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ORIGINAL ARTICLE

Queen turnover, nest usurpation and colony mortality in wild nests of the stingless bees *Tetragonula carbonaria* and *Tetragonula hockingsi* (Hymenoptera: Apidae)

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Abstract

Social bees of the tribe Meliponini (stingless bees) are used as managed pollinators of crops throughout the world's tropical and subtropical regions. On Australia's East Coast, two native species—Tetragonula carbonaria and Tetragonula hockingsi—are the most widely propagated in hives, but knowledge of their biology and ecology in natural nests remains poorly documented. Here we monitor a wild population of Tetragonula in remnant forest in south-east Queensland over a 5-year period to assess three aspects of their life history: (i) rates of colony mortality, (ii) rates of queen turnover and (iii) incidences of nest usurpation. The latter occurs when one colony usurps the nest cavity of another, installing its own gueen and enslaving the existing workers and brood. The range of T. hockingsi has increased in recent decades due to hive trade and southward range expansion. Our study area was located in the southern region of overlap with T. carbonaria. A total of 58 wild colonies were identified within the study site (1.5 nests per hectare), three-quarters of which were T. carbonaria. Colony mortality averaged 8.3% per year, such that 40% of colonies had died by the end of the 5-year study interval. Sequencing of mitochondrial-COI and microsatellite genotyping of workers at four time points was used to infer that gueen turnover (i.e., gueens replaced by daughter gueens) typically occurs every 20-30 months. Eight cases were detected consistent with interspecific nest usurpation, in all of which T. hockingsi replaced T. carbonaria. However, T. hockingsi colonies also had lower annual survivorship than those of T. carbonaria, resulting in a stable proportion of each species in the study area over time. Overall, results show that although nest occupancy by *Tetragonula* colonies is typically several years, colony death and nest usurpation are common in wild populations, and community composition is shaped by interspecific differences in both usurpation success and annual mortality.

KEYWORDS

competition, nest density, nest takeover, pollinator, population dynamics, queen longevity, queen supersedure, wild nest

INTRODUCTION

Stingless bees (Apidae, Tribe Meliponini) are abundant and important pollinators throughout the world's tropical and subtropical forests (Bueno et al. 2023b; Grüter 2020). They are eusocial bees that can be readily kept and propagated in hives and are increasingly used as managed pollinators of some fruit and vegetable crops, including macadamia, mango, avocado, blueberry and coffee, with wild stingless bees also contributing to crop pollination

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(Bueno et al. 2023b; Halcroft et al. 2015; Meléndez

Ramírez, Ayala, & Delfín González 2018; Slaa et al. 2006). On the Australian East Coast, *Tetragonula hockingsi* (Cockerel, 1929) and *Tetragonula carbonaria* (Smith, 1854) are the most commonly propagated stingless bee species (Halcroft et al. 2015; Heard 2016). These species have several characteristics that make them suitable for management as crop pollinators in tropical and subtropical eastern Australia, including generalist diets, small foraging ranges, floral constancy and large colony sizes (Heard 2016; Reynolds & Robinson 2022; Smith et al. 2017; White, Cribb, & Heard 2001).

Keeping and propagating stingless bees in hives relies on knowledge of their reproductive behaviour. In Australian *Tetragonula*, colonies consist of a single queen that lays all eggs, and up to 10 000 irreversibly sterile workers (Bueno et al. 2020; Gloag et al. 2007). Colonies also rear males equivalent to ~20% of total brood (Bueno et al. 2022a) and a small number of additional queens (Bueno et al. 2022b). Mature males leave the nest in search of virgin queens with which to mate while most young queens disappear not long after emergence, presumably because they are killed by workers (Bueno et al. 2022b). When the resident queen dies, one virgin queen will leave the colony to mate with just one male (Green & Oldroyd 2002) after which she returns to the colony to begin egg-laying and will not leave again for the rest of her life.

Virgin queens may also inherit a colony when their parent colony replicates. Australian *Tetragonula* have two modes of such colony replication. In the first, workers identify a new nest site and slowly provision it over many months with stores and propolis from the maternal nest, a strategy common to all stingless bee species (Grüter 2020). In the second, workers attack and usurp an existing colony and take over its nest, brood and resources. Nest usurpations can involve extensive battles between the colonies for days or weeks, resulting in thousands of dead bees (Cunningham et al. 2014; Gloag et al. 2008). Successful nest usurpation results in the winning colony killing and replacing the resident queen with a queen from their own colony and enslaving the resident brood (Lau et al. 2022).

Most of our understanding of *Tetragonula* biology has been made possible by the observation and experimental manipulation of colonies kept in human-made boxes (i.e., hives). Hives have several advantages in research because they can be opened or fitted with temporary plastic covers that facilitate observation of the internal working of the colony (Heard 2016). Yet knowledge of *Tetragonula* reproductive success and life history derived from wild populations is also needed. In particular, such studies can illuminate the conditions under which reproductive traits have evolved and identify ways in which beekeeping practices may impact the bees' behaviour.

Studies in natural populations also offer insights into interspecific competition. *T. carbonaria* is distributed in coastal New South Wales and south-east Queensland, with

additional isolated populations in northern Queensland (Dollin, Dollin, & Sakagami 1997) while T. hockingsi is distributed along the Queensland coast. The range of T. hockingsi has increasingly expanded southward in recent decades, aided by the human movement of hives (Law et al. 2024; Paul et al. 2023) and increasing the area of sympatry in south-east Queensland between the two species. Tetragonula hockingsi are considered by many beekeepers to defend their hives more aggressively than T. carbonaria (Heard 2016). Moreover, a previous study of more than 260 managed hives of T. hockingsi and T. carbonaria in south-east Queensland revealed that T. hockingsi were more likely to usurp T. carbonaria colonies than vice versa, with 90% of documented interspecific takeovers involving T. hockingsi usurping a hive of T. carbonaria (Cunningham et al. 2014). A guestion of interest therefore is whether T. hockingsi are capable of displacing T. carbonaria from wild populations at the advancing southern edge of their range.

In this study, we map and sample wild *Tetragonula* nests over a 5-year period in a forest patch in south-east Queensland where the ranges of *T. carbonaria* and *T. hockingsi* overlap. We then use molecular species-IDs and microsatellite genotyping of our time-series samples to better understand rates of (i) colony mortality, (ii) nest usurpation and (iii) queen turnover, in natural *Tetragonula* populations, and to determine if nest usurpations by *T. hockingsi* have increased the prevalence of this species relative to *T. carbonaria* in our study area.

MATERIAL AND METHODS

Sampling

We located 58 nest sites containing wild Tetragonula colonies in a patch of remnant bushland in Brisbane, Australia (Toohey Forest, -27.544, 153.047; Figures 1 and 2). Nests were located by searching trees and fallen logs within an area of approximately 40 ha. Three of the nests were too high to sample (5-7 m above ground) and were subsequently monitored by observation for survival only (i.e., not included in analyses of queen turnover or nest usurpation). At the remaining nests (n = 55), at least 12 workers per nest were collected either by obstructing the nest entrance with crumpled paper when foragers were active and then using an aspirator to collect returning foragers congregating on or near the obstruction or by holding a zip-lock bag over the entrance to capture exiting foragers. Workers were transferred directly onto 100% ethanol and stored in the laboratory at -20°C until DNA extraction.

We sampled the same nest sites at four time points: May 2017 (n = 53), September 2018 (n = 52), May 2019 (n = 51) and April 2022 (n = 39); Figure 3; Tables S1 and S2. The number of nests sampled each period varied because some nest sites were no longer occupied (i.e., the colony had died) and because two nests, which



FIGURE 1 Natural nest sites of *Tetragonula* in a forest fragment of south-east Queensland, Australia. White arrows indicate the position of the nest entrances in the tree (photos by Ros Gloag).

may have been newly established since the earlier sampling periods or may have overlooked them during previous searches, were only discovered and sampled for the first time in 2019 (Figure 3). All sampling was conducted with permission from Brisbane City Council Parks and Reserves, Queensland.

Genotyping

We extracted DNA from whole abdomens via the 5% Chelex method (Walsh, Metzger, & Higushi 1991). We genotyped 12 workers per colony, per sampling year, each at eight microsatellite loci using six primers designed for *T. carbonaria* (Tc3.155, Tc4.214, Tc4.287, Tc4.302, Tc4.63, Tc7.13; Green, Franck, & Oldroyd 2005) and two primers designed for *Tetragonisca angustula* (Tang60, Tang70; Brito et al. 2009). Primers were fluorescently labelled with one of four dyes (FAM, NED, PET, VIC; Sigma-Aldrich, USA). We performed polymerase chain reaction (PCR) amplifications according to Green and Oldroyd (2002), and the resulting products were analysed using a 3130xl Genetic Analyser (Applied Biosystems, Inc., USA) and visualised in GeneMapper v5.5 (Applied Biosystems, USA.).

We inferred the genotypes of the queen and her mate for each colony by examining the worker genotypes. At each locus, we assigned the single allele common to all

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FIGURE 2 Maps depicting the relative location of *Tetragonula* nests within a 40 ha area of remnant forest in south-east Queensland (Toohey Forest) at (a) the beginning of our study period (May 2017) and (b) the end of our study period (April 2022). Nests are coloured by species; nests that were unoccupied by the final sampling period (i.e., colonies had died) are in black. Geographic identifiers have been removed to protect the locations of the nests.

workers as the paternal allele (because males are haploid and queens in these species mate only once [Green & Oldroyd 2002]). The two remaining alleles were then assumed to be the maternal (queen) alleles, with each worker carrying one of these alleles at each locus (Tables S3–S7).

Allele diversity was higher in *T. carbonaria* than *T. hockingsi* for all loci (Table 1). The likelihood of two queens sharing the exact same alleles at all eight microsatellite loci, and thus the probability of a nest usurpation going undetected, was calculated using the probability of identity formula described in Paetkau and Strobeck (1994):

$$PI = \sum {p_i}^4 + \sum \sum \left(2p_i p_j \right)^2$$

This probability was ≤ 0.004 for both species.

Species identification

The workers of *T. hockingsi* are marginally larger on average than those of *T. carbonaria*, but the size range of both species overlap, and they are otherwise morphologically cryptic (Dollin, Dollin, & Sakagami 1997). We therefore assigned colonies to species by sequencing a fragment of mitochondrial cytochrome oxidase I (COI). We amplified COI using the primers *Barhock_F* (CTCCATTGTTACTGGGCATGC) and *Barhock_R* AAGGCC-GAATCCTGGAAGAA (Francoso et al. 2019). PCR amplification was performed using 94°C for 8 min, followed by 35 cycles of 94°C, 60°C and 72°C for 30 s each, then 72°C for 9 min. Reactions used $1 \times PCR$ buffer,

1.5-mM MgCl₂, 0.2-mM dNTPs, 0.4- μ M forward primer, 0.4- μ M reverse primer and 1-U Taq-Ti polymerase (Fisher Biotech). We visualised PCR products via electrophoresis on a 1.5% agarose gel to confirm the amplification, after which products were sequenced by Macrogen Inc. (South Korea). All sampled colonies were COI-sequenced in the first sampling year (2017: n = 53) and the final sampling year (2022: n = 40). In the second and third sampling periods, we performed COI sequencing only for the colonies with genotypes that indicated a queen change (2018: n = 21, 2019: n = 17).

Nest usurpation and queen turnover rates

In detecting rates of nest usurpation, we assumed that Tetragonula do not establish in nest sites where other colonies have recently died. This is consistent with reports from Australian stingless beekeepers that Tetragonula do not reuse old hives, unless they have been cleaned of most dead nest material (D. Haley and T. Heard, pers comm to R.Gloag). It is also consistent with our own data in which four sites where colonies perished in 2018 or 2019 were still unoccupied in 2022. We therefore inferred that a nest site occupied by a different species to the previous sampling interval was most likely the result of an interspecies usurpation event and that a nest site occupied by the same species with alleles inconsistent with being a descendent of the queen in the previous sampling period was most likely an intraspecific usurpation event.

For each time interval comparison (2017 vs. 2018, 2018 vs. 2019 and 2019 vs. 2022), we compared changes

QUEEN TURNOVER, NEST USURPATION AND COLONY MORTALITY IN WILD NESTS OF THE STINGLESS BEES Tetragonula carbonaria AND Tetragonula hockingsi (HYMENOPTERA: APIDAE)





FIGURE 3 Inferred queen turnovers, nest usurpations and colony deaths for monitored *Tetragonula* nest sites (nest ID) in Toohey Forest over a five-year period (may 2017–April 2022). Circles represent the species occupying the nest at each of the four sampling events: *Tetragonula carbonaria* (blue), *Tetragonula hockingsi* (orange) or unknown (white; because the nest was too high to sample, n = 3). Black circles indicate nests in which the occupying colony had died. Vertical dashes on the lines connecting sampling events indicate the number of queen turnovers that occurred in that interval (mother-to-daughter; 1, 2 or 3 turnovers per interval). Nest usurpations that occurred between sampling intervals are indicated ('usurped'). Asterisks indicate the six *T. carbonaria* nests for which we inferred three mother–daughter queen turnovers in the final sampling interval (2019–2022), though it is possible these were instead conspecific nest usurpations.

TABLE 1	The number of total alleles (and private alleles) at eight		
microsatellite	loci for Tetragonula hockingsi and Tetragonula carbonaria		
sampled in this study.			

Microsatellite	T. hockingsi	T. carbonaria	
locus	Number of alleles (number of private alleles)		
Tc3.155	5 (2)	8 (5)	
Tc4.214	4 (1)	3 (0)	
Tc4.287	5 (1)	4 (0)	
Tc3.302	3 (0)	6 (3)	
Tc4.63	7 (2)	9 (4)	
Tang60	6 (1)	7 (3)	
Tang70	3 (0)	5 (2)	
Tc7.13	7 (1)	7 (1)	
Average for all loci	5	6.25	

in the genotypes of inferred queens and their mates and scored the nest as one of five categories:

- 1. No queen change (queen and mate genotypes matched the previous sample at all loci);
- Nest inherited by daughter queen (the new queen carried the paternal allele of the previous sample, plus one of the two alleles of the previous queen, at all loci);
- Nest inherited by granddaughter queen (the new queen carries either the paternal allele of the previous sample or one of the two alleles of the previous queen at all loci);
- Intraspecific nest usurpation (species unchanged, but the new queen's genotype was not consistent with being a daughter or granddaughter of the previous queen and mate); and
- 5. Interspecific nest usurpation (species has changed).

Allowing for granddaughter queens in the dataset suggests that three generations could pass in the periods between sampling intervals, which varied for each interval: 12 months (2017–2018), 8 months (2018–2019) and 35 months (2019–2022). The minimum time required for *T. carbonaria* queens to go from egg to laying adult is approximately 80 days (5–6 weeks pre-eclosion, 2–3 weeks maturation pre-mating, 1–2 weeks post-mating until laying; Bueno et al. 2022b), and the additional time needed for the eggs laid by a new queen to become foraging workers outside the nest which we then sample is

at least 60 days (3 weeks pre-eclosion development, 4– 5 weeks in-nest as callows and nurses; Heard 2016). Therefore, we predicted that a colony would rarely turnover queens twice in a period of 12 months or less (i.e., we should see few granddaughter queens observed in the 2017–2018 and 2018–2019 sampling periods), but may do so more often in a period of 35 months (our 2019–2022 period).

Likewise, more than two natural queen turnovers (i.e., detection of great-granddaughter gueens or beyond) was highly unlikely to have occurred between our first two sampling intervals (2017-2018 and 2018-2019) but was plausible for the final sampling interval (2019–2022). As a great-granddaughter gueen is not easily distinguished from a non-relative based on eight microsatellite loci, there was some ambiguity for the assignment of nest occupancy in this final interval. In total, six nests met the criteria for Category (4) above for the 2019-2022 interval, but as the inferred queen in 2022 had a genotype that was plausibly consistent with being the great-granddaughter of the inferred gueen in 2019, we scored these colonies conservatively, as resulting from natural gueen turnovers rather than intraspecific usurpations.

Interspecies colony takeovers were confirmed by COI species IDs, and all such cases also showed microsatellite genotype changes consistent with a species change, based on private alleles (Table 1).

Queen longevity

We estimated the typical lifespan of *Tetragonula* queens in this population by first estimating the average age of queens per colony as



where *q* is the number of inferred queens that lived during time *t*, the sampling period in months. This estimate accounted for the partial age of the first and last queens by counting them as the equivalent of one queen because the duration of their reigns was unknown. In one *T. hockingsi* colony, worker genotypes were consistent with a single queen having reigned for the full 5-year period, and this queen was given an age estimate of 5 years.

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Nest usurpation and mortality by species

During the 5-year study interval, eight *T. carbonaria* colonies were usurped by *T. hockingsi* (1 between 2017 and 2018, 4 between 2018 and 2019 and 3 between 2019 and 2022). In contrast, there were no instances of *T. carbonaria* usurping *T. hockingsi*; Figure 3 (8 out of 43 *T. carbonaria* colonies usurped by *T. hockingsi* vs. 0 out of 12 *T. hockingsi* colonies usurped by *T. carbonaria*, Fisher's exact test, p = 0.178).

Conversely, *T. hockingsi* colonies died at a significantly higher rate than those of *T. carbonaria* (10 out of 16 colonies that had died by the final sampling period had been identified as *T. hockingsi* at the previous sampling point; Fisher's exact test: p = 0.003). This die-off included some of the *T. hockingsi* colonies established via usurpation; of the eight *T. hockingsi* colonies that usurped *T. carbonaria* nests, four had died by the final sampling point (Figure 3). Thus, the overall proportion of each species in the sample area was unchanged between the first and final sampling periods (species proportions at final sampling in 2022: *T. carbonaria*: 74%, n = 29 and *T. hockingsi* 26%, n = 10; Fisher's exact test: p = 0.8).

We could not confirm cases of intraspecific usurpation in either species throughout the study period. For *T. hockingsi*, all instances of queen turnover were consistent with daughter or granddaughter queens inheriting the nest (Figure 3). For *T. carbonaria*, there were no cases in which intraspecific usurpation could be unambiguously assigned, although between the longest sampling interval (2019–2022: 35 months), there were six colonies whose queen turnovers were not consistent with either daughter or granddaughter queens. We concluded these were plausibly the same maternal lineage after more than three generations (i.e., great-granddaughter queens), though it is possible some or all were in fact usurpations by another *T. carbonaria* colony.

Queen turnover and queen longevity

The proportion of colonies that had requeened between sampling intervals (excluding usurped nests) was 40% in the 12 months between sampling in 2017 and 2018, 31% in 2018–2019 (8 month interval) and 92% in 2019–2022 (35 month interval); Figure 3, Tables S1 and S2. This equates to, on average, one-third of all colonies in the population replacing their queen each year.

On average, colonies had 3.2 (\pm 0.2) queen turnovers during the five-year study period (median: 3, range 1–7; N = 33 colonies; Figure 4). We therefore estimated the average lifespan for queens in our study population as 22 \pm 2 months (median age: 20 months, range: 8–59 months) based on N = 33 colonies that did not experience nest usurpations or die (*T. carbonaria*: N = 27, *T. hockingsi*: N = 6).

For queen longevity calculations, we excluded periods of time preceding a sampling interval that detected a usurpation event or nest death, that is, queen longevity was only calculated in colonies where daughter–queen turnovers were continuous.

Species comparisons

To explore relative rates of usurpation and mortality for each species, we used Fisher's exact tests to determine (i) whether *T. carbonaria* nests were more likely to be usurped by *T. hockingsi* (interspecific usurpation) than vice versa; (ii) whether colonies of either species were equally likely to survive the 5-year sampling period; and (iii) whether the overall proportion of each species in the forest changed between the first and last sampling period.

For Test (i), we included all colonies present in the first year of sampling (2017) in addition to two colonies first sampled in 2019 (total: 43 T. carbonaria colonies and 12 T. hockingsi colonies). For Test (ii), we looked at all nests that perished during the 5-year period (i.e., no colony remained at the nest site), noted the species occupying the nest prior to death, and compared this with the number of surviving nests of each species. This test therefore considered only total nest death and did not include colony death via usurpation. This test also assumed that there was no undetected interspecific nest usurpation event prior to colony death, that is, that the last species observed occupying the nest was the species at time of colony death. The final test, (iii), compared the proportion of T. hockingsi versus T. carbonaria in the study population during the first sampling interval (May 2017) with the proportion observed in the final sampling interval (April 2022). All statistical analyses were conducted in R (v4.2.1), R Core Team (2021).

RESULTS

Species composition, nest density and mortality

At the time of initial sampling (May 2017), around threequarters of all nests were *T. carbonaria* (77%, n = 41), and one-quarter were *T. hockingsi* (23%, n = 12). Nests of each species were interspersed within the forest patch (Figure 2). Given the size of the patch we searched, the density of *Tetragonula* nests equated to approximately 1.5 nests per hectare, with the distance between nests and their nearest neighbour nest ranging from 2 to 217 m (Figure 2). Including death by nest usurpation, colony mortality was 41% over the 5-year study period (24 of 58), giving an average colony mortality rate of 8.3% per annum.



Number of queen turnovers in a colony during the five year study period

FIGURE 4 A histogram of total number of inferred laying queens per *Tetragonula* colony across our 5-year study period (n = 33 colonies that did not experience nest usurpations or death; *Tetragonula carbonaria*: n = 27, *Tetragonula hockingsi*: n = 6). Queen number was inferred based on changes in the genotypes of workers sampled from colonies at four time points (see Figure 3). The majority of colonies had 2–4 successive queens during the 5-year interval (i.e., a queen turnover every 1–2 years).

DISCUSSION

Nest usurpation and colony mortality

This study provides a snapshot of nest usurpation and colony mortality in a wild population of Tetragonula in south-east Queensland over a 5-year period. Broadly speaking, stingless bee life history is characterised by long-lived, perennial colonies and low rates of colony propagation (Slaa et al. 2006). Indeed, stingless bee colonies are potentially immortal, provided they can produce an unbroken maternal-line dynasty in which daughter queens replace their mothers to inherit the nest site (Bueno et al. 2023a). In our study, most Tetragonula colonies persisted for multiple years, but there was a steady rate of colony loss. In all, two-fifths of colonies in our study had perished within 5 years, including those that succumbed to usurpation by other colonies. This equates to an annual colony mortality of around 8%, which is similar to the rates reported for wild stingless bee populations in the Asian tropics (13.5%–15% mortality, Eltz et al. 2002) and American tropics (11% mortality, Slaa 2006; 7%–13% mortality, Silva, Ramalho, & Rosa 2021). In order to maintain a stable population size, the average Tetragonula colony at our study site must therefore successfully establish a new colony once every 12 years (i.e., 0.08 successful swarms/colony/year), or once every 10 years if we consider mortality for T. carbonaria only (0.1 swarms/colony/ year). In other words, 1 in every 12 colonies must

successfully found a daughter colony each year to maintain the current population size. Whether this rate of colony establishment occurs in our study population is unknown, as we did not search for newly established colonies.

Nest usurpation is believed to be a key feature of Australian Tetragonula's reproductive ecology, and beekeepers of both T. carbonaria and T. hockingsi regularly observe the fighting swarms that accompany attempted hive usurpation (Cunningham et al. 2014; Gloag et al. 2008; Lau et al. 2022; Stephens, Beekman, & Gloag 2017; Wagner & Dollin 1982). Our study shows that nest usurpation is also commonplace in wild Tetragonula species and that 14% of all nests were usurped over the 5-year study period. These usurpations were not evenly distributed between the two species: all eight confirmed instances involved T. hockingsi usurping T. carbonaria. A similar species bias was previously observed among managed hives from the same region of south-east Queensland (Cunningham et al. 2014). In that study, the majority (41 of 46 usurpations) observed over a 5-year period in 260 hives (18% of all hives) involved T. hockingsi as the usurper (Cunningham et al. 2014).

In both wild and managed populations therefore, T. hockingsi appears to be more likely than T. carbonaria to successfully usurp nests of the other species. This may be because they are more successful when they are the attacking colony and/or more able to repel attackers when they are the defending colony. T. hockingsi workers are marginally larger on average than those of T. carbonaria (4.4–4.7 mm and 4.1–4.6 mm respectively; Dollin, Dollin, & Sakagami 1997), which may give them an advantage during colony warfare. T. hockingsi might also be better adapted than T. carbonaria to competing for scarce resources, given the habitats occupied by the two species. T. hockingsi's range includes the hot and dry lowland regions of coastal North Queensland where floral resources will be in short supply more often than in the cooler, wetter closed forest habitats of New South Wales and south-eastern Queensland where T. carbonaria occurs (Dollin, Dollin, & Sakagami 1997).

Nevertheless, fighting swarms are also observed frequently in managed colonies of T. carbonaria, even in the parts of their range where no T. hockingsi occur. It is surprising therefore that no instance of intraspecific usurpation within the T. carbonaria population was confirmed across our 5-year study. One possibility is that some T. carbonaria/T. carbonaria usurpations did occur but could not be detected by the genetic markers we employed. In particular, queen changes during the longest sampling interval of 35 months included six T. carbonaria nests that were conservatively scored as great-granddaughters of the preceding queen (i.e., three queen successions in 35 months), but some or all of these might instead have been conspecific usurpations. Alternatively, it may be that, for T. carbonaria, intraspecific usurpations at natural sites are genuinely infrequent.

Widespread beekeeping practices, such as hive splitting (in which a colony is propagated by cutting it in half) or the aggregation of many hives in close proximity in beeyards, might increase the tendency for managed *T. carbonaria* colonies to attempt nest usurpations or produce fighting swarms (Gloag et al. 2008; Stephens, Beekman, & Gloag 2017). Further experimental studies that compare nest usurpation rates for hives under different management regimes are needed to better understand how beekeeping practices influence nest usurpation behaviour.

Importantly, the conclusions made about nest usurpation rates in this study are based on the assumption that Tetragonula in southern Queensland never or rarely reuse nest sites in which a previous colony has recently died (i.e., since the last sampling point). This assumption stems from our own observations of colony death and those of experienced Australian beekeepers who report that Tetragonula spp. do not reuse old hives unless they have been cleaned of dead nest material and that natural log cavities remain vacant once colonies have died. Unoccupied nests quickly accumulate fungal growth on any remaining pollen or unhatched brood and are over-run by native pests including larvae of the syrphid fly (Ceriana ornata), phorid fly (Dohrniphora trigonae) and hive beetles (Brachypeplus spp.; (Heard 2016). These pests covert the nest material to a pungent sludge that is presumably highly unattractive to the bees. However, nest site reuse could occur on longer time scales, where pest infestations and the decomposition of most nest material has run its course. Interestingly, while re-colonisation of nest sites appears rare in Australian *Tetragonula*, it is reported from Neotropical stingless bee species (Slaa 2006). If the species changes in nest occupancy observed in our dataset were in fact the product of rapid re-colonisation after colony death, rather than usurpations, then we might conclude that T. hockingsi are more predisposed to such re-colonisations than T. carbonaria.

The prevalence of *T. hockingsi* in the Brisbane area prior to their recent domestication in hives is not documented, but anecdotally, the species has become more common there in recent decades (T. Heard pers. comm. to R. Gloag). The genetic diversity of Brisbane's T. hockingsi population is also consistent with it being shaped by significant hive trade from regions to the north (Law et al. 2024). Might T. hockingsi's southward range expansion lead to the displacement of T. carbonaria? Despite their higher success in nest usurpation, we found that the number of colonies of T. hockingsi did not increase in proportion relative to T. carbonaria in our 5-year study period. The increase in T. hockingsi colonies due to nest usurpation was offset by the species' higher rate of colony mortality. The causes of mortality at colonies in this study are unknown but may have included predation (Kajobe & Roubik 2006), pests or parasites (Nacko et al. 2020), heat waves (Nacko et al. 2023) and/or food limitation (Slaa 2006). In tropical stingless bee populations in Costa Rica, mortality of colonies was found

to spike at the end of the wet season when food availability was lowest, suggesting that starvation can be a significant cause of colony mortality (Slaa 2006). As Tetragonula spp. are relatively small bees with limited flight ranges (500 m; Smith et al. 2017), they may be even more vulnerable to fluctuations in food supply than larger bodied Neotropical species (Silva, Ramalho, & Rosa 2021). Why Τ. hockingsi would show higher mortality than T. carbonaria, however, is unclear. One possibility is that the two species differ in their thermal physiology (Nacko et al. 2023). The minimum foraging temperature of T. hockingsi is around 20°C, compared with 18°C for T. carbonaria (Heard 2016). Though small, this difference might limit the total annual foraging hours available to each species in Brisbane, particularly for T. hockingsi in winter at the limit of their optimal climate envelope. The years of this study also spanned both El Niño (2018-2019) and La Niña (2020-2021) events in eastern Australia that might have provided stressors that more greatly affected T. hockingsi: for example, 2019 was the hottest and driest year on record in Australia and was followed by above average rainfall during the period August 2020-March 2021 (Lieber et al. 2023). Further study of T. carbonaria and T. hockingsi in regions where their ranges overlap is needed to understand how their distributions are shaped by interspecific competition.

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Queen turnover and queen longevity

The queens of social insects generally live far longer than the non-reproductive castes (Keller & Genoud 1997). Based on genotype changes in workers, we found that *Tetragonula* colonies in Toohey Forest generally requeened every 20–30 months, giving an average queen lifespan of around 1.8 years. One *T. hockingsi* colony was inferred to have maintained the same queen throughout the full 5-year period. These estimates for average queen lifespan are broadly consistent with those estimated for other stingless bee species, which range from 1.5–3 years (with maximum lifespans of 3–7 years; Grüter 2020) and for honey bees (2–5 years; Keller & Genoud 1997).

What causes queen death? Attempted nest usurpers may succeed in killing the queen, even if the takeover attempt ultimately fails. More likely, ageing queens may be killed by their own workers as egg production declines or they lay increasing proportions of maledestined (i.e., haploid, unfertilised) eggs due to a shortage of sperm. Such regicide by workers has been documented in the Neotropical stingless bee *Scaptotrigona postica* where queens have made inbred-matings and are thus producing diploid males (Vollet-Neto, Imperatriz-Fonseca, & Ratnieks 2019). In some Neotropical stingless bees, young unmated queens also participate in triggering queen supersedure (Imperatriz-Fonseca & Zucchi 1995). In a previous study, *T. hockingsi* colonies were moved outside of their range such that they had no

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nearby conspecifics to provide them with mates, which resulted in 50% of colonies having queens that had mated with their brothers and 50% failing to requeen (Paul et al. 2023). Given that we expect an average of 50% of brother-matings will result in a queen that produces a high proportion of diploid males (Hagan & Gloag 2021) while the other 50% will have no excess of diploid males, this result is consistent with *T. hockingsi* workers also killing queens that produce diploid males (Paul et al. 2023). The typical cause of death for a wellmated stingless bee queen however remains poorly understood and is very challenging to observe. In this case, behavioural observations of hived colonies are needed to shed light on how and why queen turnover is initiated (e.g., Bueno et al. 2022b).

We have provided the first estimates of rates of colony mortality, gueen turnover and nest usurpation for wild populations of T. carbonaria and T. hockingsi. Similar data from other wild populations of Australian Tetragonula across their range will help to build a more complete picture of their reproductive ecology. Moreover, the population resilience and community dynamics of social bees are determined by not only by colony mortality but also each species' fecundity. In the present study, we focused only on monitoring nests identified in the first sampling year and did not systematically search for newly founded nests each year. We recommend that future studies of wild populations aim to incorporate estimates of the typical rate of reproductive swarming and the factors that affect the survivorship of newly-founded nests, to further advance our understanding of these species.

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CONFLICT OF INTEREST STATEMENT

None of the authors have a conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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