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Main Manuscript for

Unequal reproduction early in a social transition: insights from invasive wasps

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ABSTRACT

In eusocial insects, nestmate queens can differ in their reproductive output, causing asymmetries in the distribution of mutual benefits. However, little is known about how reproductive success is partitioned in incipiently polygynous species, which would provide clues about the evolutionary forces shaping the emergence of polygyny. Here, we leverage a recent transition from predominantly single-queen (monogyne) to multiple-queen (polygyne) colonies in an invasive yellowjacket to investigate whether queens in incipiently polygyne colonies invest equally in reproductive effort or vary in their relative investment in each caste. We excavated nine polygyne *Vespula pensylvanica* colonies in Hawai'i and used RAD-sequencing to infer the parentage of worker, male, and gyne (daughter queen) pupae from each nest comb. In four colonies with at least eight gyne pupae, a single queen produced most or all gynes. These queens had no male offspring and few worker offspring, suggesting that a subset of nestmate queens might exploit the collective benefits of newly polygyne societies. In contrast to most queens, gyne-producers had offspring distributed non-randomly across nest combs. Nestmate queens generally exhibited low relatedness levels. Our results suggest that rapid, ecologically driven transitions to polygyny among unrelated queens may, at their onset, be vulnerable to reproductive asymmetries that are likely evolutionarily unstable. More broadly, this study contributes to the understanding of social evolution by uncovering asymmetric partitioning of reproduction in a population with newly evolved polygyny and raises questions about the future trajectories of introduced populations.

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Introduction

Social organisms exhibit variation in mode and extent of cooperation, relationships among group members, and societal conflicts (e.g. Bernasconi, Krieger, & Keller, 1997; Chiarati, Canestrari, Vila, Vera, & Baglione, 2011; Hughes & Boomsma, 2008; Strassmann, Zhu, & Queller, 2000). Much of this variation evolved after an initial transition from a solitary to social lifestyle. In Hymenoptera, for example, the common ancestors of eusocial lineages are thought to have possessed a monandrous and monogyne social structure, such that the initial emergence of division of labor occurred in the context of single-family groups (Boomsma, 2009; Hughes, Oldroyd, Beekman, & Ratnieks, 2008). Contemporary social insect species exhibit diverse social systems, including variation in queen and mate number (polygyny and polyandry, respectively), both of which lower average nestmate relatedness. Low relatedness, in turn, may open the door for novel intracolony conflicts to arise, raising questions about the conditions that favor the formation and maintenance of groups of non-relatives (Bourke, 2011; Keller, 1995). To understand the drivers and trade-offs associated with transitions from monogyny to polygyny or from monandry to polyandry, we ideally should study systems early in these social transitions. To date, however, the majority of research on social transitions has focused on systems with a long evolutionary history of polygyny or polyandry (reviewed in Boomsma, Huszár, & Pedersen, 2014).

Some invasive insect populations undergo rapid shifts in social organization post-introduction and therefore provide an opportunity to examine the early stages of social transitions, although we note that the roles of drift and selection may differ in evolutionary transitions associated with human-facilitated invasions. Within these

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systems, we can investigate the extrinsic and intrinsic conditions that may facilitate the social transition. We can also observe the dynamics present in colonies wherein queens and workers are experiencing novel social conditions that deviate from the conditions experienced by their recent ancestors. Research on the causes of social transitions in invasive social insects has focused predominantly on transitions to greatly expanded supercoloniality (e.g. in Argentine ants, Tsutsui, Suarez, Holway, & Case, 2000) or from monogyny to polygyny (e.g. convergent transitions in *Vespula pensylvanica, V. vulgaris,* and *V. germanica*, Gambino, 1991; Goodisman, Matthews, Spradbery, Carew, & Crozier, 2001; Leathwick & Godfrey, 1996; Loope, Millar, & Wilson Rankin, 2018). In both cases, studies have highlighted the role of resource availability and environmental clemency as extrinsic factors that may facilitate the formation of immense colonies (supercoloniality reviewed by Helanterä, 2022; polygyny: Boulay, Arnan, Cerdá, & Retana, 2014; Gambino, 1986). In parallel, many invasive populations undergo extreme bottlenecks during introduction, leading to a dramatic reduction in the ability to discriminate between nestmates and non-nestmates (e.g. Tsutsui, Suarez, & Grosberg, 2003; Foitzik, Sturm, Pusch, D'Ettorre, & Heinze, 2007; Schmidt, d'Ettorre, & Pedersen, 2010). While we are starting to develop a clear picture of the factors that may favor the initial social transitions observed in invasive social insects, we still know little about how individuals within colonies respond to novel social conditions. In newly polygynous populations, a critical question is whether queens continue to reproduce as they would in monogyne colonies, investing in worker and sexual production, or whether they modulate their reproductive effort in response to the presence of nestmate queens.

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In social insects with a long evolutionary history of polygyny, we observe substantial diversity in how queens share reproduction within their nest (Table S1; Figure 1). At one end of the spectrum, social insect nestmate queens exhibit equal contributions to worker production, such as in the ant *Pachycondyla inversa* (Heinze, Trunzer, Hölldobler, & Delabie, 2001) and the communally-nesting bee *Halictus sexcinctus* (Richards et al., 2003). At the other extreme, cheating queens – those that preferentially produce new queens instead of workers and thus obtain an unequal share of the colony's reproductive output – have been shown to form genetically distinct lineages (e.g. the ant *Pristomyrmex punctatus,* Dobata et al., 2009). The emergence of genetically distinct lineages that specialize in producing reproductive offspring could ultimately lead to incipient speciation of cheater lineages and hosts, as appears to be underway in the macrogyne/microgyne system of *Myrmica rubra* (Savolainen & Vepsäläinen, 2003; Schär & Nash, 2014). Although we highlight extreme cases, most polygyne species are likely intermediate on the reproductive partitioning continuum, as observed in the ant *Pheidole pallidula*, wherein queens vary in their contributions to different castes but most queens produce some sexual offspring (Fournier, Aron, & Keller, 2004). The degree of reproductive asymmetry among nestmate queens is expected to influence the stability and evolutionary trajectory of incipiently polygynous societies, as high asymmetry may result in very low relatedness between most colony members and the colony's reproductive offspring.

In the present study, we investigate how queens share a nest, both in terms of reproductive contributions and spatial partitioning, when they experience novel social conditions following an invasion-mediated transition to polygyny. The western

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yellowjacket, *V. pensylvanica,* spread from its native range in western North America and became established in Hawai'i in the 1970s (Gambino & Loope, 1992). Polygyne colonies have been found at low frequencies (less than 1%) in the native range, both in Washington (Akre & Reed, 1981; Reed & Landolt, 2005) and in California (Ratnieks, Vetter, & Visscher, 1996; Visscher & Vetter, 2003). However, up to 82% of Hawaiian colonies exhibit polygyny (Hanna et al., 2014; Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021). In general, little is known about reproductive asymmetries among nestmate queens in polygyne *Vespula* populations. A recent study on one of the few *Vespula* species known to be socially polymorphic within its native range, *V. squamosa*, revealed preliminary evidence that some genetic differences exist between castes in at least a subset of perennial and polygynous colonies (Dyson et al., 2022). Previous studies of reproductive competition in *Vespula* found low levels of paternity skew in some colonies of *V. germanica* (Goodisman et al., 2002) and *V. maculifrons* (Goodisman et al., 2007), but we are aware of no other studies specifically investigating reproductive asymmetries among *Vespula* matrilines. To fill this knowledge gap in *Vespula* wasps and to investigate how reproduction is shared in an incipiently polygyne population, we ask how nestmate queens share reproductive effort by sequencing dozens of age-matched worker, gyne, and male pupae from Hawaiian polygyne colonies.

Prior to the present study, we knew that *V. pensylvanica* polygyne colonies can harbor unrelated queens and that queens are multiply mated (Hanna et al. 2014; Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021). The form of polygyny observed in *Vespula* appears to be akin to that of many ant lineages in that multiple

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queens coexist and contribute to reproduction simultaneously throughout the life span of the colony. Since we had little information about how *Vespula* queens partition reproductive effort in general, we looked to previous research on reproductive partitioning in other social insects (summarized in Table S1) to develop a conceptual framework for this study (Figure 1). Table S1 provides a comparative framework of reproductive output by nestmate queens and/or males across systems with different modes of colony establishment and variation in the relatedness of nestmates. We note that the form of polygyny observed in *Vespula* differs from that observed in many other social wasps. Members of *Polistes* and most *Ropalidia* exhibit a social structure in which one queen dominates reproduction but subordinate nestmates can take over as the primary reproductive in their nest after the death of the previous queen (Hughes, Queller, Strassmann, & Davis, 1993; Sumana & Gadagkar 2001). Epiponine species can exhibit functional polygyny for an extended period, during which they produce males and workers, but future queens are usually produced when only a single queen remains in the colony (Queller, Negrón-Sotomayor, Strassmann, & Hughes, 1993). Since *Vespula* queens in polygynous colonies are known to produce offspring simultaneously, *V. pensylvanica* may fall anywhere on the reproductive continuum (Table S1). Our null hypothesis for *V. pensylvanica* is that nestmate queens without a long evolutionary history of cooperation will exhibit equal reproductive and similar caste contributions, mirroring the reproductive investments of monogyne queens. We expect this outcome to be particularly likely if nestmate queens are close relatives (e.g., sisters). Our alternate hypothesis is that some queens exploit collective benefits of incipiently polygyne societies by specializing in the production of sexuals and/or

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producing more offspring than their nestmates. This outcome would be more likely if nestmate queens were unrelated. In this case, cheating queens with lower-than-average relatedness to other queens in the colony could effectively act as social parasites of their colonies. Identifying where *V. pensylvanica* colonies fall on this continuum will reveal the degree to which conflict and exploitation among nestmate queens shape incipiently polygyne societies, with possible implications for the long-term evolutionary stability of the polygyne form in invasive populations.

Yellowjacket nests contain multiple layers of comb where eggs are laid and offspring develop. Given the spatial structure of these nests, we further hypothesize that incipiently polygyne queens could be territorial within the nest, laying their eggs in a subset of available combs. Intracolony territoriality is found in other cooperative species. In polygyne *Polybia occidentalis* wasps, brood developing on the same nest comb tend to be more closely related than brood on different combs (Queller, Negrón-Sotomayor, Strassmann, & Hughes, 1993). In the communal spider *Parawixia bistriata,* individuals share a bivouac by day and defend individual webs linked by shared scaffolding lines during the night (Uetz & Hieber 1997; Wenseleers et al., 2013). A form of reproductive territoriality has also been identified in the ant *Cardiocondyla venustula*, wherein males can defend spatial territories within their natal nest (Frohschammer & Heinze, 2009). On this basis, we ask: *does each queen produce offspring in a specific area of the nest (displaying territoriality), or do they produce offspring across different combs?* Answers to this question across species will elucidate whether physical space and nest architecture facilitate nest sharing and reproductive partitioning in incipiently polygyne populations.

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Overall, this study advances our understanding of reproductive partitioning on two levels. First, we provide a more detailed look at the composition of nestmate queens and their relative reproductive investment within the colonies of a damaging invasive yellowjacket species. Insights into intracolony dynamics, particularly how large polygyne colonies form and persist, in the invasive populations of this species may help to inform future population control efforts. More broadly, we examine whether queens experiencing a novel social environment employ different reproductive strategies, which illuminates whether intracolony conflicts are present in incipiently polygyne societies.

Materials and Methods

Study Species

Vespula pensylvanica was first reported in Hawai'i in 1919 (Williams, 1926) but was not widespread until the late 1970s, likely due to additional introductions (Hollingsworth et al., 2009). The polygyne colonies in invasive populations can reach enormous sizes, dramatically increasing pressure on their native arthropod prey (Wilson, Mullen, & Holway, 2009). Populations show little genetic structure across the native range but substantial genetic structure within and between the Hawaiian Islands, which may reflect the effects of geographic barriers and invasion history (Chau et al., 2015). Previous studies that genotyped microsatellite markers in foragers revealed that polygyne colonies can harbor several queens, some of which may be unrelated (Hanna et al., 2014). *Vespula pensylvanica* queens are polyandrous in both native and introduced populations, with average effective mate numbers of 3 and 4-6, respectively (Hanna et al. 2014; Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021). Nests

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of *V. pensylvanica* are usually subterranean or found in other dark cavities (Akre, 1981), constructed using a paper-like material made of wasp saliva and chewed wood (Spradbery, 1973), and contain multiple stacked combs of hexagonal cells in which brood are reared (Figure 2). Queen offspring are reared in cells much larger than worker cells, which occur in spatially distinct areas of the nest in native colonies (typically the lowest combs (Visscher & Vetter, 2003)).

Study Site and Nest Excavation

We excavated nests of nine large *V. pensylvanica* colonies in the vicinity of Volcano, Hawai'i (19.4 ° N, 155.2 ° W) in August 2017 (Table 1). We collected colonies on private property with permission of the owners and from Hawai'i Volcanoes National Park (permit number HAVO-2015-SCI-044). During excavation, we collected adult wasps using a vacuum and removed the nest structure, keeping the combs intact whenever possible. Four nests could not be collected intact due to their size or their position among large plant roots. We froze combs soon after collection. In three cases, the colony's size exceeded that manageable by vacuum, so we placed 5% carbaryl insecticide (Sevin, Garden Tech) at the nest entrance 24 hours before excavation. We shipped frozen nests to the University of California Riverside for further processing.

Pupa DNA Extraction and Sequencing

From each colony, we extracted DNA from age-matched (fully sclerotized) pupae that could be distinguished morphologically, sampling 48 worker pupae, as many male pupae as available up to 48, and as many gyne pupae as available (Table 1). We made

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an effort to balance replication at two levels: the colony level (n = 9 field colonies) and the individual level (n = 961 pupae extracted), in order to have sufficient within-colony resolution to assess variation in reproductive effort and to sample from all nest combs, while also being able to compare multiple field colonies. By sampling age-matched pupae, we avoided sampling individuals that may have drifted into the colony and accurately assessed each queen's investment in each of the three castes while mitigating the effects of differential brood survival during and after eclosion. We sampled pupae directly from all combs for intact nests (N = 5) in approximately equal quantities and mapped the relative location of each cell. In four nests that were not intact, we sampled in equal proportions from each comb fragment.

We extracted DNA from heads using a QIAGEN DNeasy Blood & Tissue Kit, following the insect tissue protocol with several modifications (we manually ground tissue in liquid nitrogen, used alternatively sourced spin columns (BPI-tech.com), and used 70% ethanol for the second DNA wash). We then used a customized low-density double-digest restriction site associated DNA sequencing (RADseq) approach to sequence samples (Brelsford, Dufresnes, & Perrin, 2016), with minor modifications (following Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021). We sequenced 961 wasp pupae (150 bp paired-end sequencing) on the HiSeq X Ten (Illumina) platform at Novogene Genetics.

Bioinformatics

We demultiplexed reads using the process_radtags module in Stacks version 2.0 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013), aligned reads to the *V.*

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pensylvanica genome (Harrop et al., 2020) using Bowtie 2 version 2.3.4.1 (Langmead & Salzberg, 2012), and called variants using the mpileup command of SAMtools version 1.8 (Li, 2011; Li et al., 2009). We filtered the resulting raw variants using VCFtools version 1.15 (Danecek et al., 2011), retaining genotype calls with a minimum quality score of 20 (--minGQ 20), removing loci with >10% missing data (--max-missing 0.9), and excluding individuals with more than 50% missing data. Filtering resulted in 1090 retained loci in 873 pupae. To obtain loci suitable for parentage and relatedness analysis (see below), we removed loci with a minor allele frequency <10% (--maf 0.1) and a read depth of 2 or less (--minDP 3). We then used PLINK version 1.9 (Purcell et al., 2007) to filter one locus of each pair in a sliding 200kb window using a threshold $r^2 > 0.1$ (Andrews et al., 2018), resulting in a final set of 143 unlinked loci that were distributed across the genome, with 1-19 loci on all but one of the 25 chromosomes.

Analyses

Aim 1: How numerous and how related are reproductives within each colony?

To estimate full- and half-sibling relationships and assign putative parentage, we used the program COLONY version 2.0.6.5 (Jones & Wang, 2010; Table 1), using run parameters specified in Table S2. We performed identical COLONY runs on simulated data to verify that our genotype data were powerful enough to accurately estimate parentage under various possible colony structures (see supplementary materials for details). To validate the relationships determined by COLONY, we estimated the relatedness of the predicted full- and half-sibling groups using the method of moments estimator (Huang, Ritland, Guo, Shattuck, & Li, 2014) in the program PolyRelatedness

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V1.8 (Huang et al., 2015). We compared the pairwise relatedness values of putative siblings to those of non-siblings (Figure S1).

We also used PolyRelatedness to generate relatedness matrices for inferred queen genotypes. COLONY provides probability estimates for inferred parental genotypes at each locus when the genotype is uncertain. We averaged relatedness estimates across 100 samplings from estimated queen genotypes, with each queen's potential genotype at each locus sampled in proportion to their relative probability estimated by COLONY. To assess whether nestmate queens were more likely to be related than non-nestmates, we carried out a randomization test, simulating (over 5,000 repetitions) the difference in mean relatedness between nestmate queens and non-nestmate queens, and compared the distribution to the observed difference in means. Two distinct routes to colony requeening have been suggested to occur in Hawai'i: joining of outside unrelated queens and recruitment of daughter queens (Gambino, Medeiros, & Loope, 1990). Thus, we compared the nestmate average queen relatedness to the population-wide queen average relatedness to assess the form of requeening occurring in our studied colonies. We also calculated observed mate number and effective paternity (ke3; Nielsen, Tarpy, & Reeve, 2003) for queens with at least 10 female offspring and one queen with eight offspring that exhibited significantly skewed reproduction favoring gynes.

Aim 2: Do queens vary in their investment in different castes?

To identify queens with skewed reproductive output with respect to caste, we used an exact test of goodness-of-fit (using the XNomial package in R; Engels, 2014)

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on every queen that produced six or more offspring (N = 39), according to the COLONY parentage assignments, with a Benjamini-Hochberg false discovery rate (FDR) correction for multiple comparisons (Benjamini & Hochberg, 1995). An exact test evaluates whether a set of counts fits an expected ratio. We expected each queen to produce three castes in frequencies proportional to the caste ratios of our pupal samples.

To determine if gyne- and male-producing specialist queens were less related to nestmates than average (in the five colonies where we observed differential effort in producing sexual and worker offspring), we used the relatedness matrices generated for inferred queen genotypes (see Aim 1). We compared the average pairwise relatedness values of the gyne-producing and male-producing queens to the average pairwise relatedness values between all queens in the colony using randomization tests (5000 repetitions, as above).

To complement the relatedness-based comparison of gyne- and worker-producing queens, we carried out a genome-wide association study (GWAS) to seek consistent genetic differences between gyne and worker pupae collected across all colonies. Such patterns have been observed in cases of genetic caste determination in ants (e.g. Kuhn, Darras, & Aron, 2018; Cahan & Keller, 2003). Using the initial raw output of SAMtools mpileup (Li 2011; Li et al., 2009), we imputed genotype values for all missing sites in BEAGLE 4.1 (Browning & Browning, 2016). We carried out the GWAS using the reads retained from our initial VCFtools filtering steps since these were high-quality variants present in most samples. We removed males (and loci that were only variable in the entire sample) for this analysis. We used the remaining 621 loci to

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construct a relatedness matrix and run a linear mixed-effects model to examine the relationship between each locus and female caste, with underlying genetic structure as a random effect, in GEMMA (Zhou & Stephens, 2012). We acknowledge that this is a small genetic sample for a GWAS analysis, meaning that we cannot rule out undetected genetic associations in portions of the genome not covered by our focal SNPs. However, any genome-wide patterns should be detectable with this approach.

Aim 3: Do queens partition their reproduction spatially in the nest?

To determine whether queens spatially partitioned the nest in terms of offspring production, we carried out an exact multinomial test of goodness-of-fit for every queen that produced six or more offspring, with a Benjamini-Hochberg FDR adjustment. The null hypothesis for this test was that each queen reproduces in equal proportion across all combs of their nest. Since our sample was representative of the distribution of pupae across the combs, we compared the distribution of offspring of each queen across combs to the number of pupae that we sampled from each comb in the five colonies for which we had intact nests.

Results

Aim 1: How numerous and how related are reproductives within each colony?

Polygyne *V. pensylvanica* colonies contained up to a minimum of 44 queens, each of which mated with up to a dozen males (Tables 2 & S3). Nestmate queens were rarely full- or half-siblings (Figure S2; mean nestmate queen relatedness was 0.0270), although nestmate queens exhibited slightly higher mean relatedness than non-

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nestmates (randomization test, p = 0). A similar pattern was observed in a recent study that examined temporal variation in parentage across a set of monogyne and polygyne colonies from the same invasive population in Hawaii (Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021). Low but non-zero relatedness among nestmate queens suggests that nest joining by queens produced in other colonies is likely commonplace in this invasive yellowjacket population, but that occasional sibling or mother-daughter relationships are also present.

Aim 2: Do queens vary in their investment in different castes?

In colonies with eight or more gyne pupae (four of the nine colonies), one queen significantly dominated gyne production (Figure 3). These four "gyne-producer" queens produced an average of 80.1% +/- 1.2% of gyne pupae within their own colonies and 67% (90/135) of all gynes sampled in this study.

Seven of nine colonies (78%) had queens that produced six or more sampled offspring in total (based on COLONY-based assessment of parentage). Exact tests revealed that 13 out of 39 queens (with six or more offspring) had a significantly skewed offspring caste ratio (Figure 2, Table S3). Four of these 13 queens primarily produced gynes (as mentioned above), two queens produced exclusively male offspring, and the remaining queens produced an excess of workers. Queens were so numerous in two colonies (COLONY-inferred 30 and 44 queens) that no queens produced more than five offspring in our sample.

The average relatedness of the gyne-producing and male-producing queens to their nestmate queens was equal to or significantly greater than the average

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relatedness among all nestmate queens (Figure 4; randomization test results in Table S4). These patterns deviate from our initial expectation that queens with a lower-than-average relatedness to their nestmates might exploit a colony by specializing in the production of sexual offspring.

The GWAS results were consistent with our assessment of queen relatedness. Even though gynes and workers usually had different parents in our focal nests, we found no genetic differentiation between gynes and workers in the GWAS (Figure S3). This analysis surveyed 621 genetic variants, so we cannot rule out the presence of a genetic basis for female caste in a region of the genome that is not linked to these loci.

Aim 3: Do queens partition their reproduction spatially in the nest?

In the five nests with intact combs, nest combs were shared by multiple queens, and all queens used multiple combs (ranging from 2-10 combs). Just 10% of queens (3 of 30) had offspring developing on a non-random subset of combs, in a distribution significantly different from the expected null distribution (the overall distribution of all sampled pupae, Figure 5, Table S5), and all were gyne-producing queens. *Vespula* nests in the native range consist of several discrete combs, with each comb typically constructed entirely of either small cells, used for worker and male production, or large cells, used for gyne production (Spradbery, 1973). Unlike nests in their native range, *V. pensylvanica* pupae of all castes were distributed throughout the combs in the Hawaiian nests (Table S6). Twenty-one of 23 combs with gyne pupae also contained worker pupae; only one comb appeared to be entirely constructed of gyne cells (Table S6).

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Discussion

Vespula pensylvanica colonies in an invasive, incipiently polygyne population can contain dozens of unrelated queens that each mate multiple times. In the four nests that contained a relatively large number of gyne pupae (8-58 gyne pupae, Table 1), one queen dominated gyne production. This discovery supports our alternate hypothesis that a subset of nestmate queens might exploit the collective benefits of newly polygyne societies. In contrast to our predictions, however, the gyne-producing queens were no less related to nestmate queens than were worker-producing queens, and we detected no consistent genetic differences between gyne and worker offspring. Interestingly, gyne-producing queens were the only reproductive queens with non-randomly distributed offspring within the nest combs, raising the possibility that they monopolize gyne cells. Our sampling occurred in the first month of the reproductive season, which may explain in part the low number of gynes we collected, although many colonies in Hawaii apparently do not produce gynes at all (Gambino, 1991). While we cannot rule out that the high skew in gyne production we observed among queens could be reduced if additional queens start producing gynes as the season progresses, the observed near-monopoly on early-season gynes by a single queen per colony suggests that skew would remain substantial even if other queens begin reproducing later in the season. We also observed two cases of a mother producing exclusively male offspring. Further study is needed to determine whether some queens lay predominantly haploid eggs, whether male-producing queens were simply unmated, or, conceivably, whether these males were worker-produced.

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The reproductive asymmetry we observe is not unprecedented and is consistent with recent findings based on a small sample of polygynous *V. squamosa* colonies that identified genetic differences between gynes and workers in three out of six colonies assessed (Dyson et al., 2022). Across social insects, previous studies have documented patrilines that account for most gyne production in monogyne and polyandrous colonies of the ant *Acromyrmex echinatior* (Hughes & Boomsma, 2008) and the honeybee *Apis mellifera* (Withrow & Tarpy, 2018), as well as cheating among parthenogenetic queen lineages in the ant *Pristomyrmex punctatus* (Dobata et al., 2009; Dobata & Tsuji, 2013). Secondary polygyny, where new queens join established colonies, may be particularly prone to reproductive asymmetries, as the benefits of exploiting an unrelated colony are high and cheating may be difficult to police (Boomsma, Huszár, & Pedersen, 2014). The benefits of exploitation by joining colonies of non-relatives are similar to those found in intraspecific worker parasitism, whereby workers gain direct fitness and incur minimal costs by drifting to unrelated nests and laying eggs (Lopez-Vaamonde, Koning, Brown, Jordan, & Bourke, 2004; Oliveira, Oi, Vollet-Neto, & Wenseleers, 2016). In some cases, workers can also produce future gynes through thelytokous parthenogenesis; these workers may lay gyne-destined eggs in a foreign colony, as observed in the Cape bee *Apis mellifera capensis* (Jordan, Allsopp, Oldroyd, Wossler, & Beekman, 2008) and the ant *Cataglyphis cursor* (Doums, Federici, Chifflet-Belle, & Monnin, 2018). In the invasive *V. pensylvanica* case, we see caste-based reproductive asymmetries among queens in the absence of an unusual reproductive mode (e.g. parthenogenesis). Moreover, queens produced gynes with multiple patrilines (Table S8), showing that differential investment in caste is primarily

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driven by the queen and not by the complementarity between the queen and her mate, as previously observed in the harvester ant *Pogonomyrmex rugosus* (Schwander & Keller, 2008).

In social wasps and bees, the position of brood in nest combs might provide a backdrop for nepotism to occur, since workers could preferentially care for eggs in one comb or comb region, thereby directing care towards related individuals. Brood of some polygynous wasps, such as the neotropical wasp *Polybia occidentalis,* exhibit relatedness asymmetries associated with their position in the nest (Queller, Negrón-Sotomayor, Strassmann, & Hughes, 1993), while others, such as *Polybia paulista* (Kudô et al., 2005), show no evidence of spatial partitioning. *Vespula pensylvanica* seems to have a mixed strategy. Most queens laid eggs on many different combs, making spatially-mediated nepotism unlikely. However, gyne-producing queens used a subset of combs, which may point to the importance of spatial heterogeneity in mediating the reproductive output of queens. Unlike in native-range colonies, we commonly observed worker and gyne brood intermixed on multiple combs throughout the nest. This observation suggests that caste fate is not simply determined by a larva's presence on a comb of gyne cells. Instead, gyne-destined larvae may influence their trajectory through unknown means other than merely their location in the nest. More generally, whether the traits that result in unequal reproduction are the result of selection or a byproduct of preexisting behaviors applied in the new social environment warrants further investigation.

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The reproductive asymmetries observed in this study lead us to consider several potential mechanisms (Table S7), although we currently lack sufficient data to distinguish between all scenarios.

Dominance: Behavioral dominance is common in small paper wasp societies (e.g., *Polistes* wasps; Jandt, Tibbetts, & Toth, 2014) and involves physical aggression by a foundress who attacks and suppresses the reproduction of subordinate potential reproducers. Dominance is unlikely to generate a pattern where subordinates only produce workers and males but not gynes; instead, this hypothetical mechanism predicts high skew in overall offspring production (with subordinates producing few offspring). Dominance hierarchies, which often involve ritualized behaviors (Jandt, Tibbetts, & Toth, 2014), seem unlikely in *Vespula*, which has an evolutionary history of solitary queens and lethal fights between queens prior to worker emergence (Greene, 1991). However, it remains possible that such hierarchies have arisen in this novel social context. We consider this mechanism, shaped by direct aggression among reproductive individuals, distinct from the defense of a specific comb or territory within the nest (see *physical dominance of reproductive comb* below).

Variation in reproductive potential: Queens vary in their reproductive capacity, perhaps due to their age or condition. This imbalance would produce a high variance in total offspring produced by queens, but not an asymmetry in the production of castes; queens with low reproductive output would produce few gynes and few workers.

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In contrast to the preceding mechanisms, the following three mechanisms are more likely to generate the observed pattern of a single queen specializing in gyne production while other queens produce mostly workers:

Royal cheating: Some queens could "cheat" other queens by biasing caste development such that their offspring are disproportionately reared as gynes rather than workers. Possible mechanisms include replacing other queens' gyne-destined eggs with their own eggs, or genetic or epigenetic effects that cause workers to favor their larvae during gyne-rearing, perhaps through increased juvenile hormone titers or delayed molting (Greene, 1991).

Developmental switch and queen variation: Queens may intrinsically transition to becoming gyne producers late in life or in response to external cues. Old bumblebee queens produce gynes even if transplanted into younger, non-gyne-producing colonies (Alaux, Jaisson, & Hefetz, 2005), and a similar phenomenon has been suggested in *Vespula* (Potter, 1964). If queens transition to producing 'gyne destined' offspring, and queens vary in reproductive ages based on when they joined the colony, then older queens may produce gynes, while younger (or more recently joined) queens produce workers. However, a recent study on the timing of requeening in *V. pensylvanica* colonies in Hawaii based on genotyping foragers collected once per month suggests that queens join prior to colony maturity and contribute to reproduction for months (Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021). They did not detect genetic evidence of queen turnover late in the season.

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Physical dominance of reproductive comb: Queens may defend areas of comb against reproduction by other queens. If a queen uses an area of comb where workers rear gynes, that queen may produce many more gynes and fewer workers than other queens. Our observations of gyne brood throughout the nest suggest that gyne production is not restricted to specific combs (i.e. combs of queen cells at the bottom of the nest), and thus queens may not be competing for access to gyne cells. On the other hand, only offspring of gyne-producing queens were non-randomly distributed across combs within the nest, so we cannot rule out the role of spatial territoriality in shaping variation in caste investment by queens.

If reproductive cheating occurs, the collective cost would be substantially lower if nestmates gain indirect fitness benefits from the reproductive output of gyne- and male-producing queens. However, given the typically low relatedness among queens (Figure S2), worker-producing queens would likely suffer substantial costs by not producing their own gyne offspring. This finding contrasts with queen relatedness in polygyne swarm-founding epiponine wasp colonies, in which new queens are only produced when a single queen remains and so they are full sisters (Queller, Strassmann, Solis, Hughes, & Deloach, 1993). The reproductive asymmetries among the nestmate queens of our study, and the associated low relatedness between workers and the gynes they rear (0.1061, on average, Table S9) due to simultaneous polygyny and polyandry, mean that kin selection on worker traits is likely quite limited in this population. As has been suggested for unicolonial ants (Helanterä, Strassmann, Carrillo, & Queller, 2009), the evolutionary fate of this altruistic system with very low relatedness is unclear. The

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willingness to form associations with non-relatives may have been shaped by genetic bottlenecks during the introduction of *V. pensylvanica* into the invasive range (Chau et al., 2015). For example, kin recognition mechanisms present in the native range could break down in the invasive range (Loope, Millar, & Wilson-Rankin, 2018), a pattern also associated with a founder effect in the invasive supercolonial Argentine ant (Tsutsui, Suarez, Holway, & Case, 2000). Importantly, this bottleneck-mediated route to polygyny may be an outcome of human-mediated introduction into novel environments.

Nest joining behavior in invasive populations may derive from nest usurpation by foreign queens (either conspecific or heterospecific), which has been documented in at least 14 *Vespula* species (Pickett, Osborne, Wahl, & Wenzel, 2001; Akre, 1981). In most populations, nest joining usually results in a deadly fight and occurs prior to worker emergence. Since *V. pensylvanica* colonies contain unrelated queens (Hanna et al., 2014; Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021; this study), we speculate that this behavior may be modified in invasive populations such that foreign queens are adopted by established nests instead of triggering a deadly fight. There may also be advantages to accepting foreign queens. Colonies with more queens survive longer (Scarparo, Sankovitz, Loope, Wilson-Rankin, & Purcell, 2021), and thus joining queens could expand the window of reproductive opportunity for all queens. A small percentage of such colonies can survive into a second season, achieving large colony sizes and potentially a tremendous reproductive output (Wilson, Mullen, & Holway, 2009; Loope & Wilson-Rankin, 2021).

In addition to uncovering patterns of reproductive partitioning, our investigation better characterized novel life history characteristics of these wasp societies by

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excavating nests and sequencing large numbers of brood from each. We note that this approach yielded a small colony-level sample, limiting our ability to detect inter-colony variation in reproductive strategy. However, sequencing a substantial number of pupae provides good resolution on the composition of these colonies. Our analyses reveal that polygyne *V. pensylvanica* colonies can contain up to an estimated minimum of 44 simultaneously reproducing queens, which is in line with estimates for the non-invasive, socially polymorphic *V. squamosa* (Stewart, Fritz, Fritz, & Deets, 2017). Hanna et al. (2014) found a median effective mating frequency of 2.28, ranging from 1.1 to 14.9 for 20 colonies. We found similar effective mating frequencies, but with several high outliers, suggesting that COLONY in these cases is oversplitting patrilines (Table S8). However, given the reliability of maternal sibship assignment in our simulations even when patriline membership was incorrect (see 'Simulations to validate COLONY parentage assignment' results in Supplemental Information), we believe our queen parentage assignments to be accurate. None of these suspicious mating frequency values were found in the four queens with biases toward gyne production, suggesting reliable parentage assignments for these queens. Although some nests were small enough to excavate entirely, we attempted to excavate two nests that were orders of magnitude larger in size and impossible to extract from the ground intact or accurately describe from a sample of 96 pupae. As such, we have yet to fully understand the nestmate queen dynamics in the truly immense polygyne yellowjacket colonies in Hawaii.

The findings of this study increase our understanding of the social structure of newly introduced populations of social insects and raise the question of the evolutionary

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trajectory, given a seemingly unstable fitness asymmetry. We provide a unique in-depth snapshot of colony reproduction in an invasive yellowjacket where polygyny has likely only emerged during the last ~40 generations. Invasive species offer the opportunity to study recent or ongoing evolutionary transitions. However, the evolutionary pressures faced by species introduced by human movements into non-native habitats may differ from similar evolutionary transitions that occurred millions of years ago. For example, the founder effect, enemy release, and access to naive prey could all facilitate rapid evolutionary changes in *V. pensylvanica* and other invasive social insects.

Overall, a small number of reproductive queens are responsible for the majority of gyne production, while other queens produce mostly workers and males. This discovery reveals that a form of exploitation, via unequal investment in different castes, may be present shortly after polygyny arises, placing the *V. pensylvanica* system toward the 'cheating' end of the reproductive partitioning continuum. However, gyne- and male-producing queens were not outliers displaying low nestmate relatedness or a genetic basis for caste production. These discoveries raise the possibility that early evolutionary transitions toward polygyny can include conflict (in the form of cheating queens), which may be an unstable state. As this species expands in its introduced range over time, selection may favor more extreme genetic differentiation of cheaters and hosts or mechanisms to reduce cheating, such as worker policing, that could stabilize conflict and lock in polygyny. Conversely, the seemingly unstable asymmetry may lead to selection against polygyny and a return to monogyny. Many species have unusual reproductive changes during invasions, such as clonal forms, that are thought to be unstable in the longer term as the selective pressures for dispersal and quick growth

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shift to long-term maintenance and competition (e.g. Foucaud et al., 2006; Song et al. 2013). Interestingly, this transition to polygyny does not appear to occur via philopatry of daughter queens. The presence of groups of unrelated queens suggests that polygyny yields group augmentation (Kingma, Santema, Taborsky, & Komdeur, 2014) or other synergistic benefits in this invasive population (e.g. Wilson, Mullen, & Holway, 2009) that could offset the high reproductive asymmetry among queens. We suggest in general that studying recent evolutionary transitions in social organization in invasive social insect populations could provide an unprecedented look at the conditions early in social transitions, illuminating understudied questions in social evolution.

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Statement of Authorship

J. P., E. E. W. R., and K. J. L. designed research; all authors performed research; K. J. L., J. P., and M. S. analyzed data; and K. J. L., J. P., and M. S. wrote the paper with feedback from E. E. W. R.

Data and Code Accessibility

Data have been deposited in the Dryad Digital Repository (https://doi.org/10.6086/D1566Z; Sankovitz et al. 2022). Genetic sequences reported herein have been deposited at the NCBI Sequence Read Archive with the accession number PRJNA848002.

Literature Cited

Akre, R. D. 1981. The Yellowjackets of America North of Mexico (No. 552), US Department of Agriculture, Science and Education Administration.

Akre, R. D., and H. C. Reed. 1981. A polygynous colony of *Vespula pensylvanica* (Saussure) (Hymenoptera: Vespidae). Entomological News 92:27-31.

Alaux, C., P. Jaisson, and A. Hefetz. 2005. Reproductive decision-making in semelparous colonies of the bumblebee *Bombus terrestris*. Behavioral Ecology and Sociobiology 59:270-277.

Andrews, K. R., J. R. Adams, E. F. Cassirer, R. K. Plowright, C. Gardner, M. Dwire, … and L. P. Waits. 2018. A bioinformatic pipeline for identifying informative SNP panels for parentage assignment from RADseq data. Molecular Ecology Resources 18:1263–1281.

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Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological) 57:289-300.

Bernasconi, G., M. J. Krieger, and L. Keller. 1997. Unequal partitioning of reproduction and investment between cooperating queens in the fire ant, *Solenopsis invicta*, as revealed by microsatellites. Proceedings of the Royal Society B 264:1331-1336.

Boomsma J. J. 2009. Lifetime monogamy and the evolution of eusociality. Proceedings of the Royal Society B 364:3191-3207.

Boomsma, J. J., D. B. Huszár, and J. S. Pedersen. 2014. The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. Animal Behavior 92:241-252.

Boulay, R., X. Arnan, X. Cerdá, and J. Retana. 2014. The ecological benefits of larger colony size may promote polygyny in ants. Journal of Evolutionary Biology 27: 2856-2863.

Bourke, A. F. 2011. The validity and value of inclusive fitness theory. Proceedings of the Royal Society B 278:3313-3320.

Brelsford, A., C. Dufresnes, and N. Perrin. 2016. High-density sex-specific linkage maps of a European tree frog (*Hyla arborea*) identify the sex chromosome without information on offspring sex. Heredity 2:177-181.

Browning, B. L., and S. R. Browning. 2016. Genotype imputation with millions of reference samples. The American Journal of Human Genetics 98:116-126.

This is the author's accepted manuscript without copyediting, formatting, or final corrections. It will be published in its final form in an upcoming issue of The American Naturalist, published by The University of Chicago Press. Include the DOI when citing or quoting: https://doi.org/10.1086/722514. Copyright 2022 The University of Chicago.

Cahan, S. H., and L. Keller. 2003. Complex hybrid origin of genetic caste determination in harvester ants. Nature 424:306-309.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22:3124-3140.

Chau, L. M., C. Hanna, L. T. Jenkins, R. E. Kutner, E. A. Burns, C. Kremen, and M. A. D. Goodisman. 2015. Population genetic structure of the predatory, social wasp *Vespula pensylvanica* in its native and invasive range. Ecology and Evolution 5:5573-5587.

Chiarati, E., D. Canestrari, M. Vila, R. Vera, and V. Baglione. 2011. Nepotistic access to food resources in cooperatively breeding carrion crows. Behavioral Ecology and Sociobiology 65:1791-1800.

Danecek, P., A. Auton, G. Abecasis, C. A. Abers, E. Banks, M. A. DePristo, … and R. Durbin. 2011. The variant call format and VCFtools. Bioinformatics 27:2156-2158.

Dobata, S., T. Sasaki, H. Mori, E. Hasegawa, M. Shimada, and K. Tsuji. 2009. Cheater genotypes in the parthenogenetic ant *Pristomyrmex punctatus*. Proceedings of the Royal Society B 276:567-574.

Dobata, S., and K. Tsuji. 2013. Public goods dilemma in asexual ant societies. Proceedings of the National Academy of Sciences of the USA 110:16056-16060.

Doums, C., P. Federici, P. Chifflet-Belle, and T. Monnin. 2018. Worker thelytoky allows requeening of orphaned colonies but increases susceptibility to reproductive cheating in an ant. Animal Behavior 135:109-119.

This is the author's accepted manuscript without copyediting, formatting, or final corrections. It will be published in its final form in an upcoming issue of The American Naturalist, published by The University of Chicago Press. Include the DOI when citing or quoting: https://doi.org/10.1086/722514. Copyright 2022 The University of Chicago.

Dyson, C. J., H. G. Crossley, C. H. Ray, and M. A. Goodisman. 2022. Social structure of perennial *Vespula squamosa* wasp colonies. Ecology and Evolution 12 e8569.

Engels, B. 2014. XNomial: Exact goodness-of-fit test for multinomial data with fixed probabilities. *R package version*, *101*.

Foitzik, S., H. Sturm, K. Pusch, P. D'Ettorre, and J. Heinze. 2007. Nestmate recognition and intraspecific chemical and genetic variation in *Temnothorax* ants. Animal Behaviour 73:999-1007.

Foucaud, J., H. Jourdan, J. L. Breton, A. Loiseau, D. Konghouleux, and A. Estoup. 2006. Rare sexual reproduction events in the clonal reproduction system of introduced populations of the little fire ant. Evolution 60:1646-1657.

Fournier, D., S. Aron, and L. Keller. 2004. Significant reproductive skew in the facultatively polygynous ant *Pheidole pallidula*. Molecular Ecology 13:203-210.

Frohschammer, S., and J. Heinze. 2009. Male fighting and “territoriality” within colonies of the ant *Cardiocondyla venustula*. Naturwissenschaften 96:159-163.

Gambino, P. 1986. Winter prey collection at a perennial colony of *Paravespula vulgaris* (L.) (Hymenoptera: Vespidae). Psyche 93:331-340.

Gambino, P. 1991. Reproductive plasticity of *Vespula pensylvanica* (Hymenoptera: Vespidae) on Maui and Hawaii Islands, USA. New Zealand Journal of Zoology 18:139-149.

Gambino, P., and L. L. Loope. 1992. Yellowjacket (*Vespula pensylvanica*): biology and abatement in the National Parks of Hawaii.

This is the author's accepted manuscript without copyediting, formatting, or final corrections. It will be published in its final form in an upcoming issue of The American Naturalist, published by The University of Chicago Press. Include the DOI when citing or quoting: https://doi.org/10.1086/722514. Copyright 2022 The University of Chicago.

Gambino, P., A. C. Medeiros, and L. L. Loope. 1990. Invasion and colonization of upper elevations on East Maui (Hawaii) by *Vespula pensylvanica* (Hymenoptera: Vespidae). Annals of the Entomological Society of America 83:1088-1095.

Goodisman, M. A., J. L. Kovacs, and E. A. Hoffman. 2007. Lack of conflict during queen production in the social wasp *Vespula maculifrons*. Molecular Ecology 16:2589-2595.

Goodisman, M. A., R. W. Matthews, J. P. Spradbery, M. E. Carew, and R. H. Crozier. 2001. Reproduction and recruitment in perennial colonies of the introduced wasp *Vespula germanica*. Journal of Heredity 92:346-349.

Goodisman, M. A., R. W. Matthews, and R. H. Crozier. 2002. Mating and reproduction in the wasp *Vespula germanica*. Behavioral Ecology and Sociobiology 51:497-502.

Greene, A. 1991. Dolichovespula and Vespula. In K. G. Ross & R. W. Matthews (Eds.), *The Social Biology of Wasps* (pp. 263–305).

Hanna, C., E. D. Cook, A. R. Thompson, L. E. Dare, A. L. Palaski, D. Foote, and M. A. D. Goodisman. 2014. Colony social structure in native and invasive populations of the social wasp *Vespula pensylvanica*. Biological Invasions 16:283-294.

Harrop, T. W., J. Guhlin, G. M. McLaughlin, E. Permina, P. Stockwell, J. Gilligan, … and E. J. Duncan. 2020. High-quality assemblies for three invasive social wasps from the *Vespula* genus. G3: Genes, Genomes, Genetics 10:3479-3488.

Heinze, J., B. Trunzer, B. Hölldobler, and J. H. C. Delabie. 2001. Reproductive skew and queen relatedness in an ant with primary polygyny. Insectes Sociaux 48:149-153.

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Helanterä, H. 2022. Supercolonies of ants (Hymenoptera: Formicidae): ecological patterns, behavioural processes and their implications for social evolution. Myrmecological News 32:1-22.

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going?. Trends in Ecology & Evolution 24:341-349.

Hollingsworth, R. G., G. A. Chastagner, N. J. Reimer, D. E. Oishi, P. J. Landolt, and R. E. Paull. 2009. Use of shaking treatments and preharvest sprays of pyrethroid insecticides to reduce risk of yellowjackets and other insects on Christmas trees imported into Hawaii. Journal of Economic Entomology 102:69-78.

Huang, K., S. T. Guo, M. R. Chattuck, S. T. Chen, X. T. Qi, P. Zhang, and B. G. Li. 2015. A maximum-likelihood estimation of pairwise relatedness for autopolyploids. Heredity 114:133-142.

Huang, K., K. Ritland, S. Guo, M. Shattuck, and B. Li. 2014. A pairwise relatedness estimator for polyploids. Molecular Ecology Resources 14:734-744.

Hughes, C. R., D. C. Queller, J. E. Strassmann, and S. K. Davis. 1993. Relatedness and altruism in *Polistes* wasps. Behavioral Ecology 4:128-137.

Hughes, W. O., and J. J. Boomsma. 2008. Genetic royal cheats in leaf-cutting ant societies. Proceedings of the National Academy of Sciences of the USA 105:5150-5153.

Hughes, W. O., B. P. Oldroyd, M. Beekman, and F. L. Ratnieks. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213-1216.

This is the author's accepted manuscript without copyediting, formatting, or final corrections. It will be published in its final form in an upcoming issue of The American Naturalist, published by The University of Chicago Press. Include the DOI when citing or quoting: https://doi.org/10.1086/722514. Copyright 2022 The University of Chicago.

Jandt, J. M., E. A. Tibbetts, and A. L. Toth. 2014. *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. Insectes Sociaux 61:11-27.

Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.

Jordan, L. A., M. H. Allsopp, B. P. Oldroyd, T. C. Wossler, and M. Beekman. 2008. Cheating honeybee workers produce royal offspring. Proceedings of the Royal Society B 275:345-351.

Keller, L. 1995. Social life: the paradox of multiple-queen colonies. Trends in Ecology & Evolution 10:355-360.

Kingma, S. A., P. Santema, M. Taborsky, and J. Komdeur. 2014. Group augmentation and the evolution of cooperation. Trends in Ecology & Evolution 29:476-484.

Kudô, K., S. Tsujita, K. Tsuchida, W. Goi, S. Yamane, S. Mateus, … and R. Zucchi. 2005. Stable relatedness structure of the large-colony swarm-founding wasp *Polybia paulista*. Behavioral Ecology and Sociobiology 58:27-35.

Kuhn, A., H. Darras, and S. Aron. 2018. Phenotypic plasticity in an ant with strong caste–genotype association. Biology Letters 14:20170705.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9:357-359.

Leathwick, D. M., and P. L. Godfrey. 1996. Overwintering colonies of the common wasp (*Vespula vulgaris*) in Palmerston North, New Zealand. New Zealand Journal of Zoology 23:355–358.

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Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27:2987-2993.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, … and R. Durbin. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.

Loope, K. J., J. G. Millar, and E. E. Wilson-Rankin. 2018. Weak nestmate discrimination behavior in native and invasive populations of a yellowjacket wasp (*Vespula pensylvanica*). Biological Invasions 20:3431-3444.

Loope, K. J., and E. E. Wilson-Rankin. 2021. Viral load, not food availability or temperature, predicts colony longevity in an invasive eusocial wasp with plastic life history. Scientific Reports 11:1-12.

Lopez-Vaamonde, C., J. W. Koning, R. M. Brown, W. C. Jordan, and A. F. Bourke. 2004. Social parasitism by male-producing reproductive workers in a eusocial insect. Nature 430:557-560.

Nielsen, R., D. R. Tarpy, and H. K. Reeve. 2003. Estimating effective paternity number in social insects and the effective number of alleles in a population. Molecular Ecology 12:3157-3164.

Oliveira, R. C., C. A. Oi, A. Vollet-Neto, and T. Wenseleers. 2016. Intraspecific worker parasitism in the common wasp, *Vespula vulgaris*. Animal Behavior 113:79-85.

Pickett, K. M., D. M. Osborne, D. Wahl, and J. W. Wenzel. 2001. An enormous nest of *Vespula squamosa* from Florida, the largest social wasp nest reported from North

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America, with notes on colony cycle and reproduction. Journal of the New York Entomological Society 109:408-415.

Potter, N. B. 1964. *A study of the biology of the common wasp, Vespula vulgaris L., with special reference to the foraging behavior* (University of Bristol). PhD Dissertation. Dept of Zoology.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferriera, D. Bender, … P. C. Sham. 2007. PLINK: A tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81:559–575.

Queller, D. C., J. A. Negrón-Sotomayor, J. E. Strassmann, and C. R. Hughes. 1993. Queen number and genetic relatedness in a neotropical wasp, *Polybia occidentalis*. Behavioral Ecology 4:7-13.

Queller, D. C., J. E. Strassmann, C. R. Solis, C. R., Hughes, and D. M. Deloach. 1993. A selfish strategy of social insect workers that promotes social cohesion. Nature 365:639–641.

Ratnieks, F. L. W., R. S. Vetter, and P. K. Visscher. 1996. A polygynous nest of *Vespula pensylvanica* from California with a discussion of possible factors influencing the evolution of polygyny in *Vespula*. Insectes Sociaux 43:401-410.

Reed, H. C., and P. J. Landolt. 2005. Late season polygynous *Vespula pensylvanica* (Hymenoptera: Vespidae) colonies in a northern temperate area. Pan-Pacific Entomologist 81:164-170.

Richards, M. H., E. J. von Wettberg, and A. C. Rutgers. 2003. A novel social polymorphism in a primitively eusocial bee. Proceedings of the National Academy of Sciences 100:7175-7180.

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Savolainen, R., and K. Vepsäläinen. 2003. Sympatric speciation through intraspecific social parasitism. Proceedings of the National Academy of Sciences of the USA 100:7169-7174.

Scarparo, G., M. Sankovitz, K. J. Loope, E. E. Wilson-Rankin, and J. Purcell. 2021. Early queen joining and long-term queen associations in polygyne colonies of an invasive wasp revealed by longitudinal genetic analysis. Evolutionary Applications 14:2901-2914.

Schär, S., and D. R. Nash. 2014. Evidence that microgynes of *Myrmica rubra* ants are social parasites that attack old host colonies. Journal of Evolutionary Biology 27:2396-2407.

Schmidt, A. M., P. d'Ettorre, and J. S. Pedersen. 2010. Low levels of nestmate discrimination despite high genetic differentiation in the invasive pharaoh ant. Frontiers in Zoology 7:1-12.

Schwander, T., and L. Keller. 2008. Genetic compatibility affects queen and worker caste determination. Science 322:552-552.

Spradbery, J. P. 1973. Wasps. University of Washington Press.

Strassmann, J. E., Y. Zhu, and D. C. Queller. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. Nature 408:965-967.

Stewart, S., G, Fritz, A. Fritz, and A. J. Deets. 2017. Reproductive partitioning in *Vespula squamosa* (Hymenoptera: Vespidae). Florida Entomologist 100:266-269.

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Sumana, A., and R. Gadagkar. 2001. The structure of dominance hierarchies in the primitively eusocial wasp *Ropalidia marginata*. Ethology Ecology & Evolution 13:273-281.

Tsutsui, N. D., A. V. Suarez, and R. K. Grosberg. 2003. Genetic diversity, asymmetrical aggression, and recognition in a widespread invasive species. Proceedings of the National Academy of Sciences 100:1078-1083.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, and T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Society of the USA 97:5948-5953.

Uetz, G. W., and C. S. Hieber. 1997. Colonial web-building spiders: balancing the costs and benefits of group-living (p. 458). Cambridge: Cambridge University Press.

Visscher, P. K., and R. S. Vetter. 2003. Annual and multi-year nests of the western yellowjacket, *Vespula pensylvanica*, in California. Insectes Sociaux 50:160-166.

Wenseleers, T., J. P. Bacon, D. A. Alves, M. J. Couvillon, M. Kärcher, F. S. Nascimento, ... and F. L. Ratnieks. 2013. Bourgeois behavior and freeloading in the colonial orb web spider *Parawixia bistriata* (Araneae, Araneidae). American Naturalist 182:120-129.

Williams, F. X. 1926. Notes on the habits of the bees and wasps of the Hawaiian Islands. Proceedings of the Hawaiian Entomological Society 6:425-464.

Wilson, E. E., L. M. Mullen, and D. A. Holway. 2009. Life history plasticity magnifies the ecological effects of a social wasp invasion. Proceedings of the National Society of the USA 106:12809-12813.

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Withrow, J. M., and D. R. Tarpy. 2018. Cryptic “royal” subfamilies in honey bee (*Apis mellifera*) colonies. PloS one 13:e0199124.

Zhou, X., and M. Stephens. 2012. Genome-wide efficient mixed-model analysis for association studies. Nature Genetics 44:821–824.

References Cited Only in the Online Enhancements

Chéron, B., A. L. Cronin, C. Doums, P. Fédérici, C. Haussy, C. Tirard, and T. Monnin. 2011. Unequal resource allocation among colonies produced by fission in the ant *Cataglyphis cursor*. Ecology 92:1448-1458.

Heinze, J. 1993. Habitat structure, dispersal strategies and queen number in two boreal *Leptothorax* ants. Oecologia 96:32-39.

Hölldobler, B., and E. O. Wilson. 1977. The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8-15.

Langer, P., K. Hogendoorn, M. P. Schwarz, and L. Keller. 2006. Reproductive skew in the Australian allodapine bee *Exoneura robusta*. Animal Behaviour 71:193-201.

Michener, C. D. 1974. The social behavior of the bees: a comparative study. Harvard University Press.

Premnath, S., A. Sinha, and R. Gadagkar. 1996. Dominance relationship in the establishment of reproductive division of labour in a primitively eusocial wasp (*Ropalidia marginata*). Behavioral Ecology and Sociobiology 39:125-132.

Reeve, H. K. 1991. *Polistes* In: Ross KG, Matthews RW (eds) The social biology of wasps.

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Richards, M. H., and L. Packer. 1998. Demography and relatedness in multiple-foundress nests of the social sweat bee, *Halictus ligatus*. Insectes Sociaux 45:97-109.

Schwarz, M. P., N. J. Bull, and K. Hogendoorn. 1998. Evolution of sociality in the allodapine bees: a review of sex allocation, ecology and evolution. Insectes Sociaux 45:349-368.

Seppä, P., I. Fernández-Escudero, N. Gyllenstrand, and P. Pamilo. 2008. Colony fission affects kinship in a social insect. Behavioral Ecology and Sociobiology 62:589-597.

Tschinkel, W. R. 1992. Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. Ecological Entomology 17:179-188.

West-Eberhard, M. J. 1978. Polygyny and the evolution of social behavior in wasps. Journal of the Kansas Entomological Society 832-856.

Zinck, L., P. Jaisson, R. R. Hora, D. Denis, C. Poteaux, and C. Doums. 2007. The role of breeding system on ant ecological dominance: genetic analysis of *Ectatomma tuberculatum*. Behavioral Ecology 18:701-708.

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Tables

Table 1. *Colony information.*

Colony	Number of gyne pupae sequenced	Number of male pupae sequenced	Number of worker pupae sequenced	Number of nest combs	Nest characteristics
BU	30	48	48	8	Large
CR	6	13	48	-	Small (only two combs, not intact)
J1	0	48	48	-	Very large (could not complete excavation)
J2	0	48	48	-	Large (could not complete excavation)
LE	8	48	48	5	Small
TA	4	45	48	5	Small
T1	29	48	48	9	Large
T2	0	48	48	-	Medium (could not complete excavation)
T3	58	48	48	10	Large

Note: Small nests were approximately 0.3 m^3, the medium nest (T2) was approximately 0.5 m^3, large nests were approximately 1-2 m^3, and the very large nest (J1) was so big that we could not reach its boundaries during excavation. Only some of the colonies contained a substantial number of gynes; this may be due to the fact that we excavated these colonies early in the reproductive cycle of queens, although explicit study of the timing of queen production is needed.

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Table 2. *Colony characteristics based on our estimations using COLONY.*

Colony	Minimum number of queens	Number of offspring (sum of all castes) genotyped per queen	Number of queens producing at least 6 genotyped offspring
BU	15	1-19	9
CR	5	1-22	4
J1	44	1-5	0
J2	30	1-5	0
LE	2	8-88	2
TA	6	2-26	5
T1	8	1-32	5
T2	23	1-19	5
T3	23	1-37	9

Note: The minimum queen number per colony ranged from 2-44 queens, and the average queen number per colony was 17.3 +/- 4.6 (SD).

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Figure Legends

Figure 1. Conceptual framework of this study, including three aims with their guiding questions and associated predictions or explanations. Created with BioRender.com.

Figure 2. *Vespula pensylvanica* nest layout. Nests are composed of comb layers. Each comb has many cells that hold a variety of developmental stages, from egg to emerging adult. Photographs by Madison Sankovitz.

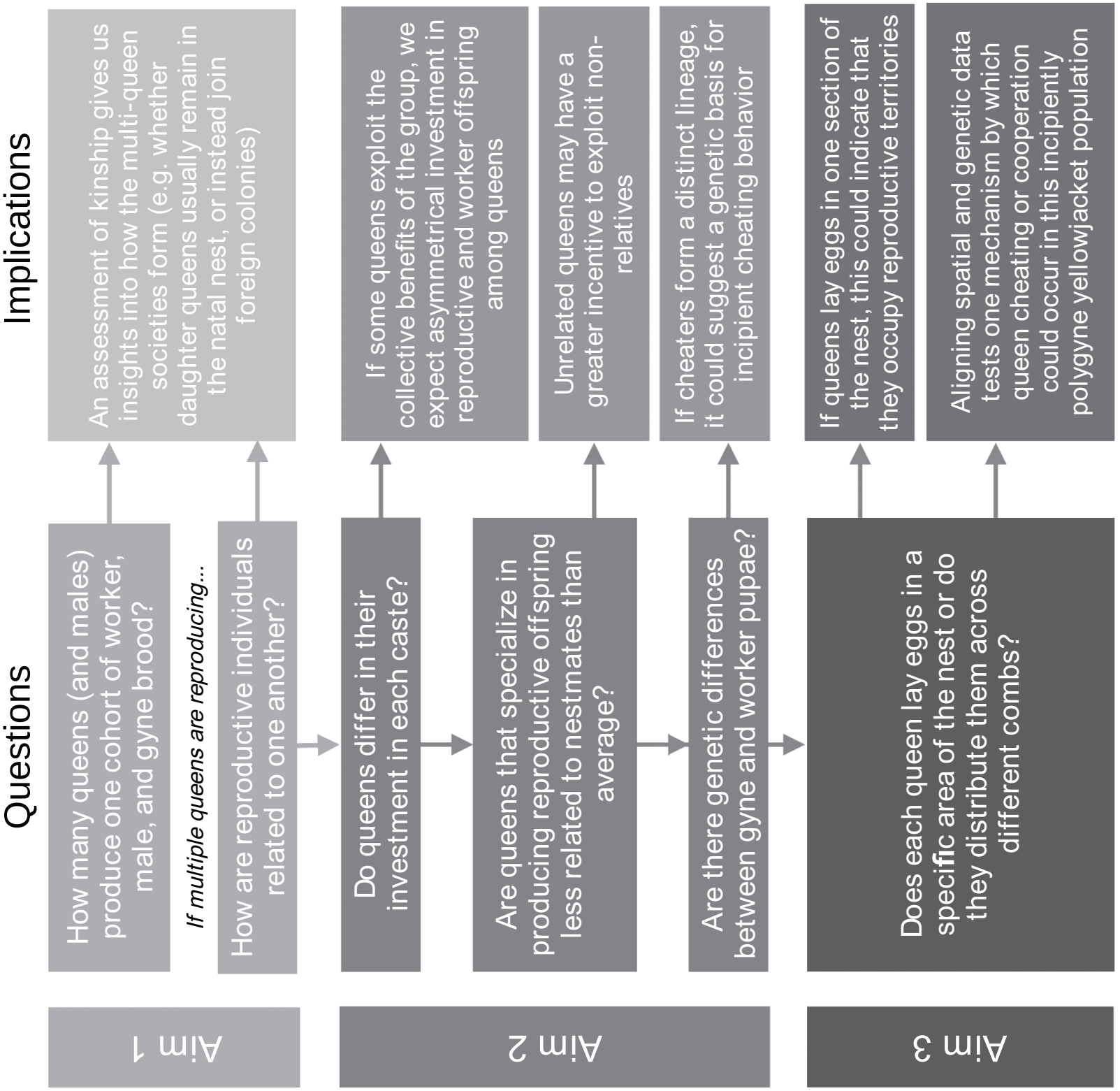
Figure 3. The number of offspring per caste per putative queen (N) for queens with six or more sampled offspring. Each graph represents a colony, and each color represents offspring produced by one queen. Bars with diagonal lines indicate queens with a significantly skewed offspring caste ratio. Asterisks indicate gynes produced by a queen that specialized in gyne production. Two analyzed colonies (J1 and J2) were omitted because all queens had fewer than six sampled offspring. Colonies are ordered by the number of gyne pupae collected.

Figure 4. Density plot showing pairwise relatedness values of all queens (grey) and pairwise relatedness values of gyne-producing queens (red) and exclusive male producers (blue) to the other queens in the colony. The vertical lines indicate the average for each group. Although the colony 'LE' had a gyne-producing queen, it is not shown because it only had two putative queens in total (relatedness = -0.171). The inferred number of queens (N) is indicated for each colony. Asterisks denote colonies in which the gyne-specialists are significantly more related to nestmates than the average colony relatedness.

Figure 5. (a) Profile view of layered combs of one western yellowjacket nest (BU), expanded into view of all combs. Colored shapes show sample locations of pupa from queens with six or more offspring. Circles=gynes, stars=workers, and triangles=males. Each putative queen is represented by a different color. (b) Number of queens with six or more offspring using each comb for egg-laying. Each graph represents one comb (layered from bottom to top), and each bar represents a colony. Note that the right-most colony (LE) had a tiny bottom comb that contained no pupae. All other combs are represented by bars. Photograph by Madison Sankovitz.

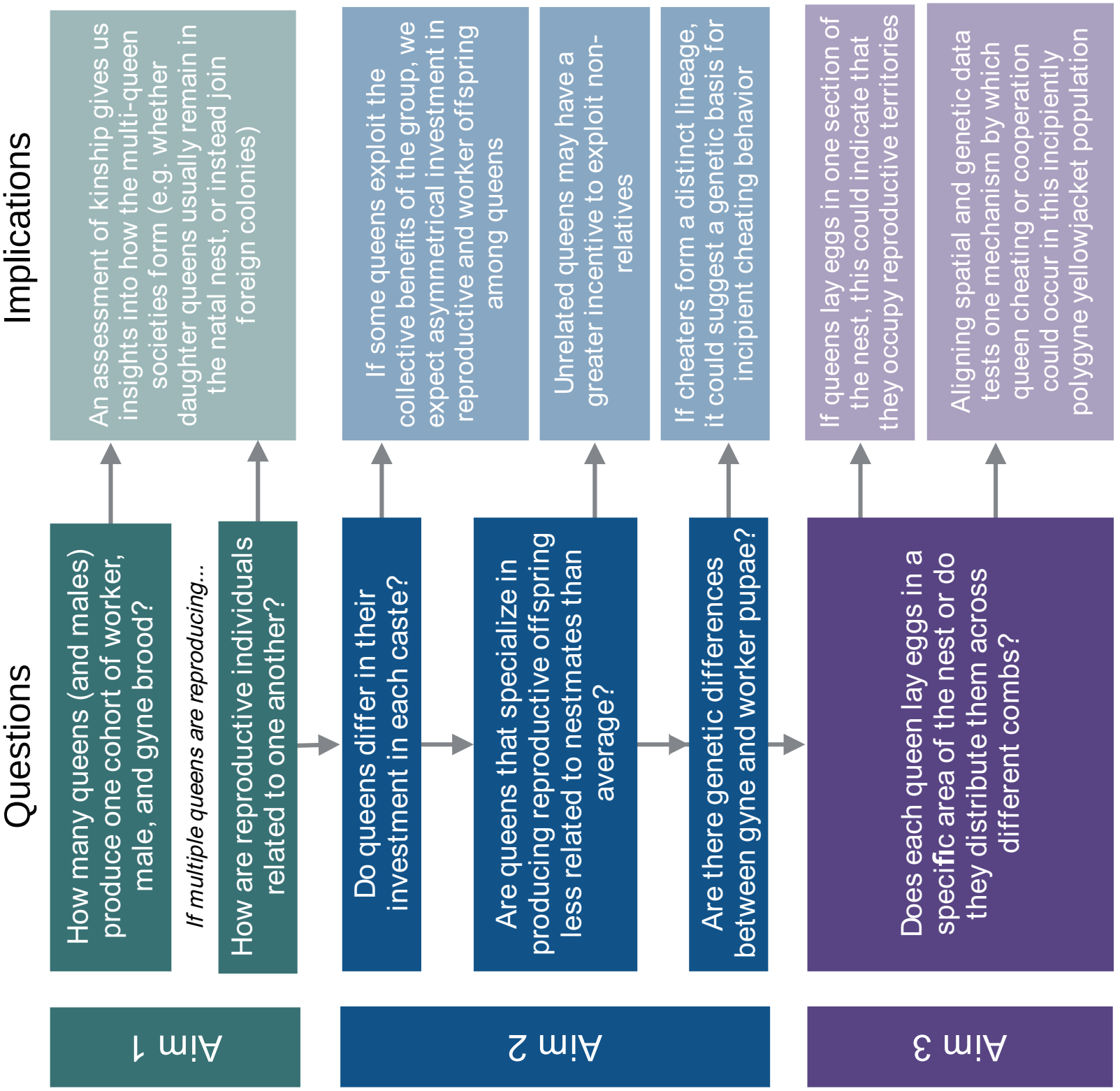
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Figure 1 (print only)



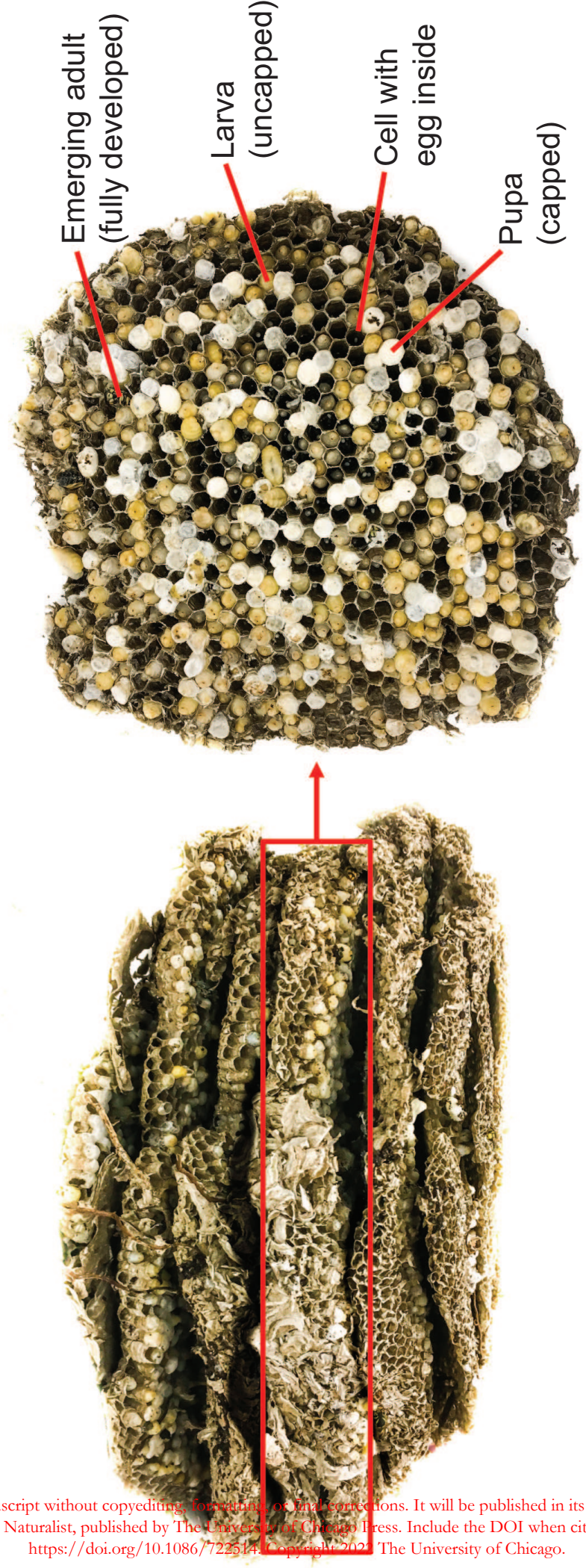
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Figure 1



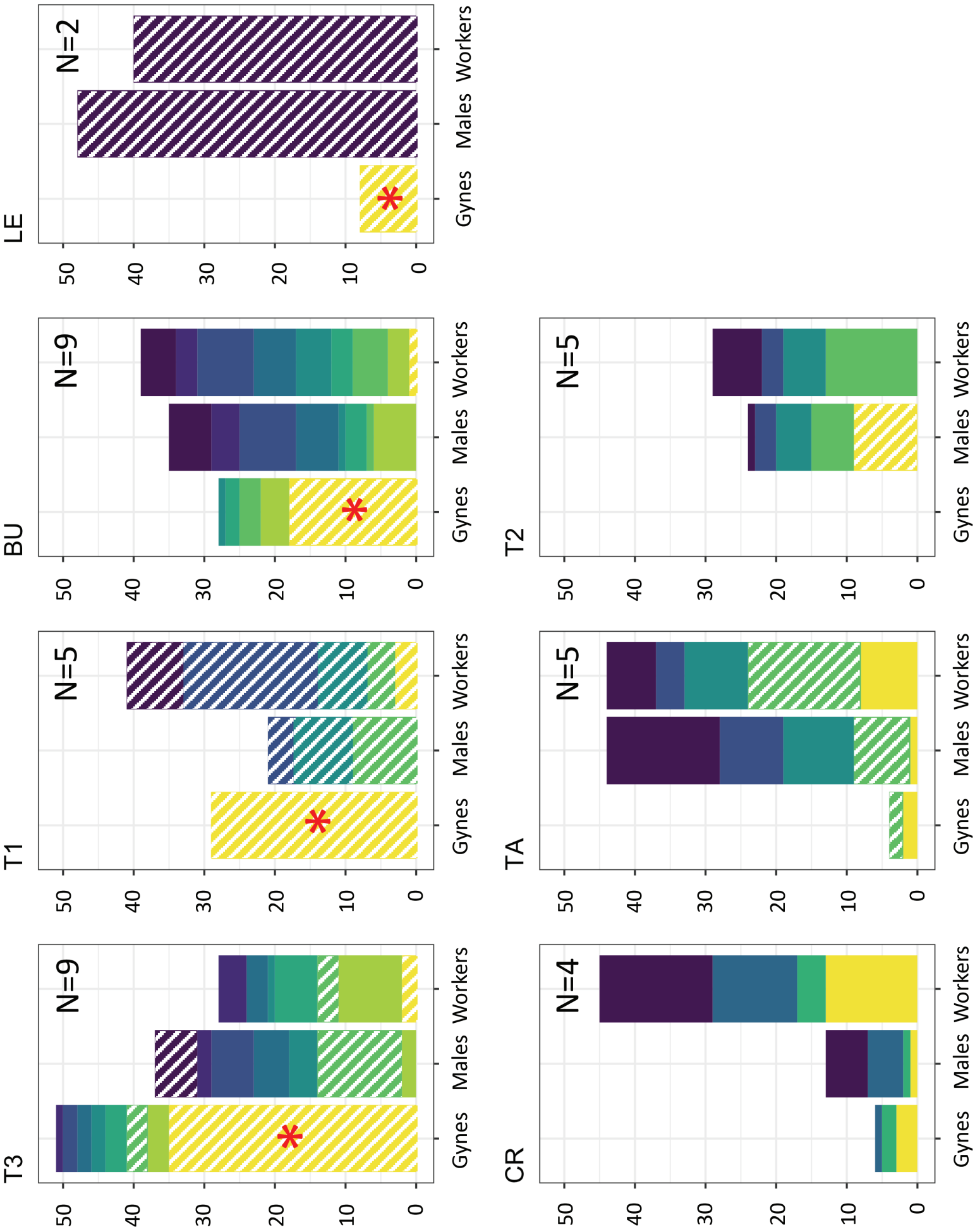
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Figure 2



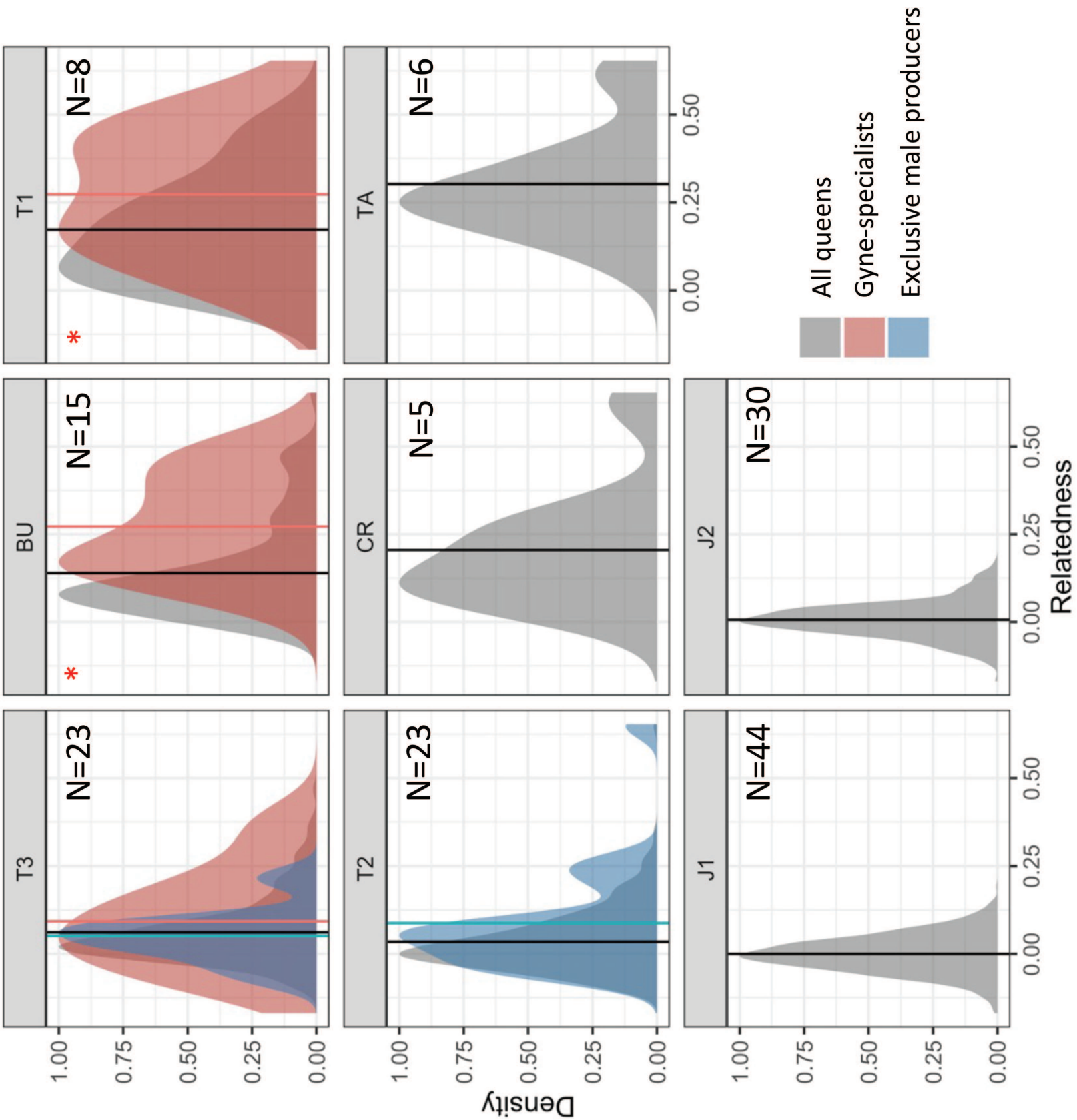
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Figure 3



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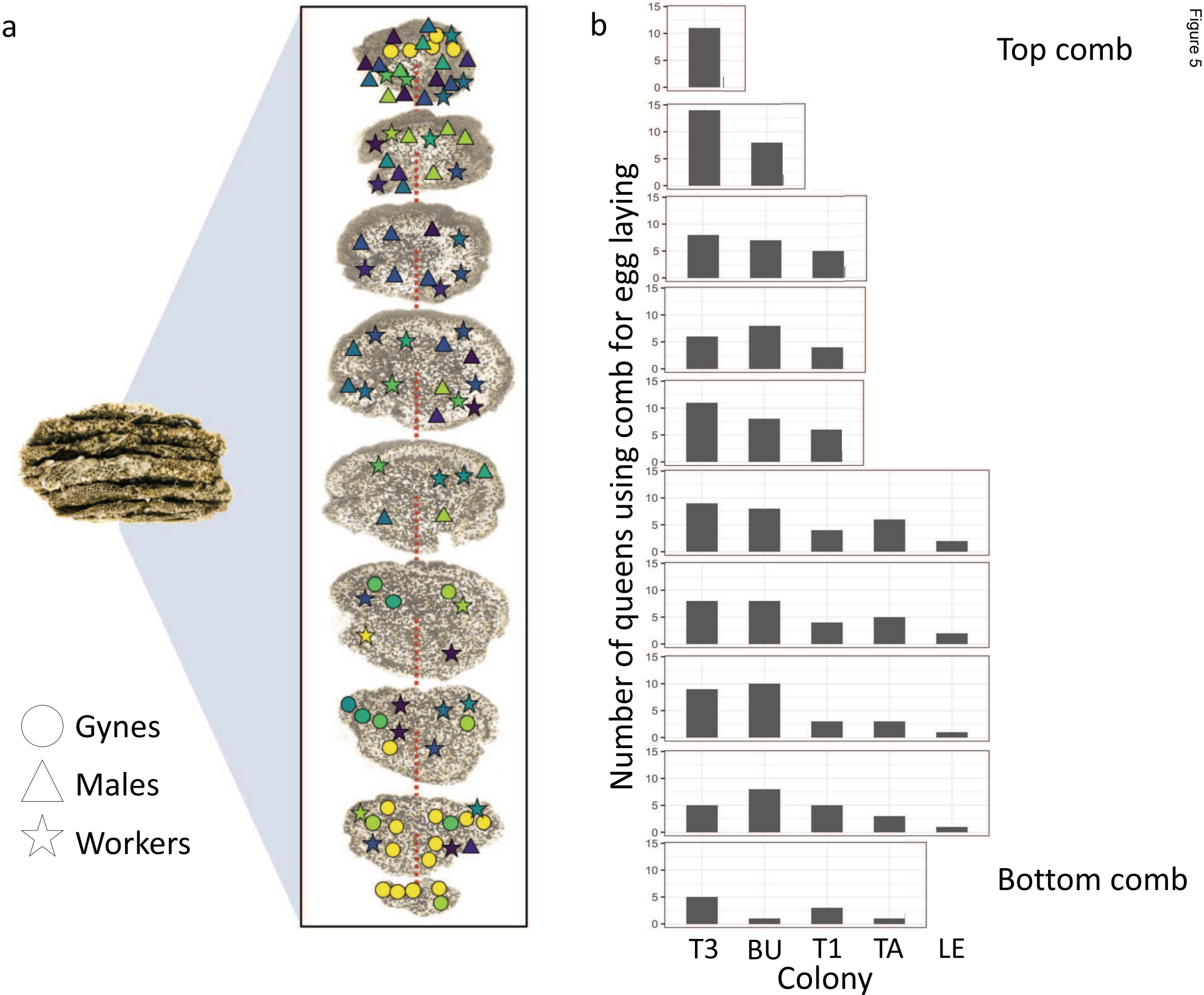
Figure 4



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Figure 5



Supplementary Files (PDF, Word, TeX, figures)

Supplementary Information for

Unequal reproduction early in a social transition: insights from invasive wasps

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Supplementary Information

Simulations to validate COLONY parentage assignment.

Although COLONY (Jones & Wang 2010) has been increasingly applied to large-scale SNP datasets and populations with complex family structures, it is important to validate the program's output for a given dataset to ensure that genotype data are sufficiently powerful to accurately estimate colony structure. This is particularly important for complex family structures like those expected in polygynous and polyandrous *Vespula* colonies, where nestmate queens may also be related. We wrote R code (code file will be deposited to Dryad with supplementary data) to generate genotypes of parents and offspring. Although COLONY provides a simulation mode, we used custom scripts to allow us to include the scenario of related queens.

Loci and allele frequencies. To generate datasets similar to our real dataset with various colony structures, we used the same number of loci as in our study (143) and used the allele frequencies from our 143 single nucleotide polymorphisms (SNPs). Simulations assume loci are completely unlinked. We randomly recoded 6.5% of genotypes as unknown to match the missing data frequency in our real dataset and added a 3% error rate by randomly assigning an incorrect allele to 3% of genotypes.

Queen number and queen relatedness scenarios. We simulated a total of 19 possible scenarios, including monogyne colonies and two types of polygynous colonies, with unrelated and related queens.

1. Monogyne colony (single queen)
2. Unrelated queen polygyne colonies: 2 or more unrelated queens produce daughters and sons
3. Related queen polygyne colonies: 2 or more half-sibling queens produce daughters and sons. Half siblings were created by drawing a random female from the population to act as the mother. This maternal genotype was combined with a new randomly drawn male genotype to produce each half-sibling.

Queen number for monogyne colonies: 1
Queen number for unrelated-queen polygyne colonies: 2, 5, 10, 15, 20, 25, 30, 40, 50
Queen number for related-queen polygyne colonies: 2, 5, 10, 15, 20, 25, 30, 40, 50
Total: 19 colony structure scenarios

Mating. Each queen was assumed to use the sperm of five unrelated males, based on an independent estimate of paternity frequency from monogyne colonies in Hawaii and Washington (Hanna et al. 2014).

Sampling colonies. To conservatively approximate our actual sampling, a genotype dataset was created for each colony consisting of 60 daughters and 40 sons. Each queen was assumed to be equally fecund, and thus equally likely to mother each offspring. To create each offspring genotype a mother was randomly selected, and (if the offspring was a daughter) one of the mother's five mates was randomly selected. Offspring genotypes were then generated by randomly drawing one of the maternal alleles and pairing it with the (haploid) father's allele at each locus.

Creating populations. For each of the 19 colony structure scenarios (i.e., combination of queen number and nestmate queen relatedness), we generated 8 independent, simulated colonies, each with the same number of queens and queen relatedness (half-sib or unrelated), constituting a genetic dataset for that scenario. We thus generated a total of 19 * 8 = 720 simulated colonies, each with 60 female and 40 male offspring. One eight-colony genotypic dataset was used for each of the 19 COLONY runs (one for each of the colony structure scenarios).

COLONY runs. COLONY run parameters were identical to those used for our real data, except for the total number of offspring (Table S2). We included a maternal sibship exclusion that tells COLONY that all individuals collected from different colonies cannot be maternal siblings. We specified multiple mating for queens but single mating for males (this assumes queens in our sampled colonies all mate with different

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males in nature, which is reasonable given the large number of males present at our field sites). Each of the 19 simulated datasets of 8 colonies was run separately on the Unix Cluster of Georgia Southern University.

Results. We first checked if COLONY could accurately estimate relationships between individuals. To do so, we generated a matrix of all pairwise relationships between colony members, identifying each relationship as either full-sibling, half-sibling, or unrelated. We then compared the true relationships (determined by the parents used to generate the simulated offspring) to the relationships estimated by COLONY (Figure S4).

In simulated colonies harboring up to 15 queens, COLONY succeeds in getting even the half-siblings correct. The key components of our analysis concern queen offspring, regardless of the father. Thus, we examined maternal siblings vs. unrelated colony-mates. To look at this, we can plot the percentage of estimated sibling relationships (maternal sibs or non-sibs) that are correct (Figure S5). We plotted accuracy as a function of the estimated number of queens in the colony from COLONY analysis, rather than the true number, as this is what is observed from the analysis of our real dataset.

We also checked the accuracy of predicted queen number by comparing the number of estimated queens per colony from the COLONY output to the true number of queens in that colony (defined in the simulation; Figure S6).

Conclusions from simulations. Our simulations show that COLONY results are not perfect for these colony structures. However, both full-sib relationships and maternal-sib relationships are likely true if predicted (Figures S4 & S5). When COLONY fails, it does so by splitting off individuals into new patrilines or matrilines but does not falsely attribute individuals to true patrilines or matrilines that they don't belong to. This means it's unlikely to be erroneously generating the observed patterns in our data. For up to 25 observed queens per colony, >90% of predicted maternal sib relationships are correct. This means we can be confident for all but two of our colonies (J1 and J2) that those individuals that are identified as daughters of the same queen are indeed sibs. For colonies with 15-25 queens, we can't be so confident in the patriline membership. However, this is not central to our question of queen caste investment. For the two colonies in which we observed >25 estimated queens, we are likely not sampling enough pupae to accurately describe the relationships between offspring.

COLONY did have trouble with the single queen scenario, often splitting off some of the patrilines into a second matriline (Figure S4). This is a known problem with COLONY when the number of sampled offspring from individual parents is very large and can be addressed using the sibship size scaling option. We only have one possible colony that could have been affected by this (colony LE with two estimated queens). We verified that the two estimated matrilines are not the result of an erroneously split single matriline using relatedness values of offspring: the offspring of putative mother 1 are related on average by 0.39. The offspring of putative mother 2 are related by 0.25. The offspring of mother 1 to the offspring of mother 2 are related by -0.07. This indicates that these two groups are truly different matrilines and not an erroneously split single matriline.

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Figures and tables

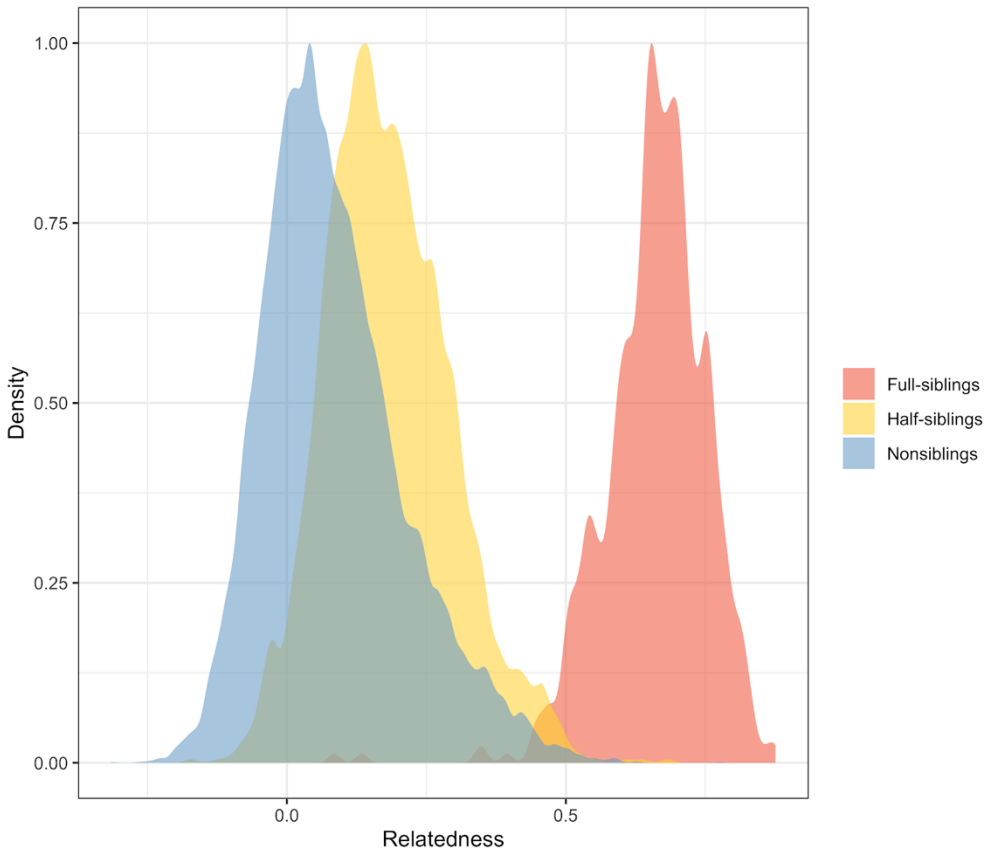


Fig. S1. Density plot (depicting a kernel density estimate) of pairwise relatedness values for worker siblings (full and half) vs. non-siblings across all colonies, as inferred by COLONY.

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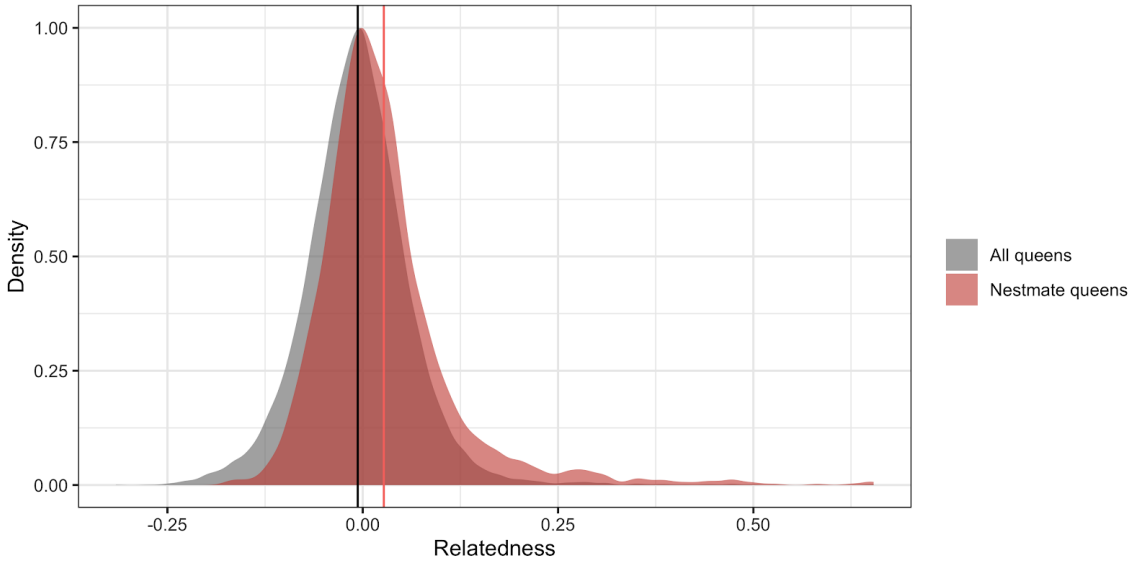


Fig. S2. Density plot (depicting a kernel density estimate) showing relatedness of nestmate queens compared to the relatedness of all queens in the population. Although the distribution of nestmate queen relatedness is positively skewed due to some rare full- and half-siblings (randomization test, p=0), most nestmate queens had a low relatedness (mean: 0.0270). The vertical lines indicate the mean relatedness of all the putative queens in the two groups.

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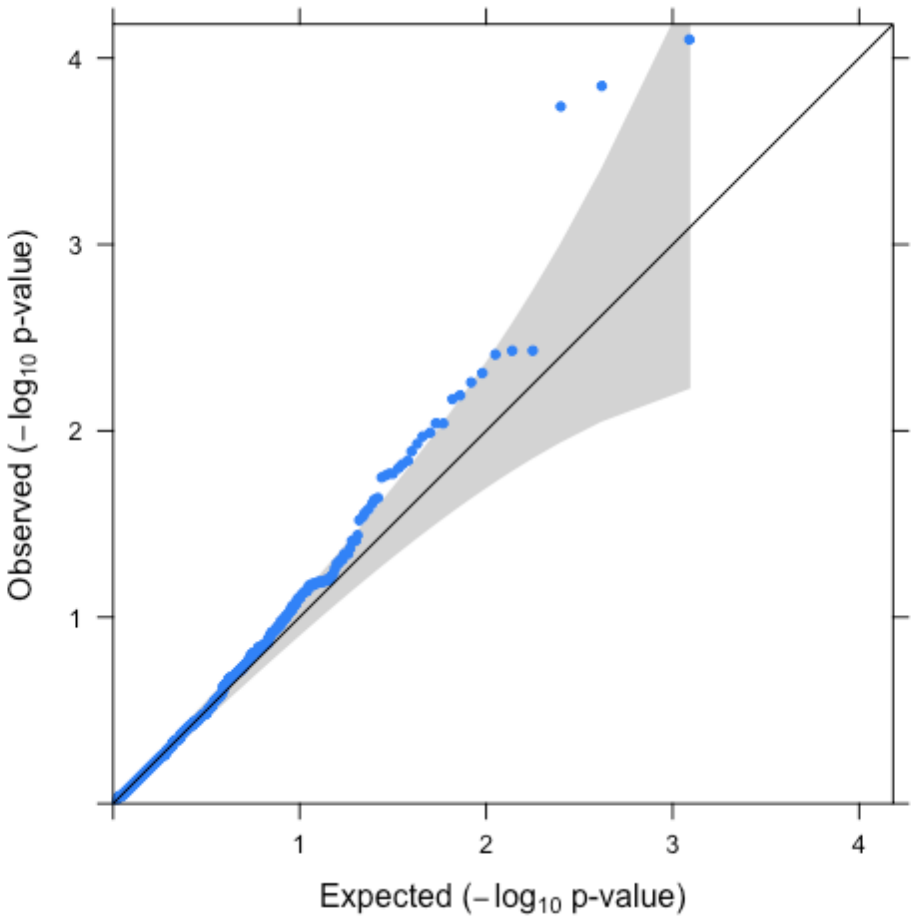


Fig. S3. A quantile-quantile plot of p-values obtained from the GEMMA analysis reveals that most p-values are within the expected distribution based on a false discovery rate of 5%, although we see some intermediate p-values outside of the 95% confidence intervals. We conclude that we have detected no genomic regions that we can confidently attribute to distinguishing gyne and worker offspring.

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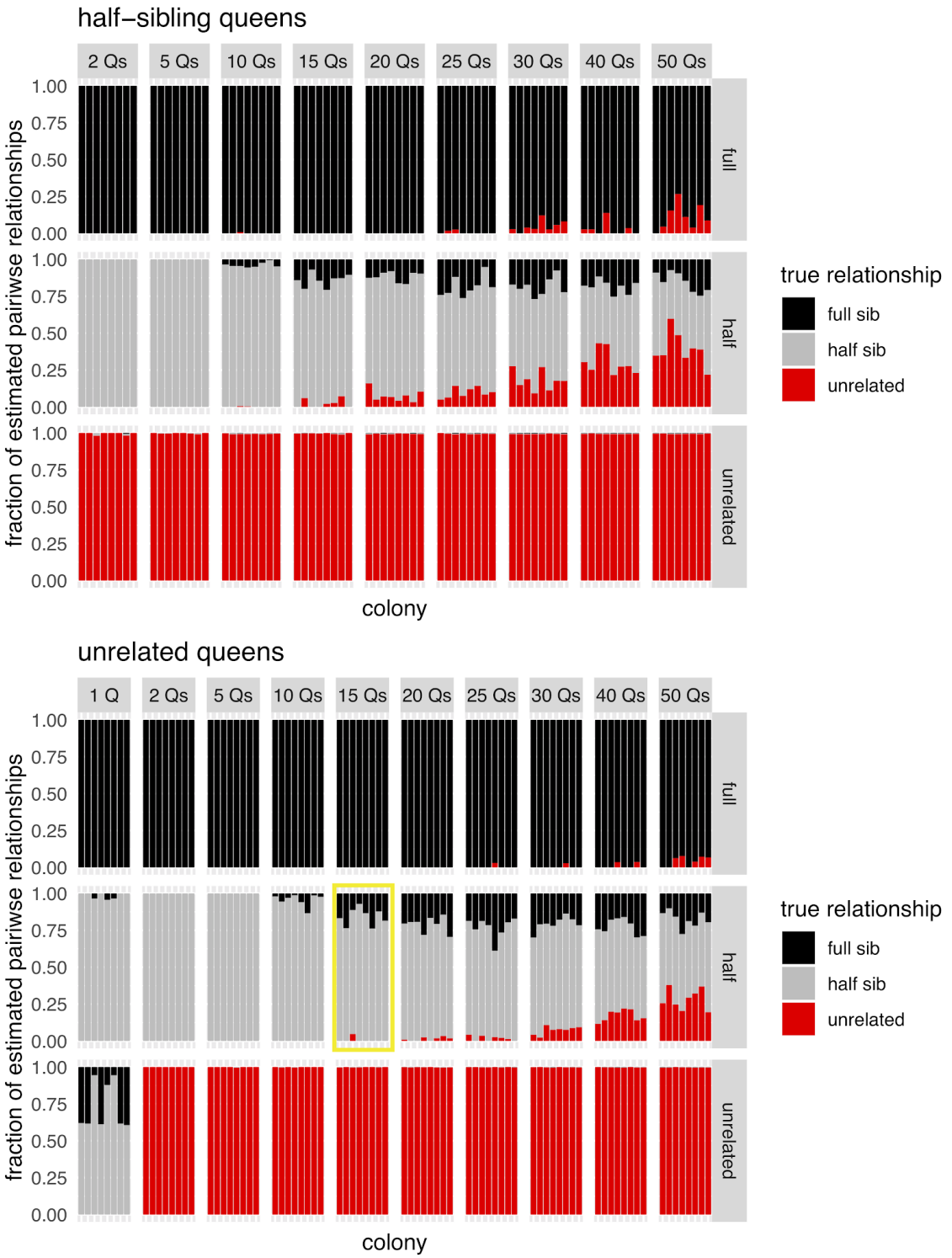


Fig. S4. The accuracy of COLONY analysis on simulated data. Horizontal panels show the types of estimated relationships in the COLONY output. Each group of 8 vertical bars represents the 8 colonies in a simulation, and there is a “full sibling”, “half sibling” and “unrelated” group for each simulation (identified by queen number at the top). The color of the bars depicts the percentage of the true relationships for

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each of the estimated relationships in each category, for each colony. For example, consider the half-siblings in the 15 unrelated queens scenario (yellow box). Approximately 10-20% of COLONY-estimated half siblings were actually full siblings in each of the colonies, and in one colony, ~5% of COLONY-estimated half-siblings were actually unrelated. The remaining relationships were correctly identified as half-siblings.

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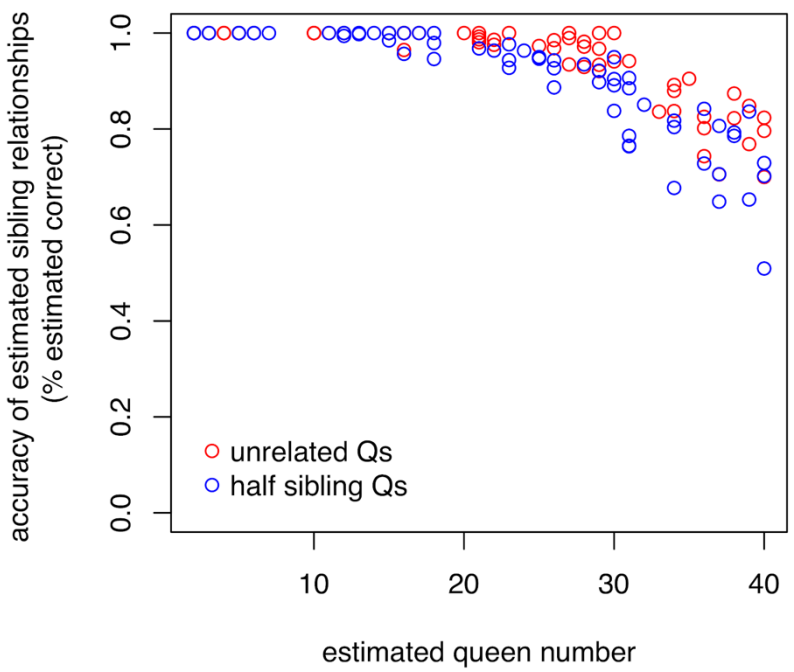


Fig. S5. The percentage of estimated sibling relationships that are correct following COLONY analysis of simulated data. Each point represents the fraction of all pairwise relationships (maternal sibling or non-sibling) that were correctly predicted in a colony under simulations of different queen numbers and queen relatedness.

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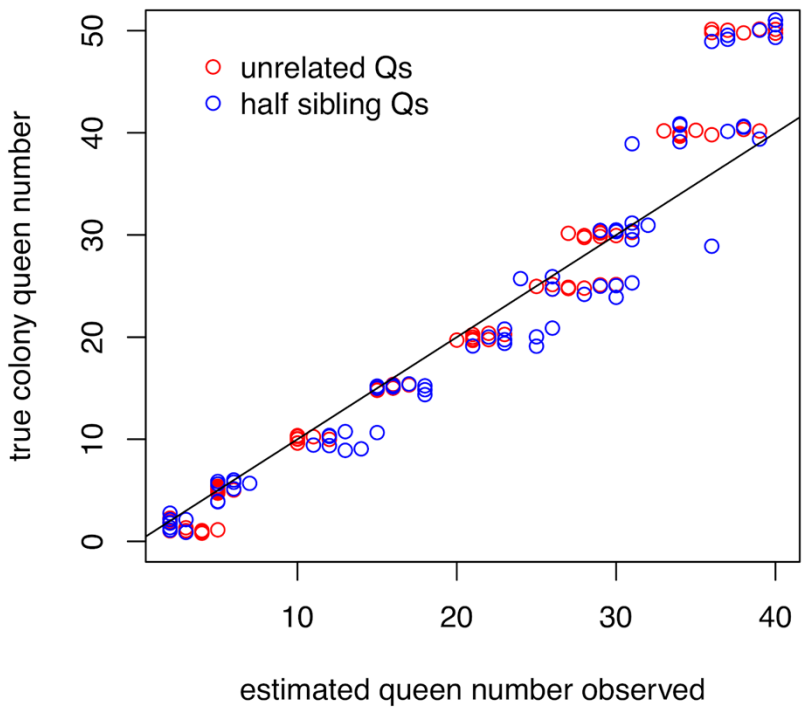


Fig. S6. Estimated queen number predicts true queen number in COLONY analysis of simulated data. The line indicates y=x. COLONY overestimates queen number for colonies with fewer than 25 queens by sometimes splitting certain patrilines of queens into additional queens, particularly in simulations with related queens. For 30+ queens, queen number is underestimated due to sampling (i.e. some queens are not represented in the sample due to the limited sample size).

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Table S1. Previous studies highlight the continuum of reproductive output by nestmate queens and/or males across systems with different modes of colony establishment and variation in the relatedness of nestmates. Little is known about reproductive asymmetries in polygyne *Vespula* populations, so we incorporate other social Hymenoptera species in this comparative framework. For additional background, see Cronin et al. 2013, which explains the evolution and characteristics of independent and dependent colony foundation.

Mode of colony foundation	Definition	Reproduction shared equally?	Example	Notes	Citation
Independent colony founding (ICF): solitary founding (= haplometrosis)	Solitary founding queen, subsequent queens join an established nest	Yes	*Leptothorax canadensis*	Daughter queens remain in the natal nest	Heinze 1993
			Formica polyctena	Local populations are comprised of networks of intercommunicating aggregations of workers, brood, and fertile queens (= unicolonial), many queens contribute to reproduction	Hölldobler & Wilson 1977
			Linepithema humile	Unicolonial	Hölldobler & Wilson 1977
		No	*Leptothorax sp. A*	Wingless queens engage in aggressive interactions and establish dominance hierarchies	Heinze 1993
ICF: cooperative founding (= pleometrosis)	Founding queens disperse alone before cooperating, usually in small groups, to establish a new nest	Yes	*Pachycondyla inversa*	Unrelated queens contribute equally to worker production	Heinze et al. 2001
		No	*Solenopsis invicta*	Queens contest for dominance once the first workers emerge	Tschinkel 1992
			Ropalidia marginata	There is evidence of pheromones suppressing other female workers from overtaking queenship	Premnath et al. 1996

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			Polistes species	Typically, the alpha female monopolizes reproduction, dominates nestmates	Reeve 1991
			Halictus ligatus	Dominant foundresses behave in a manner similar to haplometrotic queens while subordinates behave like mid-summer workers	Richards & Packer 1998
			Exoneura robusta	Once a nest enters its second or later year of use, the first female to eclose takes on a reproductively dominant status	Schwarz et al. 1998; Langer et al. 2006
Dependent colony founding: fission, budding, or swarming	One or more founding queens plus nestmate workers	Yes	Epiponine wasps: male and worker production	Male and worker production is likely equal among queens	Strassmann et al. 1991
			Proformica longiseta	Workers tend to segregate along kin lines	Seppä et al. 2008
			Ectatomma tuberculatum	Equal contributions by nestmate queens to the production of workers	Zinck et al. 2007
		No	Honey bees, stingless bees	Monogynous, colonies form by swarming to maintain monogyny. Patrilines may contribute unequally to reproduction.	Michener 1974
			Cataglyphis cursor	Queens produced through parthenogenesis, bud to form new colonies	Chéron et al. 2011
			Epiponine wasps, queen production	Only one or a few initial queens survive to produce new queens	West-Eberhard 1978; Strassman et al. 1991

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Table S2. Parameters used to run COLONY v.2.0.6.5 on the Unix Cluster at Georgia Southern University.

Value	Parameter
800	Number of offspring in the sample
143	Number of loci
1234	Seed for random number generator
1	0/1=Not updating/updating allele frequency
2	2/1=Dioecious/Monoecious species
0	0/1=Inbreeding absent/present
1	0/1=Diploid species/HaploDiploid species
1 0	0/1=Polygamy/Monogamy for males & females
0	0/1 = Clone inference = No/Yes
0	0/1=Scale full sibship=No/Yes
0	0/1/2/3/4=No/Weak/Medium/Strong sibship prior; 4=Optimal sibship prior for Ne
0	0/1=Unknown/Known population allele frequency
1	Number of runs
3	1/2/3/4 = Short/Medium/Long/VeryLong run
1	0/1=Monitor method by Iterate#/Time in second
1	Monitor interval in Iterate# / in seconds
0	0/1=DOS/Windows version
1	0/1/2=Pair-Likelihood-Score(PLS)/Full-Likelihood(FL)/FL-PLS-combined(FPLS) method
1	0/1/2/3=Low/Medium/High/VeryHigh precision
0	0/1=Codominant/dominant loci (for all markers)
0.03	dropout rate (for all markers)
0.03	other error rate (for all markers)

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Table S3. Offspring per caste for queens with at least six offspring and p-values from exact tests, with multiple comparisons corrected for using the Benjamini-Hochberg procedure. Asterisks denote p-values for queens that had skewed offspring caste production. The Benjamini-Hochberg procedure entails ordering the individual p-values and comparing them to their Benjamini-Hochberg critical value ((*i/m)Q*, where *i* is the rank, *m* is the total number of tests, and *Q* is the false discovery rate - in this case, 0.05). The largest p-value that has p<(i/m)Q is significant, and all of the p-values smaller than it are also significant. Highlighted queens are those that laid eggs in an unequal distribution across combs.

Colony	Queen	Gynes	Males	Workers	p
T3	T3-3	35	0	2	4.414×10^{-13}*
T1	T1-1	29	0	3	9.913×10^{-13}*
BU	BU-1	18	0	1	1.822×10^{-10}*
LE	LE-1	8	0	0	2.326×10^{-9}*
T1	T1-4	0	4	19	0.0001*
T1	T1-2	0	9	4	0.0006*
LE	LE-2	0	48	40	0.0009*
T1	T1-3	0	8	7	0.0018*
T3	T3-16	0	6	0	0.0018*
T2	T2-3	0	9	0	0.0039*
T1	T1-7	0	0	8	0.00412*
T3	T3-4	3	12	3	0.0107*
TA	TA-1	2	1	8	0.012*
BU	BU-8	0	8	8	0.0188
T3	T3-8	2	6	0	0.0213
T3	T3-14	3	0	6	0.0268
T2	T2-7	0	1	7	0.0371
T3	T3-1	3	2	9	0.0399
BU	BU-7	0	6	6	0.0607
BU	BU-10	0	6	5	0.0610
TA	TA-5	0	16	7	0.0672
T2	T2-2	0	6	13	0.1118
CR	CR-4	0	6	16	0.1445
BU	BU-9	0	4	3	0.1758
TA	TA-2	2	8	16	0.2061
CR	CR-1	3	1	13	0.2563
TA	TA-4	0	9	4	0.2636
T3	T3-13	1	2	4	0.2773
BU	BU-5	3	1	5	0.2948
BU	BU-6	1	1	5	0.3165
T3	T3-2	2	4	1	0.4003
T3	T3-6	2	5	3	0.415

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CR	CR-2	2	1	4	0.4401
BU	BU-4	4	6	3	0.4643
TA	TA-3	0	10	9	0.6635
CR	CR-3	1	5	12	0.6921
T2	T2-4	0	5	6	0.7721
BU	BU-2	2	3	3	1
T2	T2-1	0	3	3	1

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Table S4. Mean relatedness of reproductive specialist queens to nestmate queens.

Colony	Queen	Mean relatedness of queen	Standard deviations from mean relatedness of all queens (0.027)	Specialization	Randomization test p-value
BU	BU-1	0.2720	0.9553	Gyne	0.0006***
LE	LE-1	-0.171	NA	Gyne	N/A
T1	TI-1	0.2730	0.6674	Gyne	0.037*
T3	T3-3	0.0927	0.3413	Gyne	0.1026
T2	T2-3	0.0876	0.5932	Male	0.2981
T3	T3-16	0.0509	0.1116	Male	0.6038

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Table S5. Genotyped offspring per comb for queens with at least six offspring and p-values from exact tests, with multiple comparisons corrected for using the Benjamini-Hochberg procedure. Asterisks denote p-values for queens that reproduced in an unequal distribution across combs. Highlighted queens are unequal reproducers (their offspring caste ratio is significantly skewed).

Colony	*Queen*	*Comb (bottom to top)*										*p*
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	
T3	T3-3	0	0	0	0	0	21	0	0	16	0	2.69×10^{-5}*
BU	BU-1	4	8	1	1	0	0	0	0	5	NA	0.0004*
T1	T1-1	8	7	7	0	0	10	0	0	NA	NA	0.0005*
TA	TA-1	0	0	0	10	1	NA	NA	NA	NA	NA	0.0088
LE	LE-1	NA	0	0	7	1	NA	NA	NA	NA	NA	0.0096
TA	TA-5	2	7	5	5	4	NA	NA	NA	NA	NA	0.0195
TA	TA-4	0	0	7	4	2	NA	NA	NA	NA	NA	0.0284
TA	TA-2	0	1	5	4	16	NA	NA	NA	NA	NA	0.0404
T3	T3-8	0	0	0	1	1	1	3	1	1	0	0.0509
BU	BU-4	1	2	1	1	1	1	0	5	1	NA	0.0685
T3	T3-4	3	3	2	1	3	2	1	1	1	1	0.1399
BU	BU-6	0	1	2	0	2	0	0	1	1	NA	0.1456
T3	T3-6	0	2	3	0	0	1	0	1	2	1	0.1650
T1	T1-4	1	2	1	2	4	2	6	5	NA	NA	0.1673
T3	T3-14	0	1	3	1	0	0	1	1	1	1	0.1927
T3	T3-13	0	0	0	0	3	1	0	1	2	0	0.2252
TA	TA-3	0	4	0	8	7	NA	NA	NA	NA	NA	0.2341
T3	T3-1	2	2	1	1	1	1	2	2	2	0	0.2624
T1	T1-3	0	0	1	2	4	3	3	2	NA	NA	0.2680
T3	T3-2	2	0	1	0	1	1	0	0	2	0	0.3373
BU	BU-8	0	1	1	1	0	4	5	1	3	NA	0.3612
BU	BU-2	0	0	1	2	1	1	0	1	2	NA	0.5296
T1	T1-7	0	2	0	0	2	3	0	1	NA	NA	0.5418
BU	BU-10	0	0	1	0	1	3	1	1	4	NA	0.5695
BU	BU-9	0	1	0	0	0	1	2	2	1	NA	0.6614
T1	T1-2	1	1	0	1	1	4	4	1	NA	NA	0.7437
T3	T3-16	0	0	0	0	1	3	0	1	1	0	0.7892
LE	LE-2	NA	30	18	25	15	NA	NA	NA	NA	NA	0.7992
BU	BU-5	0	1	1	1	1	2	0	0	3	NA	0.8047
BU	BU-7	0	1	2	1	0	2	1	1	4	NA	0.9572

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Table S6. Counts of pupae of each caste collected from each comb for the five colonies with intact nest collection. Our comb numbering begins with the lowest comb (i.e., comb 1 is at the bottom of the nest).

		Comb number									
		bottom									top
colony	caste	1	2	3	4	5	6	7	8	9	10
BU	G	5	11	5	4	0	0	0	0	5	-
	M	0	1	1	0	3	7	10	7	14	-
	W	0	5	10	5	3	9	6	5	5	-
LE	G	0	0	0	7	1	-	-	-	-	-
	M	0	17	4	18	9	-	-	-	-	-
	W	0	13	14	7	6	-	-	-	-	-
T1	G	8	7	7	0	0	7	0	0	-	-
	M	1	0	0	3	5	4	9	1	-	-
	W	1	6	2	3	6	13	5	9	-	-
T3	G	5	3	3	2	2	23	2	3	14	1
	M	3	4	5	2	7	10	3	2	8	4
	W	0	1	6	4	6	3	4	4	12	6
TA	G	0	0	1	3	0	-	-	-	-	-
	M	2	11	11	12	9	-	-	-	-	-
	W	0	1	5	16	23	-	-	-	-	-

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Table S7. Summary of potential mechanisms that may lead to asymmetrical reproduction.

Potential mechanism	Between-queen variation in total offspring produced	Between-queen variation in gyne:worker ratio	Additional predictions
Dominance	High	Low	
Variation in reproductive potential	High	Low	
Cheating	Low or High	High	
Developmental switch and queen variation	Low or High	High	Fecundity and gyne production correlated
Physical dominance of reproductive comb	Low or High	High	Strong spatial partitioning in queen reproduction

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Table S8. Queen mating statistics. We calculated queen mating statistics for all queens with 10 or more female offspring, as well as for the four gyne-producing queens. Values indicated with an asterisk are unusually high effective mating frequencies based on a previous study (Hanna et al. 2014). These could be the result of incorrect COLONY assignments splitting true patrilines into multiple estimated patrilines, as was observed in simulations for colonies with large numbers of queens. All gyne-producing queens had estimated mate numbers within the expected range, and thus are likely reliable. Gyne cohorts produced by one mother always had multiple patrilines. n = number of female offspring; k_0 = observed mate number; k_{e3} = effective mate number, *sensu* (Nielsen et al. 2003), which accounts for sample size and reproductive skew.

Colony	Queen ID	n (female offspring)	k0 (observed mate number)	ke3 (effective mate number)	Queen type
BU	BU-1	19	6	4.4	gyne-producer
LE	LE-1	8	6	9.6	gyne-producer
T1	T1-1	32	6	2.8	gyne-producer
T3	T3-3	37	5	3.5	gyne-producer
CR	CR-1	16	8	10.1	normal
CR	CR-3	13	8	11.3	normal
CR	CR-4	16	12	20.1*	normal
LE	LE-2	40	5	2.3	normal
T1	T1-4	19	15	34.4*	normal
T2	T2-2	13	11	26.2*	normal
T3	T3-1	12	8	13.4	normal
TA	TA-1	10	3	3.3	normal
TA	TA-2	18	7	5.5	normal

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Table S9. Mean pairwise relatedness between worker and gyne offspring per colony (six of nine excavated colonies contained gynes).

Colony	Mean worker-gyne relatedness
BU	0.0895
CR	0.1523
LE	-0.0769
TA	0.2029
T1	0.1721
T3	0.0964

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SI References

Chéron, B., A. L. Cronin, C. Doums, P. Fédérici, C. Haussy, C. Tirard, and T. Monnin. (2011). Unequal resource allocation among colonies produced by fission in the ant *Cataglyphis cursor*. Ecology 92:1448-1458.

Jones, O. R. and J. Wang. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.

Hanna, C. *et al.* (2014). Colony social structure in native and invasive populations of the social wasp *Vespula pensylvanica*. Biological Invasions 16:283-294.

Heinze, J. (1993). Habitat structure, dispersal strategies and queen number in two boreal *Leptothorax* ants. Oecologia 96:32-39.

Hölldobler, B. and E. O. Wilson. (1977). The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8-15.

Langer, P., K. Hogendoorn, M. P. Schwarz, and L. Keller. (2006). Reproductive skew in the Australian allodapine bee *Exoneura robusta*. Animal behaviour 71:193-201.

Michener, C. D. (1974). The social behavior of the bees: a comparative study. Harvard University Press.

Nielsen, R., D. R. Tarpy, and H. K. Reeve. (2003). Estimating effective paternity number in social insects and the effective number of alleles in a population. Molecular Ecology. 12:3157-3164.

Premnath, S., A. Sinha, and R. Gadagkar. (1996). Dominance relationship in the establishment of reproductive division of labour in a primitively eusocial wasp (*Ropalidia marginata*). Behavioral Ecology and Sociobiology 39:125-132.

Reeve, H. K. (1991). Polistes In: Ross KG, Matthews RW (eds) The social biology of wasps.

Richards, M. H. and L. Packer. (1998). Demography and relatedness in multiple-foundress nests of the social sweat bee, *Halictus ligatus*. Insectes Sociaux 45:97-109.

Schwarz, M. P., N. J. Bull, and K. Hogendoorn. (1998). Evolution of sociality in the allodapine bees: a review of sex allocation, ecology and evolution. Insectes Sociaux 45:349-368.

Seppä, P., I. Fernández-Escudero, N. Gyllenstrand, and P. Pamilo. (2008). Colony fission affects kinship in a social insect. Behavioral Ecology and Sociobiology 62:589-597.

Tschinkel, W. R. (1992). Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. Ecological entomology 17:179-188.

West-Eberhard, M. J. (1978). Polygyny and the evolution of social behavior in wasps. Journal of the Kansas Entomological Society 832-856.

Zinck, L., P. Jaisson, R. R. Hora, D. Denis, C. Poteaux, and C. Doums. (2007). The role of breeding system on ant ecological dominance: genetic analysis of *Ectatomma tuberculatum*. Behavioral Ecology 18:701-708.

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