Check for updates

Received: 28 November 2023 | Accepted: 31 January 2024

DOI: 10.1111/1365-2435.14536

Functional Ecology
BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Ontogeny of superorganisms: Social control of queen specialization in ants

Vahideh Majidifar[1,2] | **Marina N. Psalti**[1] | **Martin Coulm**[1] | **Ebru Fetzer**[1] | **Eva-Maria Teggers**[1] | **Frederik Rotering**[1] | **Judith Grünewald**[1] | **Luca Mannella**[1] | **Maxi Reuter**[1] | **Dennis Unte**[1] | **Romain Libbrecht**[1,3]

[1]Institute of Organismic and Molecular Evolution, Johannes Gutenberg University of Mainz, Mainz, Germany

[2]Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

[3]Insect Biology Research Institute, UMR 7261, CNRS, University of Tours, Tours, France

Correspondence
Romain Libbrecht
Email: romain.libbrecht@cnrs.fr

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: GRK2526/1-Project number 407023052 and LI3051/2-1

Handling Editor: Cristina Lorenzi

Abstract

1. The functioning of biological systems relies on the cooperation of specialized components and understanding the processes that produce such specialization is a major challenge in biology. Here, we study the ontogeny of biological systems at a new phenotypic level: the superorganisms (i.e. insect societies with specialized individuals).
2. We investigate how founding queens, the earliest developmental stage of ant colonies, transition from expressing behavioural pluripotency to becoming strictly specialized in egg production.
3. We demonstrate that the presence of workers both initiates and maintains this queen specialization, and propose that such a social control of queen behaviour is common in ants and regulated by ancestral mechanisms.
4. These findings contradict the traditional view of social insect queens as being intrinsically specialized in egg production and may reshape our understanding of the division of labour in insect societies.

KEYWORDS
behavioural plasticity, brood care, colony foundation, division of labour, juvenile hormone, major evolutionary transitions, social behaviour, social insects

1 | INTRODUCTION

A central question in life sciences is to understand the evolution and functioning of biological systems that are constituted by specialized components. Typical examples of such biological systems include multicellular organisms that are composed of specialized cells and insect societies that are composed of specialized individuals (Szathmáry & Smith, 1995). Social insect colonies (also called superorganisms) are analogous to multicellular organisms in that they have queens that monopolize reproduction (similar to germ cells), and functionally sterile workers that perform all non-reproductive tasks and thus act as somatic cells (Boomsma & Gawne, 2018; Wheeler, 1911). Both types of biological systems evolved from solitary and non-specialized ancestors in major evolutionary transitions (Szathmáry & Smith, 1995): multicellular organisms from unicellular organisms and insect societies from solitary insects. Interestingly, in both cases, the specialization also needs to be established in every generation during the ontogeny of these biological systems (i.e. the

---

Vahideh Majidifar, Marina N. Psalti and Martin Coulm contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2024 The Authors. *Functional Ecology* published by John Wiley & Sons Ltd on behalf of British Ecological Society.

developmental process that produces the self-assembly and specialization of their components).

In this context, it is important to understand the factors and mechanisms underlying the emergence and maintenance of specialization and reproductive division of labour in social insect colonies. The many investigations that tackled this question can be separated in three broad categories. First, studies of caste determination and differentiation uncovered critical information on the regulation of alternative developmental trajectories that lead to the production of queens and workers (Ashby et al., 2016; Cameron et al., 2013; Collins et al., 2020; Corona et al., 2016; Genzoni et al., 2023; Libbrecht et al., 2011; Libbrecht, Corona, et al., 2013; Libbrecht, Oxley, et al., 2013; Montagna et al., 2015; Mutti et al., 2011; Psalti & Libbrecht, 2020; Schultner et al., 2023; Schwander et al., 2008; Schwander & Keller, 2008; Wheeler et al., 2006). Second, studies comparing adult queens and workers in mature colonies revealed a suite of caste-specific phenotypic and molecular differences (Bonasio et al., 2012; Chandra et al., 2018; Corona et al., 2007, 2013; Feldmeyer et al., 2014; Grozinger et al., 2007; Kronauer & Libbrecht, 2018; Libbrecht, Oxley, et al., 2013; Patalano et al., 2015). Third, there have been investigations of condition-dependent plasticity in the reproductive activity of workers (Amarasinghe et al., 2014; Holman, 2014; Holman et al., 2016; Libbrecht et al., 2016, 2018; Macedo et al., 2016; Negroni et al., 2021; Ronai et al., 2016; Ulrich et al., 2016). Overall, studies of reproductive division of labour between queens and workers primarily aimed to understand the development and functioning of queens as egg-laying machines, the worker-specific expression of a variety of non-reproductive behaviours, and the plasticity of worker reproduction. In contrast, there is limited research on the regulation of non-reproductive behaviours in queens (Chouvenc, 2022; Woodard et al., 2013).

By reducing queens to their reproductive activity, studies of division of labour amplified the notion, also predominant in popular science, that queens are intrinsically specialized in egg production once they reach the adult stage and that this robust specialization does not depend on environmental conditions. However, the maturation process of social insect queens is not completed by the time they emerge as adults. This is best exemplified by the behaviour of queens in the process of colony foundation, especially in the many species of social insects where mated queens found their colony independently (Peeters, 2020). These pluripotent founding queens are not specialized in egg production yet, as they express a broad repertoire of both reproductive and non-reproductive behaviours to produce the first workers (Augustin et al., 2011; Brossette et al., 2019; Cassill, 2002; Haskins & Haskins, 1955; Helms Cahan & Fewell, 2004; Jeanson & Fewell, 2008; Norman et al., 2016; Rissing & Pollock, 1986; Rüppell et al., 2002; Walsh et al., 2018; Wheeler, 1932, 1933; Woodard et al., 2013). For example, while workers provide brood care in mature colonies, founding queens need to groom and feed the larvae during the founding stage (Schultner et al., 2017). Founding queens may also dig the first chamber of the nest (Haskins & Haskins, 1955; Helms Cahan & Fewell, 2004; Rissing & Pollock, 1986) or cultivate the mutualistic fungus in leaf-cutting ant species (Augustin et al., 2011). It is only once the colonies are established (i.e. they contain workers) that the queens stop expressing non-reproductive behaviours and become strictly specialized in egg production (Augustin et al., 2011; Chouvenc, 2022; Hölldobler & Wilson, 1990; Wilson, 1971; Woodard et al., 2013).

While the process of queen specialization is central to the ontogeny of superorganisms, the factors and mechanisms that control the specialization of pluripotent founding queens remain poorly understood. It is also unclear whether queen specialization is condition-dependent, and whether queens become permanently specialized once the colonies are established (i.e. they lose the ability to express non-reproductive behaviours). In this study, we aimed to address these questions by investigating queen specialization in ants. We showed that the presence of workers was necessary and sufficient to inhibit brood care behaviour in founding queens—and thus initiate their specialization in egg production—and we identified potential mechanisms underlying these behavioural modifications. We found the queen specialization to be reversible and dependent on the social environment, as queens reverted to expressing brood care upon the experimental removal of their workers in two ant species. This continuous social regulation of queen specialization contradicts the prevailing notion of ant queens as intrinsically specialized egg-laying machines.

2 | METHODS

2.1 | General procedures

To investigate the factors and mechanisms that control the queen specialization in egg production, we conducted a series of experiments using the black garden ant *Lasius niger* as our main study system. In this section, we describe the general procedures that apply to all experiments regarding the collection and keeping conditions of queens, the use of workers for experimental manipulation of worker presence, the experimental setup and behavioural observations, as well as the statistical analyses.

2.1.1 | Founding queen collection and keeping conditions

All *Lasius niger* founding queens used in the experiments were collected after their nuptial flights in 2017, 2018, 2019 and 2020 around Mainz and Ingelheim, Germany (Table S1 in Supporting Information). After collecting them in fluon-coated boxes containing humid paper towel, we transferred the founding queens individually into glass tubes (10 cm length × 1 cm diameter) half filled with water blocked by cotton and plugged with another piece of cotton. The tubes were placed in boxes (18 cm × 12 cm × 7 cm) kept in darkness. Most queens were kept in a climate chamber at

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

21°C and 80% humidity. Founding queens were not provided with food, since *L. niger* queens found their colonies without foraging (claustral colony founding; Janet, 1907; Keller & Passera, 1989; Peeters, 2020). Once the first workers emerged, each tube containing the queen, brood and workers was transferred into a small plastic box (15 cm × 11 cm × 12 cm) with walls coated with fluon to prevent the ants from escaping. These colonies were also kept in a climate chamber at 21°C and 80% humidity, but fed every second week with a drop of honey, one frozen cricket and a piece of artificial food with a 2:1 (carbohydrate: protein) ratio (Dussutour & Simpson, 2008). Every winter, the established colonies were hibernated for 3–4 months at 5°C. They entered and left hibernation via a 2-week gradual decrease and increase in temperature, respectively. Table S1 provides information on queen collection, keeping conditions and sample sizes in all experiments. This study did not require ethics approval.

2.1.2 | Experimental manipulations of worker presence

To experimentally provide workers to founding queens, we used 'callow' workers (Errard, 1984; Julian & Fewell, 2004; O'donnell, 1998; Psalti et al., 2021; Stuart, 1988; Stuart & Page, 1991; Teggers et al., 2021; Woodard et al., 2013). We sampled brood from *L. niger* field colonies and kept it in laboratory colonies that we monitored regularly to collect workers that recently emerged from the pupae (<12 h). These callow workers did not elicit aggression from foreign individuals and were readily accepted by founding queens, possibly because they did not possess the signature chemical profile of their own colony yet (Dahbi et al., 1998; Isingrini et al., 1985; Signorotti et al., 2014). Callow workers were easily recognizable due to their light grey colour. The laboratory colonies used for callow worker production were kept between 21°C and 28°C, and between 80% and 100% humidity, depending on the timing and availability of climate cabinets. In cases where callow workers were kept at a different temperature than the temperature of the experiment, they were moved to the room of the experiment at least 1 h before it started.

2.1.3 | Experimental setup

All behavioural analyses were conducted based on observations of videos that were recorded for 1 h with cameras standing ca. 50 cm over the observation arenas. Two white LED light bars illuminated the arenas approximately 70 cm above the observation arenas. One camera recorded one tray, which could contain up to 12 observation arenas simultaneously (Figure S1). Whenever multiple cameras were used simultaneously, the recordings belonged to the same batch. All experiments were filmed in a climate chamber at 21°C and 80% humidity, with 12 h/12 h light/dark cycle. The observation arenas consisted of airtight petri dishes (50 mm diameter, 9 mm height; Falcon) half filled with moistened, and blue plaster for better contrast and visibility (Figure S2). We used soft forceps and brushes to manipulate the ants. We ensured that all treatments of an experiment were represented and equally distributed on each tray. We assigned random numbers to the observation arenas to ensure that the experimenters were blind to the treatment during the experiments and video analyses.

2.1.4 | Behavioural analyses

To assess the behavioural specialization of queens, we observed their brood care behaviour towards larvae. We defined a brood care event as an active manipulation of the brood, with frequent contacts with the antennae and touches with the front legs. We also counted as a brood care event the placement of a brood item into a different position. However, merely carrying the brood item in the mouth parts was not scored as brood care. In the experiments 'Worker emergence' and 'Worker presence and feeding status', we counted the total number of brood care events and divided it by the duration of the videos. In all other experiments, we scored the brood care behaviour via scan sampling. We watched the first 10 s of every minute of each video and recorded the presence or absence of brood care behaviour. We performed 51 such scans per 1 h video, as we did not analyse the first and last 5 min of the videos to avoid potential disturbances due to the experimental manipulations. The videos were analysed using the software BORIS (version 8.0.5) or Pot player (version 1.7.21212).

2.1.5 | Statistical analyses

We performed all statistical analyses (Appendix S1) using R v. 4.0.4 and RStudio v. 1.4.1106. Table S2 provides the input data, syntax and outputs of all statistical models. We used the lm() command from R base to build linear models, the lmer() command from the *lme4* package (Bates et al., 2015) for linear mixed-effect models, the nls() command from R base for non-linear regressions, and the glmer() command from the *lme4* package to build generalized linear mixed-effect models. We used the lsmeans() command from the *emmeans* package (Lenth et al., 2019) for post hoc pairwise comparisons. To test the effect of the response variables, we either used the summary() command from R base or an ANOVA with the Anova() function of the *car* package (Fox & Weisberg, 2019). Whenever needed, we used a square root transformation to ensure that the residuals of the models followed a normal distribution. Table S2 provides for each experiment the fixed and random variables that were included in the models. We included time as a fixed variable in the analyses of all the experiments that involved the collection of data at multiple time points. Whenever the analyses could not detect an interaction between time and the treatment of interest, we pooled the numbers of scans with brood care over the multiple time points. Whenever the analyses could detect

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

an interaction between time and the treatment of interest, we investigated the effect of time separately for the different levels of the treatment variable (Table S2).

2.2 | Detailed experimental descriptions

In this section, we provide context for each experiment, as well as experiment-specific information that deviates from the general procedures. Table S1 provides an overview of the collection and keeping conditions of all queens.

2.2.1 | Brood production of founding queens

To verify that *L. niger* founding queens raise their first cohort of workers independently, we collected newly mated queens right after their nuptial flight and transferred them to closed, individual glass tubes. We used 24 founding queens collected in July 2019 on the campus of the Johannes Gutenberg University of Mainz, Germany (from now on referred to as JGU Mainz). We used a stereomicroscope to count for each queen the number of eggs, larvae, pupae and workers three times per week for 93 days after the nuptial flight.

2.2.2 | Effect of worker emergence

To confirm that *L. niger* founding queens express brood care behaviour, we used 12 founding queens collected in July 2017 on the campus of the JGU Mainz. We kept the queens in their collection boxes with humid paper towel for 7 days before transferring them into individual glass tubes. The queens were filmed inside their tubes with all their brood for 15 min before worker emergence (35 days after the nuptial flight), and after worker emergence (56 days after the nuptial flight).

2.2.3 | Effect of experimental manipulations of worker presence and feeding status

To experimentally manipulate worker presence and feeding status, we used 54 founding queens collected in June 2018 near the campus of the JGU Mainz. The queens were kept in glass tubes at 25°C and approximately 80% humidity with a 12 h/12 h light/dark cycle. Just before the start of the experiment, all pupae were removed to prevent the emergence of workers during the experiment (all eggs and larvae were left in the tubes). The experiment started 30 days after collection. The queens were divided into four treatments: (i) queens were fed and were given five callow workers ($n = 14$), (ii) queens were fed and received no workers ($n = 11$), (iii) queens were not fed and were given five callow workers ($n = 15$) and (iv) queens were not fed and received no workers ($n = 14$). For the feeding treatment, queens were hand-fed for 5–10 min with artificial food with a 2:1 (carbohydrate: protein) ratio (Dussutour & Simpson, 2008). We filmed the queens in their tubes for at least 30 min twice a day (morning and afternoon) over seven consecutive days (14 time points per queen).

2.2.4 | Effect of worker removal on established queens that had workers for 3 days

To test whether the presence of workers maintains queen specialization, we first used 48 founding queens collected in July 2019 in three locations in and around Mainz, Germany (Mainz-Bretzenheim, Mainz-Marienborn and campus of the JGU Mainz). The experiment started 55 days after collection, and all pupae were removed once 37 days after collection to ensure that no workers emerged before the start of the experiment. We assigned the queens to three different treatments: (i) queens with five larvae and five added callow workers ($n = 16$), (ii) queens with five larvae and without workers ($n = 16$) and (iii) queens with five larvae that formerly had five callow workers (workers removed; $n = 15$). In the 'workers removed' treatment, queens had been given five callow workers in their glass tubes 3 days prior to the experimental setup when the workers were removed, and all tubes were filmed just before the experimental setup. Then, the queens were filmed in the observation arenas 24 and 48 h after the experimental setup.

2.2.5 | Effect of worker removal on established queens that had workers for 2 years and 6 months

To further investigate the role of worker presence in maintaining queen specialization, we used 12 colonies that were established in the laboratory by founding queens collected in July 2017 on the campus of the JGU Mainz. The experiment started 932 days after collection. All colonies were treated the same way. First, we filmed each queen with five of its larvae and five of its workers to allow direct observations of the queen behaviour and standardize worker numbers across colonies. Then, we removed the last five workers, and filmed the queens 24 and 48 h after the experimental setup.

2.2.6 | Effect of worker removal on established queens that had workers for 3 years and 2 months

To complete our investigations of whether the presence of workers maintains queen specialization, we used 24 colonies that were established in the laboratory by founding queens collected in July 2017 on the campus of the JGU Mainz. The experiment started 1162 days after collection. The experimental setup consisted of one queen, five of its larvae and five of its workers in observation arenas. Then, we gradually removed one worker per day over the course of 6 days, until reaching zero workers. We filmed the observation arenas every day and removed the workers after each video recording.

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.2.7 | Effect of workers in unmated queens

To test whether mating is necessary for queens to become specialized in the presence of workers, we collected 27 unmated queens from field colonies in June and July 2021 near the campus of the JGU Mainz, and kept them at 24°C and 80% humidity in darkness. The experiment started right after collection. Each unmated queen was observed with five foreign larvae and either five ($n = 14$) or zero ($n = 13$) callow workers. We filmed the unmated queens six and 24 h after the experimental setup.

2.2.8 | Effect of worker number

To investigate whether the effect of workers on queen specialization is dose-dependent, we used 76 founding queens collected in July 2019 on the JGU campus. The experiment started 35 days after collection. We set up one queen with five larvae and either zero ($n = 14$), one ($n = 18$), two ($n = 15$), three ($n = 13$) or five ($n = 16$) callow workers. We recorded the queens 24, 48 and 72 h after the start of the experiment. A similar experiment was conducted using 80 founding queens collected in July 2020 in several locations in Mainz, Germany (Marienborn, Mainz-Hechtsheim and Mainz-Oberstadt). This second experiment started 47 days after collection. For the last experimental setups of this second experiment, all pupae were removed to ensure that no workers naturally emerged before the experiment was completed. The queens were provided with 15 larvae and either zero ($n = 16$), one ($n = 16$), two ($n = 16$), three ($n = 16$) or five ($n = 16$) callow workers, and were also filmed 24, 48 and 72 h after the experimental setup.

2.2.9 | Effect of worker cuticular hydrocarbons (CHC)

To test the effect of worker CHC on the brood care behaviour of founding queens, we used 36 queens collected in July 2019 on the campus of the JGU Mainz. The experiment started 40 days after collection. We produced five CHC extracts, each from 100 workers collected in a field colony around Mainz, Germany. The workers were sedated with CO_2, transferred into glass vials and immersed in *n*-hexane 10 min while occasionally swaying the vials. The liquid was transferred to a micro insert and completely exhausted under a gentle nitrogen stream. The CHC were then re-dissolved in 50 μL of hexane and stored at 4°C until used in the experiments. We applied either 10 μL of the worker CHC extracts (treatment) or 10 μL of pure *n*-hexane (control) on glass beads (~1.5 mm, Roth GmbH). Five glass beads were placed on a bowl made of aluminium foil (5 mm diameter) to ensure that the CHC extracts would not soak into the plaster below. One queen and five larvae were placed in each observation arena, together with either treatment ($n = 18$) or control ($n = 18$) beads. We filmed the observation arenas 3, 6 and 24 h after the experimental setup.

2.2.10 | Effect of former presence of workers

To investigate the effect of the presence of worker cues on queen specialization, we first used 36 founding queens collected in July 2019 on the campus of the JGU Mainz. The experiment started 42 days after collection. Each queen was placed together with five larvae in an observation arena that used to contain 20 field-collected workers for 48 h (workers removed just before the start of the experiment, $n = 18$) or in a clean observation arena ($n = 18$). We filmed the observation arenas six and 24 h after the experimental setup.

2.2.11 | Effect of the presence of dead workers

To further test whether cues of worker presence affect queen specialization, we then used 54 founding queens collected in July 2020 in Ingelheim and Mainz, Germany. The experiment started 65 days after collection. The tubes containing the queens were regularly checked prior to the experiment to remove any pupae; thus, ensuring that no workers had emerged before the experimental setup. We assigned the queens to four treatments: one queen with five larvae and (i) five dead workers ($n = 18$), (ii) five living workers ($n = 18$) and (iii) without any workers ($n = 18$). Dead workers were obtained by placing 90 workers at −80°C for 2 h, and then at −20°C for 24 h before the experimental setup. Freshly killed, frozen ants show a similar CHC profile as living ants (Wilson et al., 1958). We recorded the observation arenas 2, 6 and 24 h after the experimental setup.

2.2.12 | Effect of workers separated by a wire mesh

To investigate whether the queen brood care behaviour changed in response to workers separated from the queen and brood by a wire mesh, we used 43 founding queens collected in July 2020 in Ingelheim and Mainz, Germany. The experiment started 52 days after collection. The tubes containing the queens were regularly checked prior to the experiment to remove any pupae; thus, ensuring that no workers had emerged before the experimental setup. In this experimental setup, we modified the observation arenas by adding a wire mesh cylinder (0.2 mm thick, 0.2 mm mesh size and 2 cm cylinder diameter) in the middle of the arena (Figure S3). The wire mesh enabled antennation between the queen and the separated workers, but the workers had no close physical interactions with either the queen or the larvae. We assigned the queens to the following treatments: one queen and five larvae were placed outside the wire circle and the three callow workers were placed (i) on the inside of the circle, thus separated from the queen and brood (workers separated; $n = 13$), or (ii) on the outside of the circle together with the queen and brood (workers present; $n = 14$) or (iii) without any workers (workers absent; $n = 16$). We filmed the observation arenas 2, 6 and 24 h after the experimental setup.

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.2.13 | Effect of methoprene and precocene I and II

To investigate the potential implication of the juvenile hormone (JH) pathway in the regulation of brood care in queens, we subjected founding queens to treatments with a JH analogue (methoprene) or with chemical inhibitors of JH production (precocene I and II). We used 160 queens collected in July 2020 in Ingelheim, Mainz-Bretzenheim and the city centre of Mainz, Germany. The experiment started 52 days after collection for the methoprene experiment and 66 days after collection for the precocene I and II experiment. For the methoprene experiment, queens were treated with (i) methoprene at 1.5 µg/µL (PESTANAL®, a commonly used JH analogue (Henrick, 2007); $n = 35$) or (ii) acetone (solvent; $n = 35$). For the precocene I and II experiment, queens were treated with (i) precocene I at 1.5 µg/µL (Sigma; JH inhibitor; $n = 30$), (ii) precocene II at 1.5 µg/µL (Cayman; JH inhibitor; $n = 30$) or (iii) acetone (solvent; $n = 30$). To treat the queens, we attached them to an eraser with a fishing line and applied 1 µL of the treatment on their thorax with a glass pipette. Each queen was treated every morning for four consecutive days. In the first 2 days, we recorded the observation arenas without workers. On the third day, before the application of the treatments, we added five callow workers to half of the queens and kept them with these workers for another 2 days. The observation arenas were recorded between 2 and 6 h after each treatment.

2.2.14 | Effect of larvae on egg production in founding and established queens

To test the influence of brood presence on egg production in founding and established queens, we used 66 *L. niger* queens collected in July 2019 on the campus of the JGU Mainz. The queens were distributed into the following treatments: (i) founding queens with larvae ($n = 18$), (ii) founding queens without larvae ($n = 18$), (iii) established queens with larvae ($n = 15$) and (iv) established queens without larvae ($n = 15$). Founding queens were queens that had not yet produced pupae or workers and were tested 8 days after the nuptial flight. Established queens were queens that had produced at least five workers and were tested 82 days after the nuptial flight. To test the effect of larvae, we used five larvae collected in field colonies near the campus of the JGU Mainz. We recorded the number of eggs once a day for five consecutive days.

2.2.15 | Effect of worker removal on established *Temnothorax nylanderi* queens

To investigate whether the presence of workers induces the queen specialization in another species of ants, we collected 21 *T. nylanderi* colonies in April 2021 in the Lenneberg forest of Mainz, Germany. In the laboratory, each colony was transferred into plastered nest boxes containing an artificial nest consisting of a Plexiglas perimeter (3 mm high) with an entrance, sandwiched between two microscope slides (7.5 cm × 2.5 cm × 0.5 cm). The colonies were kept according to the general procedures and were fed twice a week with a drop of honey, half a cricket and water. The experiment started 2 weeks after collection. The treatments consisted of queens with five larvae and (i) five of their own workers ($n = 11$) and (ii) no workers ($n = 10$). We used CO_2 to anaesthetise and transferred the ants into the observation arenas. We filmed the observation arenas every 24 h for five consecutive days.

3 | RESULTS

3.1 | Queens become specialized after the emergence of the first workers

To verify that *L. niger* founding queens produce workers independently, we collected newly mated queens right after their nuptial flight and monitored brood production and development over the next 93 days (Figure S4). 91.67% of the queens (22/24) survived the experiment, and 77.28% of the surviving queens (17/22) produced workers within the observation time (5.26 ± 3.95 workers; mean ± sd; Figure S4). This indicates that founding queens provide care to the brood, as ant larvae cannot develop independently (Schultner et al., 2017). We confirmed this by direct observations of brood care behaviour (defined as active manipulation of the brood) in founding queens before and after worker emergence. We found that founding queens expressed brood care before they produced the first workers, but that the emergence of workers was associated with a sharp decrease in queen brood care behaviour ($\chi^2 = 35.63$, $p < 0.0001$, Figure 1a). Therefore, the emergence of workers correlates with the behavioural specialization of queens in *L. niger*.

3.2 | The presence of workers triggers the specialization of queens

The emergence of workers is confounded with the age and nutrition of queens, as established queens—defined as queens with workers—are older and fed by their workers. To disentangle the effects of age, nutrition and worker presence, we manipulated worker presence and feeding status of same-age founding queens that had not produced workers yet, and quantified their brood care behaviour for 7 days. We detected an interaction between time and worker presence ($\chi^2 = 17.24$, $p < 0.0001$), as queens with workers showed a stronger decrease in brood care over time ($\chi^2 = 49.69$, $p < 0.0001$, Figure 1b) than queens without workers ($\chi^2 = 8.99$, $p = 0.0027$, Figure 1b). In addition, we found very strong evidence that queens with workers performed less brood care overall than queens without workers ($\chi^2 = 596.33$, $p < 0.0001$, Figure 1b). This inhibition of brood care by workers was already detected 20 h

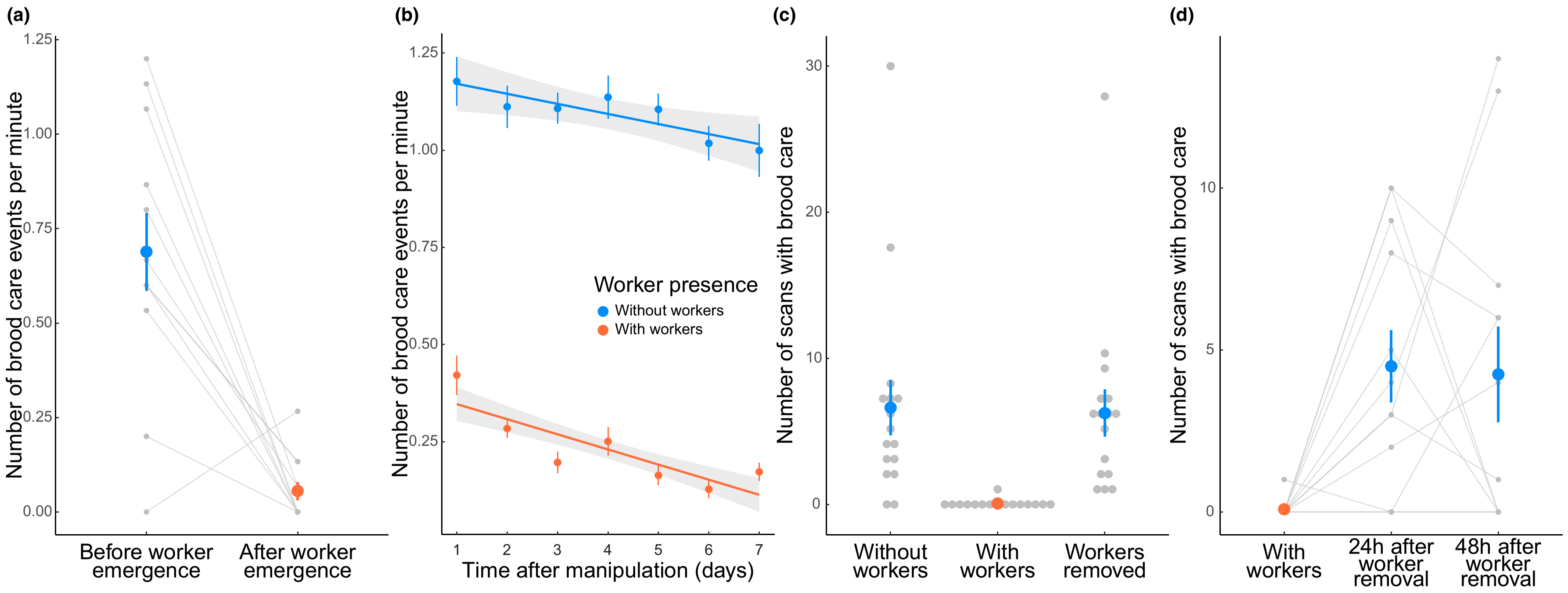

FIGURE 1 Presence of workers both initiates and maintains the queen behavioural specialization. (a) The emergence of workers correlates with the behavioural specialization of queens. Founding queens ($n=12$) reduced brood care after producing the first workers ($\chi^2=35.63$, $p<0.0001$). (b) The experimental addition of workers drives the queen specialization. Founding queens provided with workers ($n=29$) showed lower brood care levels than founding queens kept without workers ($n=25$, $\chi^2=596.33$, $p<0.0001$). The effect of worker presence was already detected 20 h after the addition of workers ($\chi^2=221.28$, $p<0.0001$). (c) The queen specialization is reversible after 3 days. Founding queens that had their workers removed ($n=16$) performed more brood care than queens that just received workers ($n=16$, $t=6.19$, $p<0.0001$), but similar brood care as queens that never had any workers ($n=16$, $t=0.15$, $p=0.99$). (d) The queen specialization is reversible after 2 years and 6 months. Established queens ($n=12$) showed increased brood care both 24 h ($t=3.69$, $p=0.0035$) and 48 h ($t=3.07$, $p=0.015$) after their workers were removed. Coloured dots and error bars represent means and standard errors, respectively, and grey dots show individual data points.

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

after the addition of workers ($\chi^2 = 221.28$, $p < 0.0001$, Figure S5). We could not detect any effect of the feeding status, neither as a main effect ($\chi^2 = 1.10$, $p = 0.29$) nor as an interaction with time ($\chi^2 = 1.90$, $p = 0.17$). This experiment demonstrates that the presence of workers is necessary and sufficient to initiate the behavioural specialization of *L. niger* queens.

3.3 | The presence of workers maintains the specialization of queens in established colonies

Then, we investigated whether the presence of workers is necessary to maintain the queen specialization in established colonies. First, we studied this question in *L. niger* queens that were provided with workers for 3 days. As expected, those queens expressed lower levels of brood care than same-age queens that never had any workers ($\chi^2 = 12.78$, $p = 0.00035$). We then removed the workers and compared the queen brood care behaviour to queens that either never had any workers or were just provided with workers. We found that queens that had their workers removed performed more brood care than queens that just received workers ($t = 6.04$, $p < 0.0001$, Figure 1c), but similar levels of brood care as queens that never had any workers ($t = 0.15$, $p = 0.99$, Figure 1c).

We report that the worker-driven inhibition of queen brood care behaviour is reversible after 3 days, but it may be that more time is needed for the queen specialization to be permanently established. Therefore, we investigated whether the queen behavioural specialization is reversible after several years. To do so, we used *L. niger* colonies that were founded in the laboratory between 2 years and 6 months before starting the experiment. We first recorded the queen brood care behaviour after removing all but five workers. Then, we removed the last five workers, and quantified queen brood care behaviour on the next 2 days. We found that queens expressed elevated brood care levels both 24 h ($t = 3.69$, $p = 0.0035$, Figure 1d) and 48 h ($t = 3.073$, $p = 0.015$, Figure 1d) after worker removal. We did not detect any difference between the two time points ($t = 0.61$, $p = 0.81$, Figure 1d), indicating that the queens did not show further behavioural changes after 24 h. To complete these findings, we quantified changes in queen brood care behaviour in response to the sequential removal of workers in laboratory colonies that were established between 3 years and 2 months before the experiment. We found that these established queens went back to expressing brood care upon the experimental removal of their last worker (Figure S6). These experiments show that the presence of workers not only initiates the specialization of *L. niger* queens during colony foundation, but also constantly maintains it in established colonies.

3.4 | Mating is not required for queens to become specialized in response to workers

Then, we investigated whether mating and reproductive activity are prerequisites for queens to express brood care behaviour, and whether it depends on worker presence. To do so, we manipulated the presence of workers and quantified the brood care behaviour of winged unmated queens collected from their colonies of origin (thus before their nuptial flight). We found that unmated queens performed brood care behaviour, but at very low levels. We did not observe brood care behaviour for 37.04% of the unmated queens (10/27), and those that did express brood care did so in only 1.76 ± 1.52 scans (out of 102). Expression of brood care by unmated queens has been reported in other ant species (Brown, 1999; Fletcher & Blum, 1983; Ito et al., 2017; Murakami, 2020; Nehring et al., 2012; Pyenson et al., 2022; Vieira et al., 2011). In addition, we found moderate evidence that the likelihood of expressing brood care was lower for unmated queens kept with workers compared with unmated queens kept without workers ($\chi^2 = 4.50$, $p = 0.034$, Figure S7). These results indicate that the presence of workers triggers the specialization of queens independent of their mating status and reproductive activity.

3.5 | Workers induce queen specialization in a dose-dependent manner

After demonstrating that the presence of workers controls the queen specialization, we set out to characterize the inhibitory effect of workers on brood care. First, we investigated whether the effect of workers is dose-dependent, as in other instances of social control of phenotypic variation in social insects (Ulrich et al., 2016; Winston et al., 1990). We provided same-age founding queens with five larvae, and either zero, one, two, three or five workers, and found a negative, non-linear correlation between queen brood care and the number of workers ($t = 8.45$, $p < 0.0001$, Figure 2a). While the presence of a single worker was sufficient to drive a reduction in brood care behaviour, additional workers inhibited brood care further, as the correlation remained after removing the queens that did not receive any workers from the analysis ($t = 3.35$, $p = 0.0014$). We repeated the same experiment with 15 larvae instead of five—thus increasing the larval need for care—and also found a negative correlation between the number of workers and the level of brood care expressed by queens, both when queens without workers were included ($t = 4.19$, $p < 0.0001$, Figure S8) and excluded from the analysis ($t = 2.37$, $p = 0.021$). These results indicate that workers have a dose-dependent negative effect on the brood care behaviour of queens.

3.6 | Mere worker cues do not induce the specialization of queens

Dose-dependent effects of social partners on phenotypic variation are often driven by variation in the quantity of social cues. Because social insects detect social partners via the blend of hydrocarbons on their cuticle (Richard & Hunt, 2013; Sprenger & Menzel, 2020), we extracted cuticular hydrocarbons (CHC) from pools of *L. niger* workers and applied the CHC to glass beads that we provided to same-age founding queens. We did not detect any effect of the CHC treatment on the

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

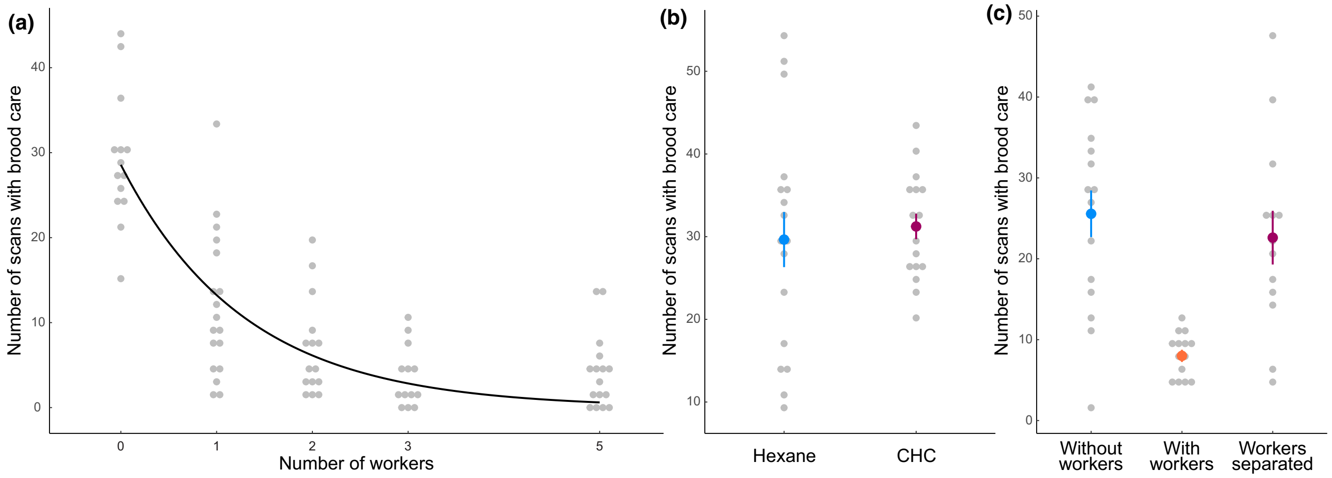

FIGURE 2 Characterization of the effect of workers on the queen behavioural specialization. (a) Workers show a dose-dependent inhibition of queen brood care. Founding queens were provided with zero ($n=14$), one ($n=18$), two ($n=15$), three ($n=13$) or five ($n=16$) workers. There was a negative, non-linear correlation between the number of workers and the queen brood care level ($t=8.5$, $p<0.0001$). The black line shows the non-linear regression curve of the model, and grey dots show individual data points. (b) Worker cuticular hydrocarbons (CHC) do not drive the queen specialization. No behavioural difference was detected depending on whether founding queens received glass beads covered in worker CHC ($n=18$) or hexane (control, $n=18$, $\chi^2=0.60$, $p=0.44$). (c) Workers separated by a wire mesh do not drive the queen specialization. The brood care behaviour of founding queens kept with workers separated by a wire mesh ($n=21$) differed from founding queens kept with workers ($n=14$, $t=3.95$, $p=0.001$), but not from founding queens kept without workers ($n=20$, $t=0.66$, $p=0.79$). Coloured dots and error bars represent means and standard errors, respectively, and grey dots show individual data points.

expression of brood care by queens ($\chi^2=0.60$, $p=0.44$, Figure 2b), indicating that queens do not modify their behaviour in response to the mere detection of worker CHC. This result was confirmed by three additional experiments. First, we did not detect any difference in brood care behaviour between founding queens depending on whether they were kept in boxes that used to contain many workers, or in boxes that never contained any ($\chi^2=0.48$, $p=0.49$, Figure S9). Second, we found that the presence of frozen workers, and thus of worker CHC (Modlmeier & Foitzik, 2011; Pamminger et al., 2011; Wilson et al., 1958), had no detectable effect on queen brood care ($t=0.57$, $p=0.84$, Figure S10). Finally, we investigated how the queen behaviour was affected by workers separated from the queen and brood by a wire mesh. This setup enabled workers to make antennal contacts with the queen and brood, but prevented closer interactions such as fluid exchange via trophallaxis (LeBoeuf, 2021). We found that queens kept with workers separated by a wire mesh expressed more brood care than queens kept together with workers ($t=3.95$, $p=0.001$, Figure 2c), but as much brood care as queens kept without workers ($t=0.66$, $p=0.79$, Figure 2c). This series of experiments shows that worker cues are not sufficient to drive the queen specialization and suggests that workers require close interactions with queens and/or larvae to inhibit the brood care behaviour of queens.

3.7 | Juvenile hormone may regulate the specialization of queens

The JH pathway is a good candidate for the regulation of brood care in queens because its function in regulating multiple worker behaviours (including brood care) has been demonstrated in many social insect species (Ferreira et al., 2023; Robinson, 1987b). To investigate the role of JH, we observed the behavioural response of same-age founding queens (kept with or without workers) to treatments with a JH analogue (methoprene) or with chemical inhibitors of JH production (precocene I and II). We found that the methoprene treatment decreased queen brood care in the first 2 days of the experiment, when brood care levels were relatively high ($\chi^2=4.43$, $p=0.035$, Figure 3a), consistent with previous reports of methoprene inhibiting brood care in *L. niger* queens (Pamminger et al., 2016). On the contrary, we found that queens treated with the JH inhibitor precocene I expressed elevated levels of brood care, although this was only detectable on the third and fourth day of the experiment, when brood care levels were overall low ($\chi^2=3.91$, $p=0.048$, Figure 3b; see Figure S11 and Table S2 for details on the effect of precocene II). These effects of methoprene and precocene I were independent of worker presence (Table S2). Finding that treatments with a JH analogue reduced brood care, while treatments with a JH inhibitor increased brood care, suggests a possible role of the JH pathway in regulating the brood care behaviour of *L. niger* queens (Ortiz-Alvarado & Rivera-Marchand, 2020).

3.8 | Founding queens show an ancestral physiological response to the presence of larvae

The JH pathway interacts closely with the insulin-signalling pathway (Al Baki et al., 2019; Libbrecht, Corona, et al., 2013; Perez-Hedo et al., 2014; Tatar et al., 2001), and both show variation between

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

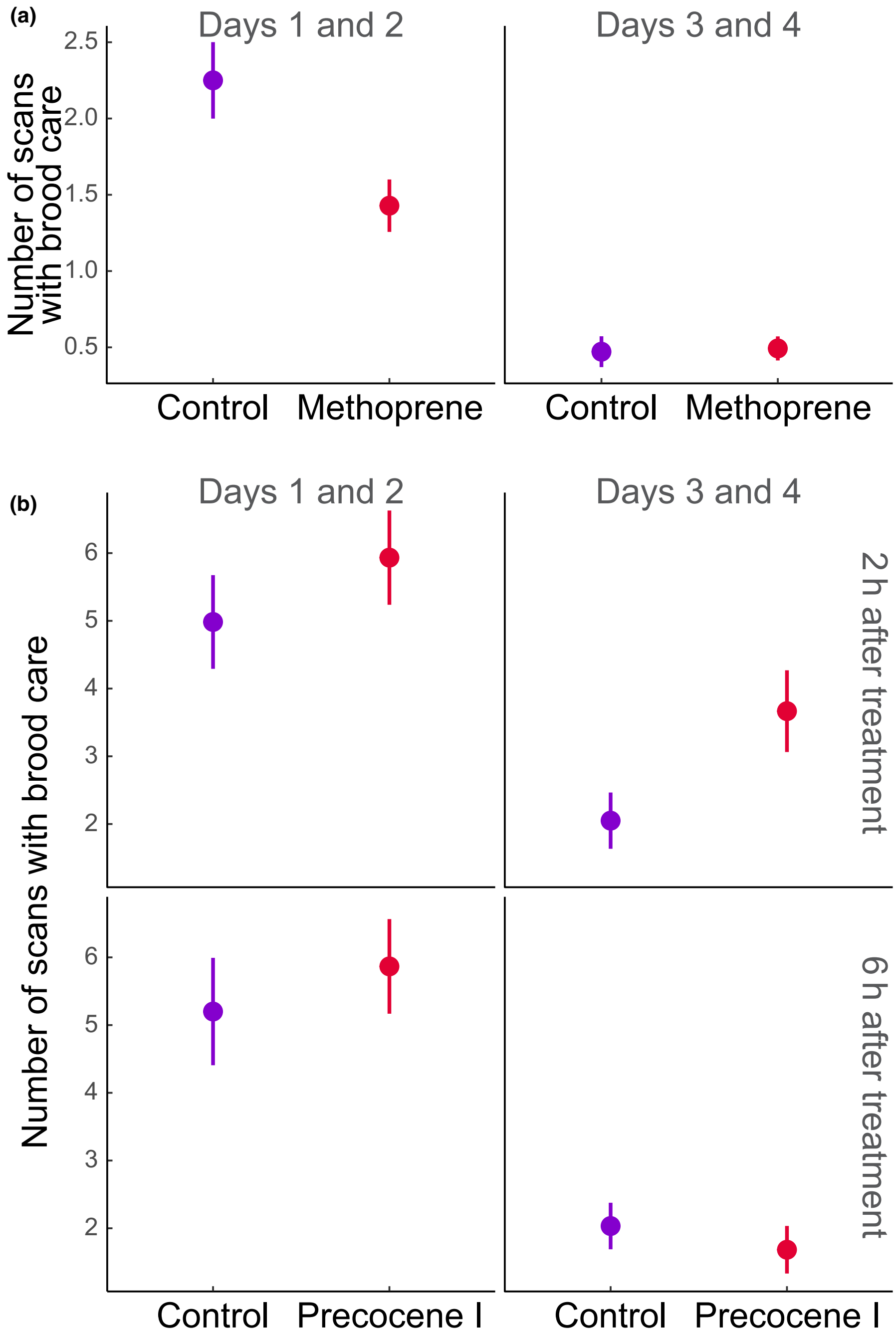

FIGURE 3 Possible role of JH in regulating the brood care behaviour of queens. (a) Founding queens treated with methoprene (JH analogue, $n=35$) showed reduced brood care levels compared with control queens (acetone, $n=35$) on Days 1 and 2 ($\chi^2=4.43$, $p=0.035$), but not on Days 3 and 4 ($\chi^2=1.05$, $p=0.31$). (b) Queens treated with precocene I (JH inhibitor, $n=30$) increased brood care levels compared with control queens (acetone, $n=30$) on Days 3 and 4 ($\chi^2=3.91$, $p=0.048$), but not on Days 1 and 2 ($\chi^2=2.34$, $p=0.13$). The effect on Days 3 and 4 was primarily driven by the difference between treated and control queens 2 h after treatment ($\chi^2=10.49$, $p=0.0012$), which was not detectable anymore 6 h after treatment ($\chi^2=0.41$, $p=0.52$).

queens and workers in various social insect species (Hartfelder et al., 2006; Libbrecht, Corona, et al., 2013; Mutti et al., 2011; Rembold et al., 1974; Röseler, 1976). In ants, the insulin-signalling pathway likely played a role in the emergence of reproductive division of labour (Chandra et al., 2018) from a subsocial ancestor that would alternate between reproduction and brood care phases (Hunt, 1999, 2012; West-Eberhard, 1987, 1996). These phases of the ancestral life cycle were controlled by the brood, which inhibited reproduction (Kelstrup et al., 2018). To investigate the effect of brood on the reproduction of founding and established queens, we quantified the effect of larvae on egg production over time in isolated *L. niger* queens before and after they produced workers. We found that, while the presence of larvae stimulated egg production in established queens (interaction between time and presence of

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

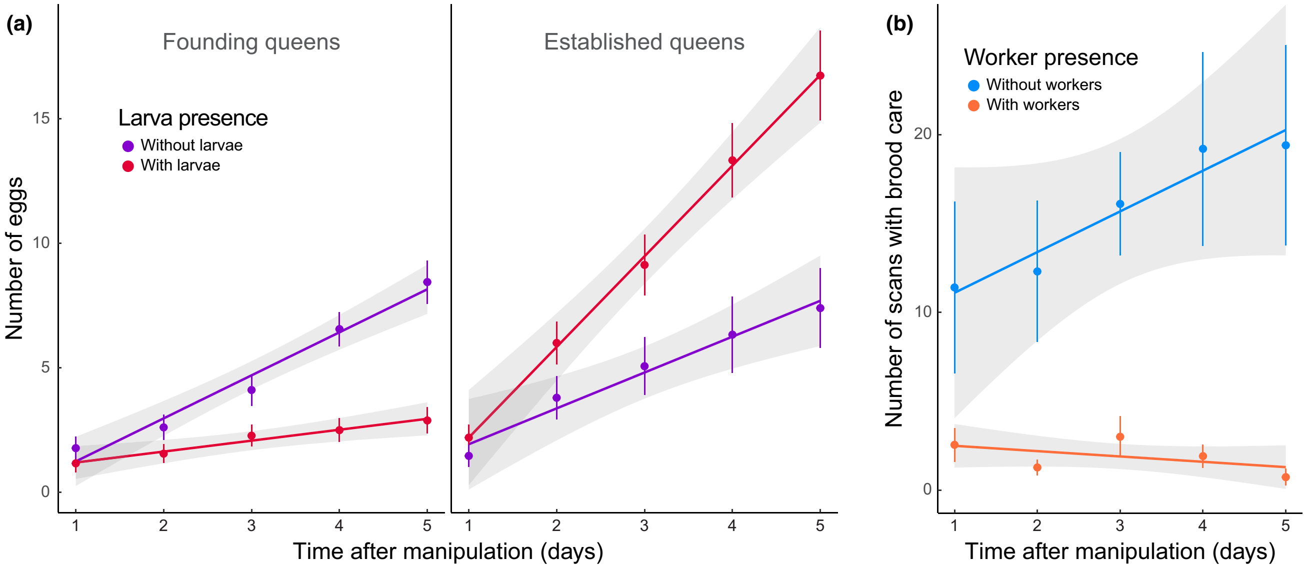

FIGURE 4 Founding queens may express an ancestral physiological response to brood and workers drive the queen specialization in another ant species. (a) Founding queens (left) kept with larvae ($n=18$) produced less eggs than founding queens kept without larvae ($n=18$; interaction between time and presence of larvae: $\chi^2=68.84$, $p<0.0001$; main effect of the presence of larvae: $\chi^2=16.47$, $p<0.0001$), while established queens (right) kept with larvae ($n=15$) produced more eggs than established queens kept without larvae ($n=15$; interaction between time and presence of larvae: $\chi^2=50.02$, $p<0.0001$; main effect of the presence of larvae: $\chi^2=9.76$, $p=0.0018$). Note that both founding and established queens were kept without workers for the duration of the experiment. (b) Established queens of the ant *Temnothorax nylanderi* expressed more brood care behaviour when kept without ($n=10$) than with workers ($n=11$; $\chi^2=32.23$, $p<0.0001$). Coloured dots and error bars represent means and standard errors, respectively.

larvae: $\chi^2=50.02$, $p<0.0001$; main effect of the presence of larvae: $\chi^2=9.76$, $p=0.0018$; Figure 4a), it had the reverse effect and inhibited egg production in founding queens (interaction between time and presence of larvae: $\chi^2=68.84$, $p<0.0001$; main effect of the presence of larvae: $\chi^2=16.47$, $p<0.0001$; Figure 4a). Because larvae inhibit their egg production, founding queens show an ancestral physiological response to the presence of brood, and are thus more similar in that respect to the subsocial ancestor of ants than to established queens. That founding and established queens show a different response to brood presence in terms of egg production may also stem from differences between these queens in their physiological condition and in the cost of expressing brood care behaviour.

3.9 | Workers induce the queen specialization in multiple species of ants

After finding that founding queens may express ancestral traits, we hypothesized that the social control of queen specialization may not be specific to *L. niger*, but rather occur across the phylogeny of ants. To test this, we used field-collected mature colonies of *Temnothorax nylanderi* (which belongs to a different subfamily from *L. niger*) and experimentally manipulated the presence of workers. In addition to worker presence affecting queen brood care changes over time (interaction between time and worker presence: $\chi^2=3.96$, $p=0.047$), we found that queens without workers generally expressed more brood care than queens with workers ($\chi^2=32.23$, $p<0.0001$, Figure 4b). This result shows that workers maintain queen specialization in *T. nylanderi* as well, and together with previous reports of social effects on queen behaviour (Cassill, 2002; Ortiz-Alvarado & Rivera-Marchand, 2020; Woodard et al., 2013), suggests that it may be a common feature in social insects (but see (Chouvenc & Su, 2017; Rüppell et al., 2002)).

4 | DISCUSSION

Social insect colonies are often viewed as superorganisms because they are intricate, complex biological systems that operate through the coordinated efforts of specialized individuals (Boomsma & Gawne, 2018; Szathmáry & Smith, 1995; Wheeler, 1911). While numerous studies explored the division of labour between fertile queens that specialize in egg production and functionally sterile workers that perform all non-reproductive tasks (Corona et al., 2016; Kronauer & Libbrecht, 2018; Libbrecht, Oxley, et al., 2013), our understanding of the establishment of this division of labour during the ontogeny of superorganisms remains limited. Specifically, it is crucial to elucidate the factors and mechanisms governing the behavioural shift from pluripotent founding queens that display a range of reproductive and non-reproductive behaviours to specialized established queens that exclusively prioritize egg laying. Our study reveals that (i) the presence of workers drives queen specialization in egg production by inhibiting brood care in a dose-dependent manner, (ii) the mere presence of worker cues does not trigger the queen specialization, (iii) this specialization is reversible upon worker removal in at least two species of ants, even after several years of specialization,

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and (iv) founding queens may express ancestral behaviour and physiology.

Our finding that queen specialization is under the strict control of the social environment is inconsistent with the traditional view of social insect queens as being intrinsically specialized in egg production (Hölldobler & Wilson, 1990; Wilson, 1971), but rather indicates that their specialization is dependent on social conditions and is more flexible than typically assumed. We argue that it is not sufficient to study caste differentiation (Ashby et al., 2016; Cameron et al., 2013; Collins et al., 2020; Corona et al., 2016; Genzoni et al., 2023; Libbrecht et al., 2011; Libbrecht, Corona, et al., 2013; Libbrecht, Oxley, et al., 2013; Montagna et al., 2015; Mutti et al., 2011; Psalti & Libbrecht, 2020; Schultner et al., 2023; Schwander et al., 2008; Schwander & Keller, 2008; Wheeler et al., 2006) and/or compare adult queens and workers (Bonasio et al., 2012; Chandra et al., 2018; Corona et al., 2007, 2013; Feldmeyer et al., 2014; Grozinger et al., 2007; Kronauer & Libbrecht, 2018; Libbrecht, Oxley, et al., 2013; Patalano et al., 2015) to fully understand division of labour in insect societies. It is necessary to also investigate the transition from pluripotent to specialized queens, as well as the maintenance of the queen specialization. Our result that queen specialization is flexible and context-dependent in two species of ants echoes previous reports that social insect species with lower degrees of morphological differentiation between queens and workers tend to exhibit behavioural flexibility within the queen caste, including in the expression of non-reproductive behaviours (Gustilo et al., 2023; Lorenzi & Turillazzi, 1986; Richards, 1971; Shell & Rehan, 2018; Theraulaz et al., 1990; Woodard et al., 2013). That established queens retain the ability to express brood care (i.e., they retain their behavioural pluripotency) raises semantic doubts on whether social insect queens are truly specialized. One may argue that because they keep the ability to express other tasks, queens show apparent, rather than true specialization. This certainly needs to be taken into account when considering insect societies with reproductive division of labour as the epitome of specialization.

Several hypotheses could explain how workers influence the behaviour of queens. The first hypothesis is that queens show an active behavioural response to the presence of workers. However, this hypothesis makes the prediction that queens would modify their behaviour in response to the mere presence of workers cues, which is inconsistent with our results. The second hypothesis is that the effect of workers on queen behaviour requires close interactions between queens and workers, for example to allow an exchange of fluids via trophallaxis. Indeed, trophallactic fluids not only contain food, but also molecules that influence behaviour (LeBoeuf et al., 2016; Meurville & LeBoeuf, 2021). Our finding that the effect of workers on queen behaviour disappeared when we prevented close interactions between queens and workers is consistent with this hypothesis, but it remains unclear whether the freshly emerged workers used in the experimental manipulations of the social environment actually engaged in trophallaxis with the queens. The third hypothesis is that the queen specialization in egg production is a mere consequence of workers performing all other, non-reproductive tasks. This hypothesis is supported by all our findings, in particular, that workers show a dose-dependent inhibition of queen brood care behaviour, that cues of worker presence are not sufficient to drive queen specialization, and that established queens revert to expressing brood care relatively rapidly upon removal of their workers. According to this hypothesis, the workers reduce the larval needs by providing care to the larvae, up to the point when the larvae receive all the care they need from the workers, and the queens stop providing brood care. We thus propose that division of labour between queens and workers may be regulated similarly as the threshold-based models of behavioural division of labour that exists within the worker force (Oldroyd & Fewell, 2007; Waddington et al., 2010; Weidenmüller, 2004). In these models of division of labour, workers have distinct thresholds for task-specific stimuli, and they only respond when a stimulus exceeds their threshold, which in turn reduces the stimulus for the other workers (Bonabeau et al., 1997; Duarte et al., 2012; Robinson, 1987a, 1992). Therefore, queen specialization may simply be explained by queens having a higher response threshold to stimuli associated with non-reproductive tasks (e.g. cues emitted by the brood) than workers.

Several lines of evidence indicate that the behavioural flexibility and physiological response to brood of the subsocial ancestor of ants are still apparent during colony foundation. First, the diverse behavioural repertoire of founding queens can be found in many species of ants (Augustin et al., 2011; Brown, 1999; Cassill, 2002; Dejean & Lachaud, 1992; Haskins & Haskins, 1955; Helms Cahan & Fewell, 2004; Jeanson & Fewell, 2008; Wheeler, 1932, 1933). Second, our results suggest that the brood care behaviour expressed by founding queens may be regulated by the JH pathway, a very conserved and pleiotropic hormonal pathway with ancestral functions (Robinson & Vargo, 1997). Third, we find that larvae inhibit egg production in founding queens, indicating that the physiological response to brood of founding queens parallels that of the subsocial ancestor of ants (Kelstrup et al., 2018). The similarities between founding queens and the subsocial ancestor of ants echo recent reports of molecular and structural parallels between pluripotent stem cells in multicellular organisms and their unicellular ancestors (Brunet & King, 2017; Mikhailov et al., 2009; Naumann & Burkhardt, 2019; Sogabe et al., 2019). Not having access to the subsocial ancestor of ants has hampered the study of the evolution of ant sociality (Kronauer & Libbrecht, 2018), and investigating pluripotent founding queens may provide a window into these ancestral functions. In that perspective, the transition from pluripotency to specialization that underlies colony foundation may even mirror the evolutionary transition from subsocial pluripotency to division of labour between specialized castes.

Finally, we report that queen specialization is reversible in two ant species that diverged more than 100 million years ago (Borowiec et al., 2020). Finding such flexibility in the queen caste is particularly unexpected in *L. niger* and *T. nylanderi*, as both are derived ant species with high degrees of social complexity and differentiation between the queen and worker castes. Future research should explore the flexibility of queen specialization in other species of ants,

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

including species where queens found their colonies dependently (i.e. with the help of workers) and thus are not required to express non-reproductive behaviours at the founding stage of the colony cycle. We propose that, although it remained relatively unnoticed so far that workers continuously maintain queen specialization, this social control may actually be a common feature of ant colonies, and possibly of other insect societies (Cassill, 2002; Ortiz-Alvarado & Rivera-Marchand, 2020; Woodard et al., 2013). By revealing such an underappreciated feature of division of labour and behavioural specialization, our study could reshape our understanding of the evolution and functioning of insect societies.

AUTHOR CONTRIBUTIONS

RL conceived the ideas and designed the methodology; VM, MNP, MC, EF, E-MT, FR, JG, LM, MR, DU and RL collected the data; VM, MNP, MC and RL analysed the data; VM, MNP, MC and RL led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank former and current members of the Behavioural Ecology and Social Evolution group at the JGU Mainz for help with the collection of founding queens, and fruitful discussions on the project. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—LI3051/2-1 and GRK2526/1-Project number: 407 023052 (RL).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data used in this study are publicly available from the Dryad Digital Repository https://doi.org/10.5061/dryad.pnvx0k6wd (Majidifar et al., 2024), and the code for all analyses is provided as Supporting Information.

ORCID

Romain Libbrecht https://orcid.org/0000-0003-4397-000X

REFERENCES

Al Baki, M. A., Lee, D.-W., Jung, J. K., & Kim, Y. (2019). Insulin signaling mediates previtellogenic development and enhances juvenile hormone-mediated vitellogenesis in a lepidopteran insect, *Maruca vitrata*. *BMC Developmental Biology*, *19*(1), 14. https://doi.org/10.1186/s12861-019-0194-8

Amarasinghe, H. E., Clayton, C. I., & Mallon, E. B. (2014). Methylation and worker reproduction in the bumble-bee (*Bombus terrestris*). *Proceedings of the Royal Society B: Biological Sciences*, *281*(1780), 20132502. https://doi.org/10.1098/rspb.2013.2502

Ashby, R., Forêt, S., Searle, I., & Maleszka, R. (2016). MicroRNAs in honey bee caste determination. *Scientific Reports*, *6*(1), 18794. https://doi.org/10.1038/srep18794

Augustin, J. O., Santos, J. F. L., & Elliot, S. L. (2011). A behavioral repertoire of *Atta sexdens* (Hymenoptera, Formicidae) queens during the claustral founding and ergonomic stages. *Insectes Sociaux*, *58*(2), 197–206. https://doi.org/10.1007/s00040-010-0137-7

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Bonabeau, E., Theraulaz, G., & Deneubourg, J. (1997). Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proceedings of the Royal Society B: Biological Sciences*, *263*(1376), 1565–1569. https://doi.org/10.1098/rspb.1996.0229

Bonasio, R., Li, Q., Lian, J., Mutti, N. S., Jin, L., Zhao, H., Zhang, P., Wen, P., Xiang, H., Ding, Y., Jin, Z., Shen, S. S., Wang, Z., Wang, W., Wang, J., Berger, S. L., Liebig, J., Zhang, G., & Reinberg, D. (2012). Genome-wide and caste-specific DNA methylomes of the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Current Biology*, *22*(19), 1755–1764. https://doi.org/10.1016/j.cub.2012.07.042

Boomsma, J. J., & Gawne, R. (2018). Superorganismality and caste differentiation as points of no return: How the major evolutionary transitions were lost in translation. *Biological Reviews of the Cambridge Philosophical Society*, *93*(1), 28–54. https://doi.org/10.1111/brv.12330

Borowiec, M. L., Moreau, C. S., & Rabeling, C. (2020). Ants: Phylogeny and classification. In C. K. Starr (Ed.), *Encyclopedia of social insects*. Springer International Publishing.

Brossette, L., Meunier, J., Dupont, S., Bagnères, A.-G., & Lucas, C. (2019). Unbalanced biparental care during colony foundation in two subterranean termites. *Ecology and Evolution*, *9*(1), 192–200. https://doi.org/10.1002/ece3.4710

Brown, M. J. F. (1999). Semi-claustral founding and worker behaviour in gynes of *Messor andrei*. *Insectes Sociaux*, *46*(2), 194–195. https://doi.org/10.1007/s000400050133

Brunet, T., & King, N. (2017). The origin of animal multicellularity and cell differentiation. *Developmental Cell*, *43*(2), 124–140. https://doi.org/10.1016/j.devcel.2017.09.016

Cameron, R. C., Duncan, E. J., & Dearden, P. K. (2013). Biased gene expression in early honeybee larval development. *BMC Genomics*, *14*(1), 903. https://doi.org/10.1186/1471-2164-14-903

Cassill, D. (2002). Brood care strategies by newly mated monogyne *Solenopsis invicta* (Hymenoptera: Formicidae) queens during colony founding. *Annals of the Entomological Society of America*, *95*(2), 208–212. https://doi.org/10.1603/0013-8746(2002)095[0208:BCSBNM]2.0.CO;2

Chandra, V., Fetter-Pruneda, I., Oxley, P. R., Ritger, A. L., McKenzie, S. K., Libbrecht, R., & Kronauer, D. J. C. (2018). Social regulation of insulin signaling and the evolution of eusociality in ants. *Science*, *361*, 398–402. https://doi.org/10.1126/science.aar5723

Chouvenc, T. (2022). Eusociality and the transition from biparental to alloparental care in termites. *Functional Ecology*, *36*(12), 3049–3059. https://doi.org/10.1111/1365-2435.14183

Chouvenc, T., & Su, N.-Y. (2017). Irreversible transfer of brood care duties and insights into the burden of caregiving in incipient subterranean termite colonies. *Ecological Entomology*, *42*(6), 777–784. https://doi.org/10.1111/een.12443

Collins, D., Wirén, A., Labédan, M., Labédan, M., Smith, M. J., Smith, M. D., Prince, D. C., Mohorianu, I., Mohorianu, I., Dalmay, T., & Bourke, A. F. G. (2020). Gene expression during larval caste determination and differentiation in intermediately eusocial bumblebees, and a comparative analysis with advanced eusocial honeybees. *Molecular Ecology*, *30*(3), 718–735. https://doi.org/10.1111/mec.15752

Corona, M., Libbrecht, R., & Wheeler, D. E. (2016). Molecular mechanisms of phenotypic plasticity in social insects. *Current Opinion in Insect Science*, *13*, 55–60. https://doi.org/10.1016/j.cois.2015.12.003

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Corona, M., Libbrecht, R., Wurm, Y., Riba-Grognuz, O., Studer, R. A., & Keller, L. (2013). Vitellogenin underwent subfunctionalization to acquire caste and behavioral specific expression in the harvester ant *Pogonomyrmex barbatus*. *PLoS Genetics*, *9*(8), e1003730. https://doi.org/10.1371/journal.pgen.1003730

Corona, M., Velarde, R. A., Remolina, S. C., Moran-Lauter, A., Wang, Y., Wang, Y., Hughes, K. A., & Robinson, G. E. (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(17), 7128–7133. https://doi.org/10.1073/pnas.0701909104

Dahbi, A., Cerdá, X., & Lenoir, A. (1998). Ontogeny of colonial hydrocarbon label in callow workers of the ant *Cataglyphis iberica*. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de La Vie*, *321*(5), 395–402. https://doi.org/10.1016/S0764-4469(98)80303-X

Dejean, A., & Lachaud, J.-P. (1992). Growth-related changes in predation behavior in incipient colonies of the ponerine ant *Ectatomma tuberculatum* (Olivier). *Insectes Sociaux*, *39*(2), 129–143. https://doi.org/10.1007/BF01249289

Duarte, A., Pen, I., Keller, L., & Weissing, F. J. (2012). Evolution of self-organized division of labor in a response threshold model. *Behavioral Ecology and Sociobiology*, *66*(6), 947–957. https://doi.org/10.1007/s00265-012-1343-2

Dussutour, A., & Simpson, S. J. (2008). Description of a simple synthetic diet for studying nutritional responses in ants. *Insectes Sociaux*, *55*(3), 329–333. https://doi.org/10.1007/s00040-008-1008-3

Errard, C. (1984). Evolution, en fonction de l'âge, des relations sociales dans les colonies mixtes hétérospécifiques chez les fourmis des genres *Camponotus* et *Pseudomyrmex*. *Insectes Sociaux*, *31*(2), 185–198. https://doi.org/10.1007/BF02232714

Feldmeyer, B., Elsner, D., & Foitzik, S. (2014). Gene expression patterns associated with caste and reproductive status in ants: Worker-specific genes are more derived than queen-specific ones. *Molecular Ecology*, *23*(1), 151–161. https://doi.org/10.1111/mec.12490

Ferreira, H. M., Di Pietro, V., Wenseleers, T., & Oi, C. A. (2023). Conserved role of juvenile hormone in regulating behavioural maturation and division of labour in a highly eusocial wasp. *Animal Behaviour*, *200*, 59–69. https://doi.org/10.1016/j.anbehav.2023.03.013

Fletcher, D. J. C., & Blum, M. S. (1983). The inhibitory pheromone of queen fire ants: Effects of disinhibition on dealation and oviposition by virgin queens. *Journal of Comparative Physiology*, *153*(4), 467–475. https://doi.org/10.1007/BF00612601

Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage Publications.

Genzoni, E., Schwander, T., & Keller, L. (2023). Trophic eggs affect caste determination in the ant *Pogonomyrmex rugosus*. *bioRxiv* https://doi.org/10.1101/2023.01.28.525977

Grozinger, C. M., Fan, Y., Hoover, S. E. R., & Winston, M. L. (2007). Genome-wide analysis reveals differences in brain gene expression patterns associated with caste and reproductive status in honey bees (*Apis mellifera*). *Molecular Ecology*, *16*(22), 4837–4848. https://doi.org/10.1111/j.1365-294x.2007.03545.x

Gustilo, E. S., Fisher, K., & Woodard, S. H. (2023). Parental care behaviour in bumble bee queens is tightly regulated by the number of helpers in incipient nests. *Animal Behaviour*, *203*, 241–250. https://doi.org/10.1016/j.anbehav.2023.07.009

Hartfelder, K., Makert, G. R., Judice, C. C., Pereira, G. A. G., Santana, W. C., Dallacqua, R., & Bitondi, M. M. G. (2006). Physiological and genetic mechanisms underlying caste development, reproduction and division of labor in stingless bees. *Apidologie*, *37*(2), 144–163. https://doi.org/10.1051/apido:2006013

Haskins, C. P., & Haskins, E. F. (1955). The pattern of colony foundation in the archaic ant *Myrmecia regularis*. *Insectes Sociaux*, *2*(2), 115–126. https://doi.org/10.1007/bf02224097

Helms Cahan, S., & Fewell, J. H. (2004). Division of labor and the evolution of task sharing in queen associations of the harvester ant *Pogonomyrmex californicus*. *Behavioral Ecology and Sociobiology*, *56*(1), 9–17. https://doi.org/10.1007/s00265-003-0746-5

Henrick, C. A. (2007). Methoprene. *Journal of the American Mosquito Control Association*, *23*(sp2), 225–239. https://doi.org/10.2987/8756-971X(2007)23[225:M]2.0.CO;2

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.

Holman, L. (2014). Bumblebee size polymorphism and worker response to queen pheromone. *PeerJ*, *2*, e604. https://doi.org/10.7717/peerj.604

Holman, L., Hanley, B. P., Hanley, B., & Millar, J. G. (2016). Highly specific responses to queen pheromone in three *Lasius* ant species. *Behavioral Ecology and Sociobiology*, *70*(3), 387–392. https://doi.org/10.1007/s00265-016-2058-6

Hunt, J. H. (1999). Trait mapping and salience in the evolution of eusocial vespid wasps. *Evolution*, *53*(1), 225–237. https://doi.org/10.1111/j.1558-5646.1999.tb05348.x

Hunt, J. H. (2012). A conceptual model for the origin of worker behaviour and adaptation of eusociality. *Journal of Evolutionary Biology*, *25*(1), 1–19. https://doi.org/10.1111/j.1420-9101.2011.02421.x

Isingrini, M., Lenoir, A., & Jaisson, P. (1985). Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proceedings of the National Academy of Sciences of the United States of America*, *82*(24), 8545–8547. https://doi.org/10.1073/pnas.82.24.8545

Ito, F., Miyazaki, S., Hashim, R., & Billen, J. (2017). Colony composition and behavioral characteristics of *Myrmoteras iriodum* and *M. jaitrongi* in Ulu Gombak, Peninsular Malaysia (Hymenoptera: Formicidae). *Asian Myrmecology*, *9*, e009010. https://doi.org/10.20362/am.009010

Janet, C. (1907). Anatomie du corselet et histolyse des muscles vibrateurs, après le vol nuptial, chez la reine de la fourmi (*Lasius niger*) (Vol. 1). Ducourtieux et Gout.

Jeanson, R., & Fewell, J. H. (2008). Influence of the social context on division of labor in ant foundress associations. *Behavioral Ecology*, *19*(3), 567–574. https://doi.org/10.1093/beheco/arn018

Julian, G. E., & Fewell, J. H. (2004). Genetic variation and task specialization in the desert leaf-cutter ant, *Acromyrmex versicolor*. *Animal Behaviour*, *68*(1), 1–8. https://doi.org/10.1016/j.anbehav.2003.06.023

Keller, L., & Passera, L. (1989). Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). *Oecologia*, *80*(2), 236–240. https://doi.org/10.1007/BF00380157

Kelstrup, H. C., Hartfelder, K., Lopes, T. F., & Wossler, T. C. (2018). The behavior and reproductive physiology of a solitary progressive provisioning vespid wasp: Evidence for a solitary-cycle origin of reproductive castes. *The American Naturalist*, *191*(2), E27–E39. https://doi.org/10.1086/695336

Kronauer, D. J. C., & Libbrecht, R. (2018). Back to the roots: The importance of using simple insect societies to understand the molecular basis of complex social life. *Current Opinion in Insect Science*, *28*, 33–39. https://doi.org/10.1016/j.cois.2018.03.009

LeBoeuf, A. C. (2021). Trophallaxis. In C. K. Starr (Ed.), *Encyclopedia of social insects*. Springer International Publishing.

LeBoeuf, A. C., Waridel, P., Brent, C. S., Gonçalves, A. N., Menin, L., Ortiz, D., Riba-Grognuz, O., Koto, A., Soares, Z. G., Privman, E., Miska, E. A., Benton, R., & Keller, L. (2016). Oral transfer of chemical cues, growth proteins and hormones in social insects. *eLife*, *5*, e20375. https://doi.org/10.7554/eLife.20375

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2019). Package 'emmeans'. R package version, 1(3.2).

Libbrecht, R., Corona, M., Wende, F., Azevedo, D. O., Serrão, J. E., & Keller, L. (2013). Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates maternal effects on polyphenism in ants. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(27), 11050–11055. https://doi.org/10.1073/pnas.1221781110

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Libbrecht, R., Oxley, P. R., Keller, L., & Kronauer, D. J. C. (2016). Robust DNA methylation in the clonal raider ant brain. *Current Biology*, *26*(3), 391–395. https://doi.org/10.1016/j.cub.2015.12.040

Libbrecht, R., Oxley, P. R., & Kronauer, D. J. C. (2018). Clonal raider ant brain transcriptomics identifies candidate molecular mechanisms for reproductive division of labor. *BMC Biology*, *16*(1), 89. https://doi.org/10.1186/s12915-018-0558-8

Libbrecht, R., Oxley, P. R., Kronauer, D. J. C., & Keller, L. (2013). Ant genomics sheds light on the molecular regulation of social organization. *Genome Biology*, *14*(7), 212. https://doi.org/10.1186/gb-2013-14-7-212

Libbrecht, R., Schwander, T., & Keller, L. (2011). Genetic components to caste allocation in a multiple-queen ant species. *Evolution*, *65*(10), 2907–2915. https://doi.org/10.1111/j.1558-5646.2011.01348.x

Lorenzi, M. C., & Turillazzi, S. (1986). Behavioural and ecological adaptations to the high mountain environment of *Polistes biglumis bimaculatus*. *Ecological Entomology*, *11*(2), 199–204. https://doi.org/10.1111/j.1365-2311.1986.tb00295.x

Macedo, L. M. F., Nunes, F. M. F., Freitas, F. C. P., Pires, C. V., Tanaka, E. D., Martins, J., Piulachs, M.-D., Cristino, A. S., Pinheiro, D. G., & Simões, Z. L. P. (2016). MicroRNA signatures characterizing caste-independent ovarian activity in queen and worker honeybees (*Apis mellifera* L.). *Insect Molecular Biology*, *25*(3), 216–226. https://doi.org/10.1111/imb.12214

Majidifar, V., Psalti, M. N., Coulm, M., Fetzer, E., Teggers, E.-M., Rotering, F., Grünewald, J., Mannella, L., Reuter, M., Unte, D., & Libbrecht, R. (2024). Data from: Ontogeny of superorganisms: Social control of queen specialization in ants. *Dryad Digital Repository* https://doi.org/10.5061/dryad.pnvx0k6wd

Meurville, M.-P., & LeBoeuf, A. C. (2021). Trophallaxis: The functions and evolution of social fluid exchange in ant colonies (Hymenoptera: Formicidae). *Myrmecological News*, *31*, 1–30. https://doi.org/10.25849/myrmecol.news_031:001

Mikhailov, K. V., Konstantinova, A. V., Nikitin, M. A., Troshin, P. V., Rusin, L. Y., Lyubetsky, V. A., Panchin, Y. V., Mylnikov, A. P., Moroz, L. L., Kumar, S., & Aleoshin, V. V. (2009). The origin of Metazoa: A transition from temporal to spatial cell differentiation. *BioEssays*, *31*(7), 758–768. https://doi.org/10.1002/bies.200800214

Modlmeier, A. P., & Foitzik, S. (2011). Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behavioral Ecology*, *22*(5), 1026–1032. https://doi.org/10.1093/beheco/arr086

Montagna, T. S., Raizer, J., & Antonialli-Junior, W. F. (2015). Effect of larval topical application of juvenile hormone on caste determination in the independent-founding eusocial wasp *Mischocyttarus consimilis* (Hymenoptera: Vespidae). *Open Journal of Animal Sciences*, *5*(2), 174–184. https://doi.org/10.4236/ojas.2015.52020

Murakami, T. (2020). Non-inseminated queens have worker-like behaviors in colonies of fungus-growing ants, *Mycetomoellerius turrifex* Wheeler (Attini, Hymenoptera). *Sociobiology*, *67*(3), 358. https://doi.org/10.13102/sociobiology.v67i3.5773

Mutti, N. S., Dolezal, A. G., Wolschin, F., Mutti, J. S., Gill, K. S., & Amdam, G. V. (2011). IRS and TOR nutrient-signaling pathways act via juvenile hormone to influence honey bee caste fate. *Journal of Experimental Biology*, *214*(23), 3977–3984. https://doi.org/10.1242/jeb.061499

Naumann, B., & Burkhardt, P. (2019). Spatial cell disparity in the colonial choanoflagellate *Salpingoeca rosetta*. *Frontiers in Cell and Developmental Biology*, *7*, 231. https://doi.org/10.3389/fcell.2019.00231

Negroni, M. A., Macit, M. N., Stoldt, M., Feldmeyer, B., & Foitzik, S. (2021). Molecular regulation of lifespan extension in fertile ant workers. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *376*(1823), 20190736. https://doi.org/10.1098/rstb.2019.0736

Nehring, V., Boomsma, J. J., & d'Ettorre, P. (2012). Wingless virgin queens assume helper roles in *Acromyrmex* leaf-cutting ants. *Current Biology*, *22*(17), R671–R673. https://doi.org/10.1016/j.cub.2012.06.038

Norman, V. C., Pamminger, T., & Hughes, W. O. H. (2016). Behavioural development, fat reserves and their association with productivity in *Lasius flavus* founding queens. *The Science of Nature*, *103*(3), 23. https://doi.org/10.1007/s00114-016-1350-7

O'donnell, S. (1998). Genetic effects on task performance, but not on age polyethism, in a swarm-founding eusocial wasp. *Animal Behaviour*, *55*(2), 417–426. https://doi.org/10.1006/anbe.1997.0627

Oldroyd, B. P., & Fewell, J. H. (2007). Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology & Evolution*, *22*(8), 408–413. https://doi.org/10.1016/j.tree.2007.06.001

Ortiz-Alvarado, Y., & Rivera-Marchand, B. (2020). Worker queens? Behavioral flexibility of queens in the little fire ant *Wasmannia auropunctata*. *Frontiers in Ecology and Evolution*, *8*, 241. https://doi.org/10.3389/fevo.2020.00241

Pamminger, T., Buttstedt, A., Norman, V., Schierhorn, A., Botías, C., Jones, J. C., Basley, K., & Hughes, W. O. H. (2016). The effects of juvenile hormone on *Lasius niger* reproduction. *Journal of Insect Physiology*, *95*, 1–7. https://doi.org/10.1016/j.jinsphys.2016.09.004

Pamminger, T., Scharf, I., Pennings, P. S., & Foitzik, S. (2011). Increased host aggression as an induced defense against slave-making ants. *Behavioral Ecology*, *22*(2), 255–260. https://doi.org/10.1093/beheco/arq191

Patalano, S., Vlasova, A., Wyatt, C. D. R., Ewels, P., Camara, F., Ferreira, P. G., Asher, C., Jurkowski, T. P., Segonds-Pichon, A., Bachman, M., González-Navarrete, I., Minoche, A. E., Krueger, F., Lowy, E., Marcet-Houben, M., Rodriguez-Ales, J. L., Nascimento, F. S., Balasubramanian, S., Gabaldón, T., … Sumner, S. (2015). Molecular signatures of plastic phenotypes in two eusocial insect species with simple societies. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(45), 13970–13975. https://doi.org/10.1073/pnas.1515937112

Peeters, C. (2020). Colony foundation. In C. K. Starr (Ed.), *Encyclopedia of social insects*. Springer International Publishing.

Perez-Hedo, M., Rivera-Perez, C., & Noriega, F. G. (2014). Starvation increases insulin sensitivity and reduces juvenile hormone synthesis in mosquitoes. *PLoS One*, *9*(1), e86183. https://doi.org/10.1371/journal.pone.0086183

Psalti, M. N., Gohlke, D., & Libbrecht, R. (2021). Experimental increase of worker diversity benefits brood production in ants. *BMC Ecology and Evolution*, *21*(1), 163. https://doi.org/10.1186/s12862-021-01890-x

Psalti, M. N., & Libbrecht, R. (2020). Caste differentiation. In C. K. Starr (Ed.), *Encyclopedia of social insects*. Springer International Publishing.

Pyenson, B., Albin-Brooks, C., Burhyte, C., & Liebig, J. (2022). Worker-like behavioral and physiological phenotype in queens with removed wings in a ponerine ant. *Journal of Experimental Biology*, *225*(18), jeb243684. https://doi.org/10.1242/jeb.243684

Rembold, H., Czoppelt, C., & Rao, P. J. (1974). Effect of juvenile hormone treatment on caste differentiation in the honeybee, *Apis mellifera*. *Journal of Insect Physiology*, *20*(7), 1193–1202. https://doi.org/10.1016/0022-1910(74)90225-X

Richard, F.-J., & Hunt, J. H. (2013). Intracolony chemical communication in social insects. *Insectes Sociaux*, *60*(3), 275–291. https://doi.org/10.1007/s00040-013-0306-6

Richards, O. W. (1971). The biology of the social wasps (Hymenoptera, Vespidae). *Biological Reviews*, *46*(4), 483–528. https://doi.org/10.1111/j.1469-185X.1971.tb01054.x

Rissing, S. W., & Pollock, G. B. (1986). Social interaction among pleometrotic queens of *Veromessor pergandei* (Hymenoptera:Formicidae) during colony foundation. *Animal Behaviour*, *34*, 226–233. https://doi.org/10.1016/0003-3472(86)90027-8

Robinson, G. E. (1987a). Modulation of alarm pheromone perception in the honey bee: Evidence for division of labor based on hormonally regulated response thresholds. *Journal of Comparative Physiology A*, *160*(5), 613–619. https://doi.org/10.1007/BF00611934

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Robinson, G. E. (1987b). Regulation of honey bee age polyethism by juvenile hormone. *Behavioral Ecology and Sociobiology*, *20*(5), 329–338. https://doi.org/10.1007/BF00300679

Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annual Review of Entomology*, *37*(1), 637–665. https://doi.org/10.1146/annurev.en.37.010192.003225

Robinson, G. E., & Vargo, E. L. (1997). Juvenile hormone in adult eusocial hymenoptera: Gonadotropin and behavioral pacemaker. *Archives of Insect Biochemistry and Physiology*, *35*(4), 559–583. https://doi.org/10.1002/(SICI)1520-6327(1997)35:4<559::AID-ARCH13>3.0.CO;2-9

Ronai, I., Oldroyd, B. P., & Vergoz, V. (2016). Queen pheromone regulates programmed cell death in the honey bee worker ovary. *Insect Molecular Biology*, *25*(5), 646–652. https://doi.org/10.1111/imb.12250

Röseler, P.-F. (1976). Juvenile hormone and queen rearing in bumblebees. In M. Lüscher (Ed.), *Phase and caste determination in insects* (pp. 55–61). Elsevier. ISBN 9780080212562. https://doi.org/10.1016/B978-0-08-021256-2.50009-1; https://www.sciencedirect.com/science/article/pii/B9780080212562500091

Rüppell, O., Schäffler, L., & Hölldobler, B. (2002). Short communication: Lack of plasticity in the behavior of queens of the ant *Leptothorax rugatulus* Emery (Formicidae: Hymenoptera). *Journal of Insect Behavior*, *15*(3), 447–454. https://doi.org/10.1023/A:1016277511957

Schultner, E., Oettler, J., & Helanterä, H. (2017). The role of brood in eusocial Hymenoptera. *The Quarterly Review of Biology*, *92*(1), 39–78. https://doi.org/10.1086/690840

Schultner, E., Wallner, T., Dofka, B., Brülhart, J., Heinze, J., Freitak, D., Pokorny, T., & Oettler, J. (2023). Queens control caste allocation in the ant *Cardiocondyla obscurior*. *Proceedings of the Royal Society B: Biological Sciences*, *290*(1992), 20221784. https://doi.org/10.1098/rspb.2022.1784

Schwander, T., Humbert, J. Y., Brent, C. S., Cahan, S. H., Chapuis, L., Renai, E., & Keller, L. (2008). Maternal effect on female caste determination in a social insect. *Current Biology*, *18*(4), 265–269. https://doi.org/10.1016/j.cub.2008.01.024

Schwander, T., & Keller, L. (2008). Genetic compatibility affects queen and worker caste determination. *Science*, *322*(5901), 552. https://doi.org/10.1126/science.1162590

Shell, W. A., & Rehan, S. M. (2018). Behavioral and genetic mechanisms of social evolution: Insights from incipiently and facultatively social bees. *Apidologie*, *49*(1), 13–30. https://doi.org/10.1007/s13592-017-0527-1

Signorotti, L., Jaisson, P., & d'Ettorre, P. (2014). Larval memory affects adult nest-mate recognition in the ant *Aphaenogaster senilis*. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1774), 20132579. https://doi.org/10.1098/rspb.2013.2579

Sogabe, S., Hatleberg, W. L., Kocot, K. M., Say, T. E., Stoupin, D., Roper, K. E., Fernandez-Valverde, S. L., Degnan, S. M., & Degnan, B. M. (2019). Pluripotency and the origin of animal multicellularity. *Nature*, *570*(7762), 519–522. https://doi.org/10.1038/s41586-019-1290-4

Sprenger, P. P., & Menzel, F. (2020). Cuticular hydrocarbons in ants (Hymenoptera: Formicidae) and other insects: How and why they differ among individuals, colonies, and species. *Myrmecological News*, *30*, 1–26. https://doi.org/10.25849/myrmecol.news_030:013

Stuart, R. J. (1988). Collective cues as a basis for nestmate recognition in polygynous leptothoracine ants. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(12), 4572–4575. https://doi.org/10.1073/pnas.85.12.4572

Stuart, R. J., & Page, R. E. (1991). Genetic component to division of labor among workers of a leptothoracine ant. *Naturwissenschaften*, *78*(8), 375–377. https://doi.org/10.1007/BF01131615

Szathmáry, E., & Smith, J. M. (1995). The major evolutionary transitions. *Nature*, *374*(6519), 227–232. https://doi.org/10.1038/374227a0

Tatar, M., Kopelman, A., Epstein, D., Tu, M.-P., Yin, C.-M., & Garofalo, R. S. (2001). A mutant *drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science*, *292*, 107–110. https://doi.org/10.1126/science.1057987

Teggers, E.-M., Deegener, F., & Libbrecht, R. (2021). Fecundity determines the outcome of founding queen associations in ants. *Scientific Reports*, *11*, 2986. https://doi.org/10.1038/s41598-021-82559-9

Theraulaz, G., Pratte, M., & Gervet, J. (1990). Behavioural profiles in *Polistes dominulus* (Christ) wasp societies: A quantitative study. *Behaviour*, *113*(3/4), 223–250.

Ulrich, Y., Burns, D., Libbrecht, R., & Kronauer, D. J. C. (2016). Ant larvae regulate worker foraging behavior and ovarian activity in a dose-dependent manner. *Behavioral Ecology and Sociobiology*, *70*(7), 1011–1018. https://doi.org/10.1007/s00265-015-2046-2

Vieira, A. S., Fernandes, W. D., & Antonialli-Junior, W. F. (2011). Behavioral differentiation and ovarian development of unmated gynes, queens, and workers of *Ectatomma vizottoi* Almeida 1987 (Formicidae, Ectatomminae). *Psyche: A Journal of Entomology*, *2012*, e349896. https://doi.org/10.1155/2012/349896

Waddington, S. J., Santorelli, L. A., Ryan, F. R., & Hughes, W. O. H. (2010). Genetic polyethism in leaf-cutting ants. *Behavioral Ecology*, *21*(6), 1165–1169. https://doi.org/10.1093/beheco/arq128

Walsh, J. T., Signorotti, L., Linksvayer, T. A., & d'Ettorre, P. (2018). Phenotypic correlation between queen and worker brood care supports the role of maternal care in the evolution of eusociality. *Ecology and Evolution*, *8*(21), 10409–10415. https://doi.org/10.1002/ece3.4475

Weidenmüller, A. (2004). The control of nest climate in bumblebee (*Bombus terrestris*) colonies: Interindividual variability and self reinforcement in fanning response. *Behavioral Ecology*, *15*(1), 120–128. https://doi.org/10.1093/beheco/arg101

West-Eberhard, M. J. (1987). Flexible strategy and social evolution. In Y. Ito, J. L. Brown, & J. Kikkawa (Eds.), *Animal societies. Theories and facts*. Japan Scientific Societies Press.

West-Eberhard, M. J. (1996). Wasp societies as microcosms for the study of development and evolution. In S. Turillazzi & M. J. West-Eberhard (Eds.), *Natural history and evolution of paper wasps*. Oxford Academic Press.

Wheeler, D. E., Buck, N. A., & Evans, J. D. (2006). Expression of insulin pathway genes during the period of caste determination in the honey bee, Apis mellifera. *Insect Molecular Biology*, *15*(5), 597–602. https://doi.org/10.1111/j.1365-2583.2006.00681.x

Wheeler, W. M. (1911). The ant-colony as an organism. *Journal of Morphology*, *22*(2), 307–325. https://doi.org/10.1002/jmor.1050220206

Wheeler, W. M. (1932). How the primitive ants of Australia start their colonies. *Science*, *76*(1980), 532–533. https://doi.org/10.1126/science.76.1980.532

Wheeler, W. M. (1933). *Colony-founding among ants*. Harvard University Press.

Wilson, E. O. (1971). *The insect societies*. Harvard University Press.

Wilson, E. O., Durlach, N. I., & Roth, L. M. (1958). Chemical releasers of necrophoric behavior in ants. *Psyche*, *65*(4), 108–114. https://doi.org/10.1155/1958/69391

Winston, M. L., Higo, H. A., & Slessor, K. N. (1990). Effect of various dosages of queen mandibular gland pheromone on the inhibition of queen rearing in the honey bee (Hymenoptera: Apidae). *Annals of the Entomological Society of America*, *83*(2), 234–238. https://doi.org/10.1093/aesa/83.2.234

Woodard, S. H., Bloch, G., Band, M. R., & Robinson, G. E. (2013). Social regulation of maternal traits in nest-founding bumble bee (*Bombus terrestris*) queens. *Journal of Experimental Biology*, *216*(18), 3474–3482. https://doi.org/10.1242/jeb.087403

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitätsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. One experimental tray with 12 observation arenas, which were recorded simultaneously with a single camera.

Figure S2. Resolution of the video when zooming in on one observation arena with one queen and five larvae.

Figure S3. Experimental setup to test the effect of workers separated from the queens with a circular wire mesh.

Figure S4. Brood production of 22 founding queens.

Figure S5. Effect of workers on the brood care behaviour of founding queens 20 h after the experimental manipulation.

Figure S6. Brood care behaviour of established queens (3 years and 2 months after their nuptial flight) in response to the sequential removal of their workers over the course of 6 days.

Figure S7. Proportion of unmated queens expressing brood care behaviour in the presence or absence of workers.

Figure S8. Workers induce queen specialization in a dose-dependent manner.

Figure S9. No effect of former presence of workers on the brood care behaviour of founding queens.

Figure S10. Effect of dead worker presence on brood care of founding queens.

Figure S11. Effect of precocene II on brood care behavior in founding queens.

Table S1. Information on the collection and keeping conditions of ant queens.

Table S2. Summary of the input data, syntax, and output of all statistical models.

Appendix S1. Fully annotated R script to run the data analyses conducted in the study.

How to cite this article: Majidifar, V., Psalti, M. N., Coulm, M., Fetzer, E., Teggers, E.-M., Rotering, F., Grünewald, J., Mannella, L., Reuter, M., Unte, D., & Libbrecht, R. (2024). Ontogeny of superorganisms: Social control of queen specialization in ants. *Functional Ecology*, *38*, 1044–1060. https://doi.org/10.1111/1365-2435.14536

13652435, 2024, 5, Downloaded from https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2435.14536 by Universitatsbibliothek Mainz, Wiley Online Library on [12/12/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License