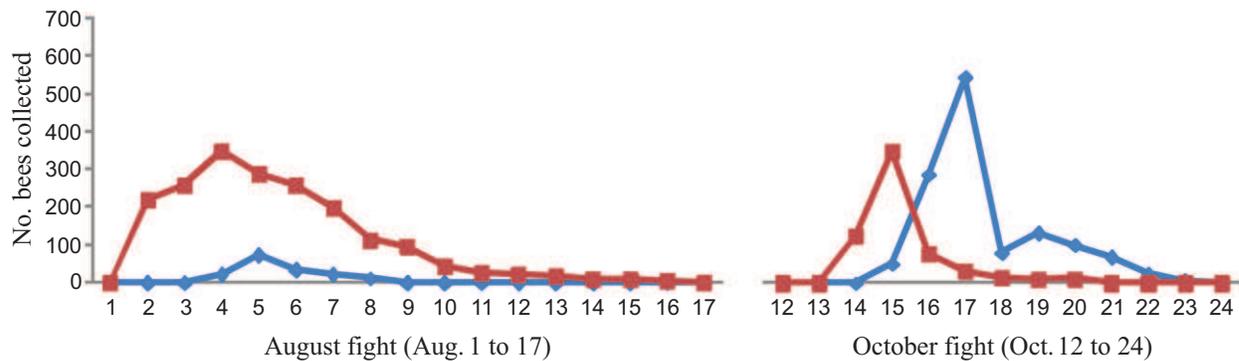
Figure 3: Fighting pairs (red lines) and ejected callow bees (blue lines) collected each day outside the focal *Tetragonula carbonaria* hive in two successive *Tetragonula hockingsi* attacks, in August 2008 and October 2008.

continued until day 15, with a total of 1,906 fighting pairs collected. Fighting pairs were found to be both *T. carbonaria* versus *T. carbonaria* and *T. hockingsi* versus *T. carbonaria* (fig. 2*d*). A continuous swarm of bees covered the hive entrance throughout, and our analysis identified these as *T. carbonaria* with the same genotypes as the defending hive in the previous fight. Callows were first observed ejected from the hive on day 3 of the fight and continued until day 7, with a total of 163 callows. All those that were genotyped were identified as *T. carbonaria* (fig. 2*d*). In two cases, where the intruder and callow were both captured, we identified these pairs as *T. hockingsi* workers ejecting *T. carbonaria* callows. Callows were sexed (by dissection and identification of genitalia) and found to be both workers (female) and drones (male).

October Fight

The next fight began on October 13 and continued for 6 days (fig. 3). Fighting occurred over the first 3 days, during which we collected a total of 664 pairs of fighting bees. Swarming activity moved to directly outside the hive entrance, and bees within the swarm ($N = 33$) were identified as drones. Ejection of callow bees began on day 2 and peaked on day 4, with a total of 1,389 ejected callows. Large (late instar) larvae and pupae were also found discarded outside the hive. Molecular analysis of the fighting pairs collected on days 1–3 (fig. 2*e*) identified the same *T. hockingsi* colony to be attacking the resident *T. carbonaria* colony as in the August fight. During this fight, a swarm of bees covered the entire surface of the hive (see video 1), and unlike the previous fight (where these bees were the resident *T. carbonaria*), analysis identified these as *T. hockingsi* (fig. 2*e*), suggesting that the attacking colony may have taken possession of the hive. All callows were identified as being *T. carbonaria* from the defending hive (thus

no evidence for usurpation from previous fights). Two workers captured dragging *T. carbonaria* callows were identified as *T. hockingsi*.

We saw no further fighting activity in this hive over the next 5 months, at which point we split the hive for brood identification. The nest architecture was typical of *T. hockingsi*, and molecular analysis identified all brood as *T. hockingsi* (fig. 2*f*), demonstrating that this species had usurped the original *T. carbonaria* colony from the hive. Further, the allele frequencies of the brood (up to three alleles per locus) were almost identical to the allele frequencies of the August and October *T. hockingsi* members of the fighting pairs, differing by only one allele at each of two loci, indicating that the queen that founded the new hive was likely to be the daughter of the attacking hive.

Species Divergence

The COI sequences did not contain any stop codons or insertions that would interrupt amino acid coding and are thus unlikely to be pseudogenes. The 14 *T. hockingsi* individuals sequenced composed only two haplotypes with two single nucleotide polymorphisms (SNPs) between them; similarly, the nine *T. carbonaria* individuals composed only three haplotypes, each separated by a single SNP. By contrast, there were 113 SNPs between the two species in the 675-bp fragment analyzed, or 16.7% difference. This equates to approximately 8 million years since divergence of the two species based on the generally accepted rate of COI divergence in insects of approximately 2% per million years (DeSalle et al. 1987).

Longitudinal Study

Each year, during the period 2008–2012, we examined changes in nest architecture in a sample population of

hives that were split open for commercial propagation (mean = 260 ± 5 hives/year), using this as an indicator for interspecific nest usurpation. Over the 5-year period, 46 hives changed occupancy from one species to the other, the majority of these changes (41) being in the direction *T. carbonaria* to *T. hockingsi* compared to *T. hockingsi* to *T. carbonaria* (5; table 1). The overall number of *T. hockingsi* colonies in the study population increased from 10 in 2008 to 29 in 2012, but it must be borne in mind that hives were predominantly *T. carbonaria*. When yearly changes in hive species occupancy were analyzed as a proportion of the total number of hives of each species, we found no evidence for a difference in usurpation events in either direction ($P > 0.05$ for all years, χ^2 test; table 1).

Table 1: Changes in the resident species of stingless beehives in a study population of man-made hives from 2008 to 2012

Year	Hive species changes		% change		
	Tc to *Th*	*Th* to *Tc*	*Tc*	*Th*	χ^2
2008	10 (253)	1 (10)	4	10	ns
2009	8 (268)	0 (21)	3	0	ns
2010	9 (252)	2 (21)	4	10	ns
2011	8 (253)	1 (26)	3	4	ns
2012	6 (274)	1 (29)	2	3	ns
Total (5 years)	41	5			

Note: Columns show changes in hive occupancy (and total hives examined) for each species, percentage changes in resident species per year for each species, and statistical (χ^2) test outcome comparing proportional changes for each species (ns = not significant at $P < .05$). Totals in parentheses differ each year as a few hives died or were added to the study population. *Tc* = *Tetragonula carbonaria*; *Th* = *Tetragonula hockingsi*.

Discussion

Using a combination of molecular analysis and behavioral observation, we have shown that intercolony battles in *Tetragonula* bees can result in usurpation of the defeated hive by the winning colony. Our study is the first report of interspecific warfare in bees, with our demonstration of nest usurpation providing an ecological and evolutionary explanation for why the lives of thousands of workers might be sacrificed in these spectacular fights. We support our molecular and behavioral study on hive usurpation with a longitudinal study on a population of *Tetragonula* hives, revealing 46 interspecific changes in hive occupation over a 5-year period, with the two species usurping each other at approximately the same rate.

In three consecutive attacks on our focal *Tetragonula carbonaria* hive, the vast majority of invading bees came from the same *Tetragonula hockingsi* colony. Although additional *T. hockingsi* and *T. carbonaria* colonies were also involved (under the assumption of single queen matings; Green and Oldroyd 2002), this was at a low rate (2%–8%; see also Gloag et al. 2008). Each fight differed markedly in length and severity and in the progression of collective behaviors toward eventual usurpation. The initial July fight had relatively few deaths and lasted only 3 days; the second fight was a major attack that continued for more than 2 weeks and led to large numbers of dead bees. Here we also observed a previously unseen behavior: that of *T. hockingsi* workers ejecting *T. carbonaria* callow (young) adults from the hive. The final battle resulted in fewer deaths from fighting and had a shorter duration than the previous fight, but here the attacking *T. hockingsi* gained control of the hive entrance and many more callows were ejected. Molecular identification of callows identified them as belonging to the resident *T. carbonaria* colony, indicating that usurpation had not occurred in the previous fight. Fighting activity then ceased entirely for 5 months, after which examination of nest architecture and molecular analysis

of the brood confirmed that the hive had been successfully usurped by *T. hockingsi.*

The capture of a fully provisioned nest (including propolis, pollen, and honey stores) provides an evolutionary explanation for why large numbers of fatalities occur during these fights. The willingness of workers to self-sacrifice in defense of the nest has evolved many times in eusocial insects (Shorter and Rueppell 2012) and is well known in bees (Breed et al. 2004), with this behavior being explained by the inclusive fitness benefits for each sterile worker of protecting the colonies reproducing adults (Hamilton 1964; Boomsma and Franks 2006). Of particular interest in the fighting swarms of *Tetragonula* is that the attacking hive also stands to lose a large proportion of its workers, since both contestants die in the fight. Enquist and Leimar (1990) propose that the evolution of fatal fighting requires the value of the resource being fought over to exceed the value to the individual's life. Under this theory, the benefits to each individual in the attacking *Tetragonula* swarm of gaining or maintaining resources and colony security (Hölldobler and Lumsden 1980) would have to outweigh the risks to its own nest through a substantial loss in workforce.

Attacking workers that emerged from the hive dragging callows (and in a few cases pupae or larvae) released these individuals within a short distance of the hive entrance, which implies that young were not taken as slaves, as with slave-maker ant species (Hölldobler and Wilson 1990). Workers exiting the hive during fights were not observed to be carrying nest material (such as pollen stores), suggesting that these were not raids on hive food stores, although we cannot discount the possibility of raided honey supplies being carried in the crop (Hölldobler et al. 2011). Very few pupae and larvae were observed being ejected from our study hive, and the fate of the remaining brood following an usurpation event remains to be tested. Suka

and Inoue (1993) demonstrated that callows of *Tetragonula minangkabau* were accepted into a conspecific hive, where they readily exchanged food with workers: it is possible that the usurping *T. hockingsi* colony in our study allowed the existing brood to mature as slaves, which could be tested by genotyping workers at regular intervals following an usurpation event. In our study we did not anticipate which battle would lead to usurpation, but our results suggest that this could be preempted by genotyping worker swarms on the hive entrance (to identify when the attackers dominate the hive) together with observations on numbers of ejected callows.

Eusocial insects can compete aggressively over nest sites when these are limiting (Foitzik and Heinze 1998; Palmer et al. 2000; Rangel et al. 2010), and the availability of suitable nest cavities or proximity of neighboring colonies could be a factor influencing the frequency of *Tetragonula* battles. Investigating this further would require experimental manipulation of the local environment (since potential nest sites could be spread over a large distance and could be high up in buildings or trees) such that colonies are forced to compete for available sites. Rangel et al. (2010) achieved this through an elegant experiment in which honeybee hives were transported to a small island devoid of nest sites. Colony takeovers aimed at securing food resources (as opposed to gaining a suitable nest site) might be expected to occur more frequently under certain environmental conditions, such as when potential foraging sites are low (Roubik 1982), and in a study system such as ours (using commercial hives that are all identical in size), seasonal food resource availability could perhaps be estimated by assessing changes in hive weight.

The diverse range of behaviors we observed during these fights suggests that the fighting swarms of *Tetragonula* have evolved as an elaborate behavioral strategy. Our molecular analysis estimated that *T. hockingsi* and *T. carbonaria* diverged around 8 million years ago, which suggests that these battles may be an ancestral trait. Usurpation may have evolved from a complex of behaviors such as reproductive swarming and nest site location (Schneider et al. 2004), nest raiding (Sakagami et al. 1993), or territorial attacks (Hölldobler and Lumsden 1980). Weak or queenless colonies may be actively selected for attack, as with Africanized honeybee attacks on European honeybees (Schneider et al. 2004), and, in the case of our study population, hive splitting events (which severely damage the nest and reduce the workforce) may have exacerbated the vulnerability of hives, making them targets for attack by wild bee colonies.

Was our observation an isolated event? Carrying out multiple combined molecular and behavioral analyses on fighting hives over extended periods was beyond the scope of this study. However, our 5-year study on an average of 260 *Tetragonula* hives per year revealed 46 interspecific changes in the hive occupation, with the most likely explanation for these changes being usurpation events. Although it is possible that interspecific changes in hive occupancy in our study population could have occurred by the original colony dying and subsequently being replaced, we do not believe this is a likely explanation. An advantage of using commercial (hobby) hives is that beekeepers generally report whether the hive is dead. Fighting events often go unreported because beekeepers witness their hives settling down, which could indicate either an unsuccessful (or intermediate) attack or an usurpation event. Changes in hive occupancy unrelated to usurpation would have to occur within a year of a hive dying (i.e., between inspections) and without the observer noticing that the hive was inactive. Additionally, we received no reports of dead hives becoming reestablished within the 5-year study period.

Do the commercial hives used in our study resemble nest sites used by these bees in the wild? *Tetragonula* colonies are commonly found in urban areas and frequently use man-made cavities as nest sites. The fact that we documented an usurpation event on a commercial hive, and found evidence for usurpation many times in our longitudinal study, strongly supports the argument that the bees recognize these hives as potential nest sites. Moreover, the wooden hives used in this study were specifically designed to resemble natural cavities in trees that are occupied by stingless bees, and thus fights over these nesting sites are likely to be representative of *Tetragonula* behavior away from human habitation.

As the majority of changes in the hive occupancy were in the direction *T. carbonaria* to *T. hockingsi* (41 changes in 5 years, compared to 5 changes in the opposite direction), the number of *T. hockingsi* colonies within the study population gradually increased in number. However, the vast majority of hives in the study population were *T. carbonaria*, and our analysis of the proportional changes in hive occupancy (i.e., changes relative to the total number of hives sampled) did not indicate that *T. hockingsi* was a more successful usurper. In 2004, when records of nest architecture for this population began, all hives were *T. carbonaria*, the appearance of *T. hockingsi* hives within this network first being recorded in 2007 (T. Heard, unpublished data). Although *T. carbonaria* and *T. hockingsi* have overlapping ranges in northern Queensland, *T. hockingsi* has only recently migrated south to the latitudes of southern Queensland and northern New South Wales, with its original distribution largely confined to the tropics (Franck et al. 2004). Their prevalence in southern Queensland might therefore be increasing for other reasons, such as climatic or habitat change or human movement of hives. The increase in natural *T. hockingsi* colonies in the surrounding environment could explain corresponding in-

creases in this species in our study population without any differences in its ability to usurp. As we used species nest architecture to identify usurpations, intraspecific usurpation could not be quantified in this study, but given the studies on fighting within *T. carbonaria* by Gloag et al. (2008), it is likely that this is also prevalent (and could be validated by molecular analyses of intraspecies fights).

Our study left us with many interesting questions to be answered. Multiple attacks were launched by the *T. hockingsi* colony before the hive was eventually usurped: Did the earlier fights cripple the defending hive? How do colonies choose which hives to attack? Is there some prior assessment of colony size or strength, as has been proposed for raiding slave-maker ants (Pohl and Foitzik 2011)? During fights, bees were also found locked together with workers from their own colony: Does this indicate an inability to recognize kin during these fights? And how does the usurping queen gain access to the hive? In the stingless bee *Melipona scutellaris*, queenless colonies are invaded by daughter queens from nearby hives (Wenseleers et al. 2011); perhaps these usurpation events create a similar scenario. Our molecular study revealed that the usurper (*T. hockingsi*) queen was from the attacking colony (and almost certainly a daughter of the attacking queen). Moreover, drone swarming around the hive entrance in the later stages of attack, combined with their congregation in the surrounding foliage, implies that these battles may be connected with mating events (Inoue et al. 1984; Cameron et al. 2004). So, is the new ruling queen escorted to the hive once the attackers are victorious? Such questions certainly provide fertile ground for future research and make *Tetragonula* battles an ideal model system for studying the evolution of fatal fighting in bees.

Acknowledgments

Literature Cited

Adams, E. S. 1994. Territory defense by the ant *Azteca trigona*: maintenance of an arboreal ant mosaic. Oecologia 97:202–208.

Batchelor, T. P., and M. Briffa. 2011. Fight tactics in wood ants: individuals in smaller groups fight harder but die faster. Proceedings of the Royal Society B: Biological Sciences 278:3243–3250.

Boomsma, J. J., and N. R. Franks. 2006. Social insects: from selfish genes to self organisation and beyond. Trends in Ecology & Evolution 21:303–308.

Breed, M. D., E. Guzman-Novoa, and G. J. Hunt. 2004. Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. Annual Review of Entomology 49:271–298.

Brito, R. M., T. M. Schaerf, M. R. Myerscough, T. A. Heard, and B. P. Oldroyd. 2012. Brood comb construction by the stingless bees *Tetragonula hockingsi* and *Tetragonula carbonaria*. Swarm Intelligence 6:151–176.

Cameron, E. C., P. Franck, and B. P. Oldroyd. 2004. Genetic structure of nest aggregations and drone congregations of the southeast Asian stingless bee *Trigona collina*. Molecular Ecology 13:2357–2364.

Cunningham, J. P., J. P. Hereward, T. A. Heard, P. J. De Barro, and S. A. West. 2014. Data from: Bees at war: interspecific battles and nest usurpation in stingless bees. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.2ng61.

DeSalle, R., T. Freedman, E. Prager, and A. Wilson. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. Journal of Molecular Evolution 26:157–164.

Dollin, A. E., L. J. Dollin, and S. F. Sakagami. 1997. Australian stingless bees of the genus *Trigona* (Hymenoptera: Apidae). Invertebrate Taxonomy 11:861–896.

Enquist, M., and O. Leimar. 1990. The evolution of fatal fighting. Animal Behaviour 39:1–9.

Foitzik, S., and J. Heinze. 1998. Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. Behavioral Ecology 9:367–375.

Foitzik, S., and J. M. Herbers. 2001. Colony structure of a slave-making ant. II. Frequency of slave raids and impact on the host population. Evolution 55:316–323.

Franck, P., E. Cameron, G. Good, J. Y. Rasplus, and B. P. Oldroyd. 2004. Nest architecture and genetic differentiation in a species complex of Australian stingless bees. Molecular Ecology 13:2317–2331.

Gloag, R., T. A. Heard, M. Beekman, and B. P. Oldroyd. 2008. Nest defence in a stingless bee: what causes fighting swarms in *Trigona carbonaria* (Hymenoptera, Meliponini)? Insectes Sociaux 55:387–391.

Green, C., P. Franck, and B. Oldroyd. 2001. Characterization of microsatellite loci for *Trigona carbonaria*, a stingless bee endemic to Australia. Molecular Ecology Notes 1:89–92.

Green, C. L., and B. P. Oldroyd. 2002. Queen mating frequency and maternity of males in the stingless bee *Trigona carbonaria* Smith. Insectes Sociaux 49:196–202.

Halcroft, M. T., R. Spooner-Hart, A. M. Haigh, T. A. Heard, and A. Dollin. 2013. The Australian stingless bee industry: a follow-up survey, one decade on. Journal of Apicultural Research 52:1–7.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I, II. Journal of Theoretical Biology 7:1–52.

Heard, T. A. 1996. The stingless bees. Nature Australia 1996:50–55.

———. 1999. The role of stingless bees in crop pollination. Annual Review of Entomology 44:183–206.

Heard, T. A., and A. E. Dollin. 2000. Stingless bee keeping in Australia: snapshot of an infant industry. Bee World 81:116–125.

Hölldobler, B. 1981. Foraging and spatiotemporal territories in the honey ant *Myrmecocystus mimicus* Wheeler (Hymenoptera, Formicidae). Behavioral Ecology and Sociobiology 9:301–314.

Hölldobler, B., B. Grillenberger, and J. Gadau. 2011. Queen number and raiding behavior in the ant genus *Myrmecocystus* (Hymenoptera: Formicidae). Myrmecological News 15:53–61.

Hölldobler, B., and C. J. Lumsden. 1980. Territorial strategies in ants. Science 210:732–739.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Harvard University Press, Cambridge, MA.

Inoue, T., S. F. Sakagami, S. Salmah, and S. Yamane. 1984. The process of colony multiplication in the Sumatran stingless bee *Trigona* (*Tetragonula*) *laeviceps*. Biotropica 16:100–111.

Johnson, L. K., and S. P. Hubbell. 1974. Aggression and competition among stingless bees: field studies. Ecology 55:120–127.

Kreuter, K., E. Bunk, A. Lueckemeyer, R. Twele, W. Francke, and M. Ayasse. 2012. How the social parasitic bumblebee *Bombus bohemicus* sneaks into power of reproduction. Behavioral Ecology and Sociobiology 66:475–486.

Maynard-Smith, J., and G. R. Price. 1973. The logic of animal conflict. Nature 246:15–18.

Murray, M. G. 1987. The closed environment of the fig receptacle and its influence on male conflict in the old-world fig wasp, *Philotrypesis pilosa*. Animal Behaviour 35:488–506.

Nagamitsu, T., and T. Inoue. 1997. Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. Oecologia 110:432–439.

Nieh, J. C., K. Kruizinga, L. S. Barreto, F. A. L. Contrera, and V. L. Imperatriz-Fonseca. 2005. Effect of group size on the aggression strategy of an extirpating stingless bee, *Trigona spinipes*. Insectes Sociaux 52:147–154.

Pohl, S., and S. Foitzik. 2011. Slave-making ants prefer larger, better defended host colonies. Animal Behaviour 81:61–68.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Rangel, J., S. R. Griffin, and T. D. Seeley. 2010. Nest-site defense by competing honey bee swarms during house-hunting. Ethology 116:608–618.

Roubik, D. W. 1982. Seasonality in colony food storage, brood production and adult survivorship: studies of *Melipona* in tropical forest (Hymenoptera, Apidae). Journal of the Kansas Entomological Society 55:789–800.

Sakagami, S. F., D. W. Roubik, and R. Zucchi. 1993. Ethology of the robber stingless bee, *Lestrimelitta limao* (Hymenoptera, Apidae). Sociobiology 21:237–277.

Scharf, I., T. Pamminger, and S. Foitzik. 2011. Differential response of ant colonies to intruders: attack strategies correlate with potential threat. Ethology 117:731–739.

Schneider, S. S., T. Deeby, D. C. Gilley, and G. DeGrandi-Hoffman. 2004. Seasonal nest usurpation of European colonies by African swarms in Arizona, USA. Insectes Sociaux 51:359–364.

Shorter, J. R., and O. Rueppell. 2012. A review on self-destructive defense behaviors in social insects. Insectes Sociaux 59:1–10.

Suka, T., and T. Inoue. 1993. Nestmate recognition of the stingless bee *Trigona* (*Tetragonula*) *minangkabau* (Apidae, Meliponinae). Journal of Ethology 11:141–147.

van Wilgenburg, E., E. van Lieshout, and M. A. Elgar. 2005. Conflict resolution strategies in meat ants (*Iridomyrmex purpureus*): ritualised displays versus lethal fighting. Behaviour 142:701–716.

Wagner, A., and L. Dollin. 1982. Swarming in Australian native bees: help solve the mystery! Australasian Beekeeper 84:15–18.

Wenseleers, T., D. A. Alves, T. M. Francoy, J. Billen, and V. L. Imperatriz-Fonseca. 2011. Intraspecific queen parasitism in a highly eusocial bee. Biology Letters 7:173–176.

Whitehouse, M. E. A., and K. Jaffe. 1996. Ant wars: combat strategies, territory and nest defence in the leaf-cutting ant *Atta laevigata*. Animal Behaviour 51:1207–1217.

Wille, A. 1983. Biology of stingless bees. Annual Review of Entomology 28:41–64.

Wilson, E. O. 1971. The insect societies. Harvard University Press, Cambridge, MA.

Zee, J., and D. Holway. 2006. Nest raiding by the invasive Argentine ant on colonies of the harvester ant, *Pogonomyrmex subnitidus*. Insectes Sociaux 53:161–167.

Zobel, M. U., and R. J. Paxton. 2007. Is big the best? queen size, usurpation and nest closure in a primitively eusocial sweat bee (*Lasioglossum malachurum*). Behavioral Ecology and Sociobiology 61:435–447.

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q1. In table A1: (1) Please define T_a and N_a. (2) What is the measurement for size range? (3) Do any of the abbreviations in the "Dye" column need to be defined?

q2. Palmer et al. 2000 is not listed in the literature cited. Please provide reference information or delete from text.

q3. OK to change Holldober 1980 to Hölldobler and Lumsden 1980 to match literature cited?

q4. Does "Their" refer to both *T. hockingsi* and *T. carbonaria* here or just *T. hockingsi*? If just the latter, OK to say "Its prevalence ..."?

q5. Generally we see primers in capital letters. OK as lowercase here?

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