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Worker task organization in incipient bumble bee nests

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Bumble bees (*Bombus*: Apidae) are a long-standing model system for understanding animal behaviour, ecology and evolution. However, how workers in this system are organized to perform fundamental tasks related to brood feeding and food collection remains unclear. Bumble bees undergo dramatically different life stages, across which the social environment, and therefore task organization, changes over time. Queens initiate nests solitarily, and when the first cohort of workers emerge, they help the queen carry out brood-feeding and food collection tasks, whereas the queen transitions to primarily egg laying. Although task organization has been studied in mature colonies, few studies to date have explored how these tasks are organized in young, incipient nests. Here, we explored how food-related tasks, including brood feeding and food collection, are organized by workers in incipient colonies. We found that food - related tasks were nested, in that the majority of workers fed brood, a subset also collected nectar and a smaller subset also collected pollen. These patterns suggest that brood feeding is a task shared by most workers, and that the distinction between pollen collecting versus nonpollen collecting might be the most important axis of division of labour in bumble bee nests, at least at the early nesting stage.

A principal difference separating the social and solitary insects is that in the former, fundamental tasks related to feeding and reproduction are performed cooperatively by social group members, rather than by a single individual. All reproductive female insects, whether solitary or social, have to perform the fundamental tasks of finding food and suitable places to deposit and/or rear their offspring, in order to survive and reproduce. However, in the social insects, these tasks often change in both form and complexity and are coordinated between multiple individuals that collectively perform a broad suite of tasks (Michener, 1969; Wilson, 1971). Identifying how tasks are coordinated between multiple individuals is a major goal in social insect research (Korb & Heinze, 2008; Oster & Wilson, 1978; Toth & Rehan, 2017; Toth & Robinson, 2007; Wheeler, 1928).

Specialization, defined as individuals repeatedly performing the same task or task repertoire, to the exclusion of other tasks, is a prevalent component of social insect societies (Beshers & Fewell, 2001; Gordon, 2016; Wilson, 1971). Specialization is considered adaptive for social groups because it can improve collective efficiency; however, groups often perform more optimally when they contain both specialized and flexible individuals (Fisher, 2019; Muller & Chittka, 2008; Oster & Wilson, 1978). Social insects can vary dramatically with respect to the degree that they specialize on tasks. Some systems are organized by morphological or age-related polyethism, where worker age or morphology are associated with specialization on specific tasks (Mildner & Roces, 2017; Wilson, 1971). For example, in honey bees, a model system for studying social insect behaviour, workers undergo an age-related transition from specialization on in-hive tasks, including brood feeding, to foraging (i.e. food collection). Other species have more flexible social organization, such as the annually or facultatively eusocial species (Brian, 1952; West-Eberhard, 1967), where individuals often move fluently along a gradient between behavioural specialization and flexibility (Gordon, 2016; Santoro et al., 2019; Wilson, 1971).

Bumble bees are annually social insects that have been shown to exhibit flexible task organization across the colony cycle. When a bumble bee nest is first initiated, typically in spring, all tasks are exclusively performed by a solitary foundress queen. In the subsequent weeks, after the first cohort of workers emerge in the nest, they assume tasks such as feeding larvae (hereafter, 'brood feeding') and food collection, and the queen transitions to specializing on egg laying and ultimately ceases foraging and brood feeding (Shpigler et al., 2013; Woodard et al., 2013). Among

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workers in mature colonies, some individuals exhibit specialization on specific tasks (Cartar, 1992; Hagbery & Nieh, 2012), but workers can also be observed performing multiple tasks, even within a single day (Brian, 1952; Crall et al., 2018; Jandt & Dornhaus, 2009). Furthermore, even when individuals exhibit a greater tendency to perform specific tasks, they usually do not perform these tasks exclusively (Jandt et al., 2009; Russell et al., 2017). There does not appear to be an association between age and task performance in bumble bee workers (Cameron, 1989). Additionally, although some studies have found that body size marginally predicts task performance (Crall et al., 2018; Goulson et al., 2002), other studies have failed to find this association (Russell et al., 2017; Smith et al., 2016). Thus, overall, bumble bee workers in mature colonies are considered to exhibit only weak task specialization. Despite a relative wealth of studies on how labour is divided among bumble bee workers in mature colonies, our understanding of how tasks are organized at the earliest stages of nest development is extremely limited. The incipient stage of the colony is important to examine with respect to how tasks are organized because this is a transitional period, when worker division of labour is only beginning to form and queens still perform some food-related tasks, then cease performing them through time (Shpigler et al., 2013; Woodard et al., 2013). Moreover, the successful performance of nests at this stage is essential for colonies to mature and ultimately produce reproductive individuals. The early nesting stage is also more experimentally tractable compared to larger (mature) colonies, which can have several hundred individuals (Cnaani et al., 2002), because it is possible to perform relatively detailed behavioural observations on every group member.

The collection of pollen and nectar resources and the progressive feeding of offspring over the course of their larval development are two of the most essential tasks required for bumble bee colonies to continuously develop and ultimately produce reproductive individuals (e.g. new queens and males) (Heinrich, 1979). Despite this, an important limitation in understanding the organization of food-related tasks in bumble bees is that brood-feeding behaviour is rarely included in experimental studies (Cartar, 1992; Crall et al., 2018; Goulson et al., 2002; Russell et al., 2017; but see Dornhaus et al., 2012; Shpigler et al., 2013; Woodard et al., 2013). This is due in large part to the fact that it is difficult to observe in-nest behaviours without disturbing the nest, compared to out-of-nest behaviours. Brood feeding also occurs relatively infrequently and thus requires long periods of observation, and the detection of this behaviour cannot be automated with currently existing technology (Shpigler et al., 2013; Woodard et al., 2013) in contrast to food collection behaviour (Crall et al., 2018; Russell et al., 2017). As a result, no contemporary study has explicitly tested the relative frequency of brood-feeding and food collection behaviours and incorporated this into analyses of task organization (but see Brian,1952). The objective of this study was to determine how food-related tasks that are essential to young nest growth and survival are organized among workers during the early nesting stage in bumble bees. To explore this, we used small, artificially assembled groups of the common eastern bumble bee, *Bombus impatiens*, that contained a queen and five workers, the typical number of workers in the first cohort in young nests (Costa et al., 2021; Watrous et al., 2019; Woodard et al., 2013). Within these nests, we observed the relative frequency of food collection, brood-feeding and egg-laying events performed by individual workers and evaluated the exclusivity of these tasks both within and among individuals. For task exclusivity, we predicted that pollen foraging would be the most exclusive food-related task, given that, under field-realistic conditions, it is a cognitively demanding (Menzel, 2012; Muth et al., 2016) and relatively risky (Rueppell et al., 2007) activity. We also examined how task performance is influenced by two factors: the number of workers in the nest (a reduction in worker number, from five to three) and the presence of the queen. This allowed us to explore the prediction that both might strongly influence worker task organization. For the former, this was predicated on the fact that, at the incipient stage, nests only contain a few workers (typically the first cohort of brood consists of five or fewer workers) and thus small changes in worker number equate to relatively large differences in group size. This manipulation reflects, for example, the loss of a subset of workers from the first cohort, or a reduction in the size of the first worker cohort due to nutritional stress (Watrous et al., 2019) or some other external factor. The latter prediction was based on previous studies in bumble bees (Orlova et al., 2020) and other social insects (Kocher & Grozinger, 2011) that have found that queens have a strong influence on worker behaviour and physiology.

METHODS

Rearing and Experimental Design

Fifteen mature *B. impatiens* colonies (consisting of a queen and > 50 female workers) were provided by Koppert Biological Systems (Howell, MI, U.S.A.) and maintained in their commercial boxes at the University of California, Riverside at 23 °C and 40% relative humidity (RH). Colonies were fed ad libitum mixed-source, honey bee-collected pollen purchased from Brushy Mountain Bee Farm (Moravian Falls, NC, U.S.A.) and a syrup solution provided by Koppert Biological Systems. Individual bees were removed from source colonies to create small, artificially constructed groups (hereafter 'nests') in plastic nestboxes (15 × 15 × 10 cm) with the following four social configurations: queenright with three workers (QW3; *N* ¼ 14 nests), queenright with five workers (QW5; *N* ¼ 15), queenless with three workers (W3; *N* ¼ 16) and queenless with five workers (W5; *N* ¼ 13). *Bombus impatiens* queens initiate nests by laying a single cohort of typically five female-destined eggs (Leza et al., 2018; Watrous et al., 2019), then colonies grow in worker number over the course of the season, before producing reproductive offspring (Cnaani et al., 2002). For the two queenright groups (QW3 and QW5), callow queens (< 24 h old; identified by their silvery appearance and inability to fly) were removed from their natal colonies, maintained in small, plastic rearing containers (7 × 7 × 5 cm), and kept in a queen-rearing room that was temperature- and humidity-controlled at 25 °C and 60% RH. All queens were unmated so that they would produce only haploid (male-destined) brood. Thus, queenright groups were more comparable with the queenless groups in that both only contained male-destined eggs, and variation introduced by mating was minimized (Baer & Schmid-Hempel, 2005). Queens were treated with CO_2 gas at adult ages 12 and 13 days (30 minutes per day) to cause them to bypass diapause and initiate egg laying (Roseler, 1985). Queens will undergo reproductive maturation irrespective of mating status, and unmated queens that are subjected to CO_2 treatment develop their ovaries and lay eggs on a similar timescale as mated queens (Amsalem et al., 2015; Amsalem & Grozinger, 2017; Woodard et al., 2019). Unmated, CO2-treated queens suppress worker reproduction equally well as mated, post-diapause queens (Amsalem et al., 2017). Workers were not subjected to this CO_2 treatment because they do not diapause, and, in queenless groups with the absence of social inhibition, will lay eggs approximately seven days after eclosion (Cnaani et al., 2002).

After the second CO_2 treatment, queens were either placed with three (to create QW3 nests) or with five (for QW5 nests) unrelated, callow workers in plastic queen rearing boxes (15 × 15 × 10 cm) purchased from Biobest USA, Inc. (Romulus, MI, U.S.A.). Queenless groups were also created with either three (W3) or five (W5) callow workers in identical rearing boxes. All workers were <24 h old at the time of group formation to prevent rejection by the queen (if applicable) and to control for age-related differences in behaviour among workers. We excluded extremely large- and small-bodied workers from these groups in order to minimize extreme differences in body sizes between nests. This resulted in size variation in our nests comparable to what is found in young nests (Shpigler et al., 2013; Watrous et al., 2019), where there is lower worker body size variation compared to mature nests (Costa et al., 2021; Couvillon et al., 2010; Shpigler et al., 2013). We determined body size using the length of the marginal wing cells, a metric that is highly correlated with body size (Owen, 1988; Shpigler et al., 2013). Within a single nest, queens and workers originated from different natal colonies; all workers originated from the same natal colony, with equal representation from 10 source colonies across social configurations; and all queens originated from six different source colonies with equal representation across social configurations.

All nests were maintained in a dark, temperature- and humidity-controlled (25 °C, 60% RH) room with infrared cameras (VIGICA Peashooter QD520) placed directly above each nest to continuously record in-nest behaviours. Each nest was provided with a synthetic nectar solution that does not spoil readily (recipe provided in Boyle et al., 2018) to minimize handling of the feeders during the experiment, and both a waxed and unwaxed pollen ball (same pollen as described above, given ad libitum).

Nests were monitored daily for egg laying. Five days after eggs were observed in a nest, which is when eggs hatch into larvae (Cnaani et al., 2002), food resources were removed from inside the nestbox. At this point, two separate, lighted (12:12 h light:dark cycle) food collection arenas were connected to the nestboxes via 30 cm translucent, polypropylene tubes (1.6 cm diameter). Nestboxes were covered in opaque black cloth such that nests remained dark (with the exception of any light entering through the tube), whereas the food collection arenas alternated between light and dark in a windowed room with supplementary artificial light during the day. Pollen (described above, ground to powder) and synthetic nectar (described above, available through a cotton wick) were placed in separate containers (clear, 6 ounce (177 ml), diameter ¼ 7 cm). Pollen was replaced every 4e5 days and synthetic nectar was replenished as needed and replaced every 2 weeks to avoid spoilage. Additional cameras were placed above each food collection chamber and continuously recorded activity until the first adult males eclosed (~25 days later). Bees in our experiment did not free-forage but instead collected food from discrete foraging chambers. Our experimental design allowed us to identify workers that were positively phototactic and motivated to collect food, which are two fundamental components of foraging, without introducing variation due to flight and interindividual differences in foraging experience.

Nests were minimally disturbed during the ~25-day experimental period, with the exception of replacing pollen, nectar or deceased workers. Any workers who died during the experiment were replaced with callow workers (from the same natal colony as the deceased bee), and the date of replacement was recorded. Nests were typically inspected daily, or at minimum every 3 days, so any worker replacement occurred within 24e72 h of mortality. Nests in which the queen died, or more than two workers died (*N* ¼ 11), were removed from the experiment and excluded from analyses (final sample sizes are reported in the Results). For each nest, on the date of first male adult eclosion, the entire nest (including any queens, workers, males and brood) was frozen over dry ice and subsequently stored in a −80 °C freezer.

Data Collection and Behavioural Methods

Videos were analysed using Behavioral Observation Research Interactive Software (BORIS; Friard & Gamba, 2016) to quantify egg-laying, brood-feeding and food collection (pollen or nectar) behaviours. Workers and queens were individually number-tagged (Betterbee, Greenwich, NY, U.S.A.) using superglue as an adhesive, in order to track individual behaviours. Egg laying was identified within videos as point events with no duration (Table 1; see example in Supplementary Video S1). Both queens and workers laid eggs, although queens primarily laid eggs in the queenright groups, and only workers laid eggs in the queenless groups. Given that egg-laying events are relatively infrequent, we scanned videos and documented a minimum of three egg-laying events per nest, including one or more events in the first 5 days of egg laying and also one or more additional events occurring a minimum of 10 days after the first recorded egg-laying event. Subordinate workers have been shown to supersede dominant egg layers in small groups of bumble bee workers (van Doorn, 1989). Thus, we chose two disparate time frames to capture as many egg-laying individuals as possible and account for any changes in dominance status and egg laying over the course of the experiment. The later time frame here (::: 10 days after the first egg-laying event) fell immediately after the early time frame described below.

To record events of brood feeding and food collection (pollen or nectar), 24 nonconsecutive hours were randomly selected over each of two time frames: 7e9 and 13e15 days after eggs were observed in the nest (hereafter referred to as the ‘early’ and ‘late’ time frames). We chose to perform more extensive observations across two discrete time frames, rather than observe nests consecutively across the study period with fewer observations per day, in order to capture more behavioural events per day. This approach allowed us to capture any behavioural differences related to circadian rhythmicity (Yerushalmi et al., 2006) and was amenable to directly comparing task organization between two discrete developmental periods. Our two time frames capture (1) the time wherein the oldest larvae are 2e4 days old and bees have just begun carrying out brood care tasks in their newly formed social groups, as well as (2) a later-stage time frame approximately halfway through the development of the first set of brood (Cnaani et al., 2002). At the later time frame, the oldest larvae and pupae are 8e10 days old and nests typically contain larvae at all stages of development and may also contain early stage pupae (Leza et al., 2018; Watrous et al., 2019). At this second time frame, workers had been carrying out brood care tasks for more than 1 week. At both time points, the ratio of workers to brood in all nests approximated that found in young nests at the stage when the first cohort of workers has eclosed. Because brood feeding occurs more

Table 1

Recorded behaviours and associated descriptions

Behaviour	Definition
Brood feeding	Brood feeding is a stereotypical, discrete behaviour (lasting 1e5 s) where individuals open a wax-covered larval cell and regurgitate into it by contracting their abdomen (described further in Free & Butler, 1959; Woodard et al., 2013)
Egg laying	Egg laying is a stereotypical, discrete behaviour where the abdomen tip in placed inside an open wax cup, with legs gripping and sliding over wax. Eggs can be observed within the egg cup following this behaviour
Nectar collection	Here defined as when a bee was completely inside the nectar chamber for :::10 s
Pollen collection	Here defined as when a bee was completely inside the pollen chamber for :::10 s

frequently than food collection trips, based on our preliminary observations, and these two tasks have different behavioural classifications (i.e. point versus state event; Altmann, 1974), we watched and scored more food collection video than in-nest video in an effort to control for large differences in numbers of observations among behaviours. For each randomly selected hour, we observed the first 5 min of in-nest video and the entire hour of video of the food collection arenas. Our methodology may have introduced ascertainment bias into our data set; specifically, we might have observed more food collection events because we allocated more time to their observation. However, because we examined exclusivity rather than time spent performing tasks, and scaled our behavioural data (see below), any biases should not have fundamentally impacted our analyses. We assume that bees located in the food collection arenas were primarily collecting the types of floral rewards they contained, given that all nests continued to successfully develop and grow, which requires pollen and nectar (Heinrich, 2004). We used a minimum threshold of time spent in the foraging chamber as a confirmation of food collection, because bees were not observed transporting pollen in their corbiculae, which is typical in laboratory studies (Woodard et al., 2014). Specifically, we excluded data for any bees who remained within the food collection arenas for $<$ 10 s in an effort to filter out exploratory or other nonfood collection behaviours in these arenas. All brood-feeding events were recorded as point events with no duration, and all putative pollen collection and nectar collection events were recorded as state events with a duration (:::10 s), start time and stop time, and later converted to point events after filtering for events :::10 s long. Descriptions of these behaviours are provided in Table 1 and in Supplementary Videos S1eS2. Total durations of in-nest observations were chosen based on previous studies that examined the frequency of these events in bumble bees (Costa et al., 2021; Shpigler et al., 2016; Woodard et al., 2014).

Following nest collections, we measured several additional factors that might impact or be associated with behaviour, to include in our statistical analyses. We dissected brood cells over dry ice to quantify the number of eggs, larvae and pupae in each nestbox. The amount of brood in a nest influences the frequency and organization of egg-laying, brood feeding and food collection tasks (Kraus et al., 2019; Nagari et al., 2019; Orlova et al., 2020; Starkey et al., 2019; Woodard et al., 2013). We dissected worker ovaries in cold 100% ethanol, and the largest terminal oocyte in each ovary was staged (IeIV) according to groupings in Duchateau and Velthuis (1989) (hereafter 'ovary stage'). We then measured marginal cell length of all bees as a proxy for body size (Costa et al., 2021; Shpigler et al., 2013).

Statistical Methods

All statistical methods were carried out in R (v.4.0.0). Plots were generated with the ggplot package (v.3.3.0; Wickham, 2016). For all statistical models, the best-fit model was selected based on the lowest Akaike's information criterion for small sample sizes (AICc), using the 'model.sel()' function from the 'car' package (v.3.0e7; Fox & Weisberg, 2019). The model with the lowest AICc score that was not rank deficient was selected for analyses. To control for differences in the relative frequency of observed behaviours carried out in each nest (e.g. brood feeding is inherently more common than egg laying), we scaled counts of all behaviours according to their relative frequency using the following equation: 1 scaled count of behaviour Y ¼ 1/((total counts of behaviour Y in the nest)/(total counts of all behaviours in the nest)).

In this way, the total scaled counts of each behaviour (i.e. brood feeding, nectar collection, pollen collection, egg laying) in a given nest were equal, and frequent behaviours did not dominate infrequent behaviours in our statistical analyses. All subsequent analyses were performed on scaled counts unless otherwise specified. All data filtering was performed on raw counts of behaviours. Egg laying was excluded from all time-specific analyses because the collection of egg-laying data did not directly correspond with the two time frames during which other behaviours were observed. Natal colony (i.e. the mature colony workers were sourced from) and nest identity were included as random effects in all possible models unless otherwise stated.

Exclusivity of tasks in nests

We first quantified the exclusivity of each task within each nest, which we defined as the number and frequency of individuals observed carrying out a given task in a given nest. For this, we calculated Shannon diversity indices for raw counts of all tasks in nests using the 'diversity()' function from the 'vegan' package (v.2.5e6; Oksanen & Blanchet, 2019), which has previously been used as a task specialization index (Gorelick et al., 2004). We only included nests with a minimum of three observations each of food collection, egg laying and brood feeding (N ¼ 33 nests). We chose this minimum threshold based on previously reported thresholds in the literature (Charbonneau & Dornhaus, 2015; Shpigler et al., 2016) and to avoid drawing conclusions about tasks in nests with limited observations. Here, the Shannon index incorporates the diversity and relative frequency of individuals carrying out each task in a given nest, thereby calculating each task's degree of exclusivity. A Shannon value of 0 indicates a highly exclusive task that was observed being carried out by only a single individual in the nest, whereas a high Shannon value indicates a task that many or all individuals in a nest were observed performing with relatively equal frequency. We compared Shannon indices among tasks to identify the most and least exclusive behaviours in nests with a generalized linear mixed model (GLMM) using the 'glmer()' function from the 'lme4' package (v.1.1e23; Bates et al., 2015), including the number of recorded instances of the task, social configuration, task identity and the interaction between social configuration and task identity as possible fixed effects.

Individual work task organization

Task organization and specialization have previously been quantified in bumble bees using various different frameworks, which can influence how individuals are characterized. To account for this in our analyses, we used four frameworks to capture task specialization. First, we quantified the (1) degree of behavioural specialization among individual bees (i.e. whether individuals specialized in performing only one or a few tasks) and (2) visualized how individual workers cluster around behaviours using a nonmetric multidimensional scaling (NMDS) analysis. For these two analyses, we used scaled counts of observed behaviours for all worker bees with a minimum of three raw behavioural observations (N ¼ 120 bees from 40 nests). Next, we categorized bees into groups using (3) K-means categorization (Hartigan, 1975; Ramette, 2007) and (4) Shannon-based categorization. For these two analyses, we used scaled counts of observed behaviours but included all worker bees (N ¼ 179), including those with fewer than three observations, because this set of methods can incorporate bees with few ($<$3) behavioural observations without bias in assigning task specialization to these individuals. For the K-means and Shannon-based categorization, we used Poisson-distributed generalized linear mixed models (GLMMs) to test whether the number of individual workers in a nest assigned to each behavioural category was influenced by social configuration. We included the number of individuals assigned to each category per nest as a response variable and behavioural category, social configuration and the interaction between behavioural category and social configuration as possible fixed effects. We also categorized in- dividuals based on single tasks they performed (see Appendix). Queens were excluded from all individual-based behavioural ana- lyses as our goal was to specifically understand worker task orga- nization in the early nesting stage.

(1) Degree of task specialization. Here, the Shannon index in- corporates the diversity and relative frequency of all tasks we observed each individual carry out, thereby calculating each in- dividual's degree of behavioural specialization. We then compared Shannon indices among social configurations to explore whether group size or presence of the queen influenced the degree of specialization of individual bees. We used a two-part GLMM including the number of scaled behaviours carried out by the in- dividual and social configuration as possible fixed effects. We used a binomial distribution in part one of the model to analyse specialization as a binary response variable comparing perfectly specialized individuals (with a Shannon index of 0) to nonperfectly specialized individuals (with a Shannon index > 0). We used a Gaussian distribution in part two of the model to analyse all non- perfectly specialized individuals along a continuous scale.

(2) NMDS clustering. To visualize how individual workers cluster around behaviours, we performed a nonmetric multidimensional scaling (NMDS) analysis on the scaled counts of observed behav- iours carried out by each individual using the 'metaMDS()' function from the 'vegan' package using Euclidean distance. NMDS takes the rows of a multidimensional matrix (here, individual bees) and plots them in two-dimensional space to enable the visualization of multidimensional data.

(3) K-means categorization. Here, we used a *K*-means clustering analysis to determine whether individual bees naturally cluster into distinct behavioural categories. *K*-means assigns individuals to *K* clusters with the lowest possible within-cluster variance. An elbow plot was used to determine the number of distinct clusters (*K*) that maximizes explanatory power while minimizing overfitting, and the 'kmeans()' function defined *K* distinct clusters and assigned all bees to one of these clusters.

(4) Shannon-based categorization. Lastly, we categorized worker bees based on their degree of specialization as calculated by the Shannon index, which incorporates the diversity and relative fre- quency of all tasks we observed each individual carry out, thereby capturing more of the continuous and complete behavioural perfor- mance of each bee. However, here, we labelled bees with a Shannon index below 0.6 to be 'specialists'. Bees who met this criterion were said to specialize on the scaled behaviour that they carried out most frequently. We also included a 'generalist' bee category to represent bees who never specialized on a single task. Bees with a Shannon index above 0.6 were labelled 'generalists'. We chose a threshold of 0.6 because the most frequently observed behaviour in our study comprised 71e100% of all behaviours carried out by specialists and 33e76% of all behaviours carried out by generalists. Bees with fewer than three raw observed behaviours were labelled as 'other'.

Correlations between behaviour and worker characteristics

To test whether any correlations existed between worker task specialization and body size or ovary development in any of our behavioural frameworks and whether these associations were consistent between frameworks, we used Gaussian-distributed GLMMs. We compared body size and ovary development across behavioural categories for each framework. These models included average oocyte stage, resorption of ovaries or body size as a response variable and behavioural category as a possible fixed effect.

Repeatability across time

We tested whether the behaviours we observed were per- formed consistently and repeatably across time, both within indi- vidual workers and within nests. We tested the repeatability of behaviours using scaled counts of behaviours, individual Shannon indices across time and NMDS coordinates.

(1) Task repeatability. To estimate the repeatability (*R*) for the scaled counts of each behaviour, we used repeatability mixed models (RMMs) in the 'rptR' package (Stoffel et al., 2017), a typical approach to measure behavioural repeatability. We did this at the individual, nest and nest configuration levels to compare between the early and the late time frames. Here, we only included workers that had at least three raw observations at both time frames (*N* ¼ 44). All models were constructed with a Gaussian error dis- tribution for scaled counts of each behaviour, with time frame as a fixed effect and with natal colony, social configuration and bee identity as random effects; we interpreted a behaviour as repeat- able if the 95% confidence intervals of the random effect did not reach 0 (Stoffel et al., 2017). *R* is defined as the total variation that is reproducible among repeated measurements of the same individ- ual (Nakagawa & Schielzeth, 2010).

(2) Average Shannon repeatability. To investigate whether the average degree of specialization was consistent over time, we recalculated Shannon indices for individual worker bees based solely on the scaled behaviours carried out at a given time frame (early or late) for each bee with at least three behavioural obser- vations at one or both of these time frames (*N* ¼ 108). We then compared the average Shannon index at each time frame using a two-part GLMM including time frame (early or late) as a possible fixed effect. Individual bee within nest identity within social configuration, natal colony and number of scaled behaviours were included as random effects.

(3) ANOSIM. Finally, to determine whether time frame influenced NMDS clustering, we performed an analysis of similarity (ANOSIM) on NMDS coordinates between the two time frames.

Animal Welfare Note

All bees were maintained under standard rearing conditions during the experiment with constant access to food resources. Bees were euthanized at the end of the experiment using dry ice, which is among the most humane methods of euthanasia. We worked only with commercially reared *B. impatiens* and thus did not negatively impact any wild populations. We made every effort to meet the high standards of animal welfare required by ASAB/ABS for the use of animals in research. We followed all legal re- quirements for working with *B. impatiens* and followed all institu- tional guidelines. Colonies were transported and maintained at the Insectary and Quarantine Facility at the University of California Riverside under California Department of Food and Agriculture permit number 3182.

We assembled small worker groups with unmated queens. This was amenable to increasing our sample size without requiring an excessive number of full-sized colonies. We based our sample size on preliminary experiments and previously published studies on bumble bee behaviour.

RESULTS

Our data analyses included a total of 43 nests (QW5 ¼ 12, QW3 ¼ 10, W5 ¼ 9, W3 ¼ 12), after removing 11 nests in which the queen died or >2 workers died from the experiment. We viewed and scored a total of 484.1 h of video data (43.6 h of in-nest video and 440.4 h of food collection video) distributed across the 43 nests, for an average (± SEM) of 1 ± 0.1 h of in-nest video and 10 ± 0.9 h of food collection video per nest. Across all workers and queens in the experiment, we recorded a total of 1096 nectar collection, 315 pollen collection, 487 brood-feeding and 157 egg- laying events (with a mean ± SEM per nest ¼ 28.1 ± 3.7 nectar collection, 14.3 ± 3.4 pollen collection, 12.2 ± 1.6 brood feeding, 4.6 ± 0.4 egg laying). Social configuration predicted the amount of brood in the nest at the end of the experiment and the frequency of nectar and pollen collection (Appendix, Table A1); thus, the factor termed 'social configuration' in our statistical analyses encom- passes these differences, in addition to differences in group size and/or the presence of the queen. Within the 43 analysed nests, eight worker bees from eight nests died and were replaced prior to the behavioural observation period. Replacement bees had fewer recorded behaviours than original bees (mean ± SEM ¼ 5.1 ± 1.7 behaviours per replacement bee versus 9.7 ± 1.0 behaviours per original bee), and none of the eight replacement bees were observed laying eggs. Replacement bees did not differ in their Shannon-based behavioural category relative to original (i.e. non-replacement) bees (Pearson's chi-square test: C^2_4 ¼ 8.292, P ¼ 0.0814). Similarly, introducing replacement bees did not result in measurable changes in Shannon-based behavioural categoriza- tion of original bees in those nests relative to bees in nests without replacements (Pearson's chi-square test: C^2_4 ¼ 4.832, P ¼ 0.305). Scaling all individual behaviours to the total behaviours in each nest resulted in 1741 nectar collection, 1285 pollen collection, 1976 brood-feeding, and 1617 egg-laying scaled events (mean ± SEM per nest ¼ 40.5 ± 0.8 nectar collection, 29.9 ± 0.9 pollen collection, 45.9 ± 0.9 brood feeding and 37.6 ± 0.9 egg laying). All subsequent results reflect analyses on scaled behavioural counts unless noted otherwise.

Exclusivity of Tasks in Nests

Shannon indices of raw counts of behaviours in each nest (here measuring the degree of exclusivity of the tasks themselves) ranged from 0 (where only a single individual was observed carrying out that task) to 1.73 (where all individuals in the colony were observed performing that task multiple times) (Fig. 1). Frequency of task performance (i.e. the number of times we observed a given task being performed by queens and workers) was positively correlated with Shannon index (GLMM: estimate ¼ 0.005 ± 0.001, t ¼ 4.61, N ¼ 530, $P <$ 0.001). Egg laying and pollen collection were per- formed more exclusively than brood feeding and nectar collection (GLMM pairwise Tukey contrasts: brood feeding e egg laying estimate ¼ 0.62 ± 0.040, z ¼ 15.33, $P <$ 0.0001; nectar collection e egg laying estimate ¼ 0.33 ± 0.050, z ¼ 6.78, $P <$ 0.0001; pollen collection e brood feeding estimate ¼ −0.58 ± 0.059, z ¼ −9.89, $P <$ 0.0001; pollen collection e nectar collection estimate ¼ −0.30 ± 0.06, z ¼ −4.77, $P <$ 0.0001). Brood feeding was the least exclusive behaviour, with 64% of all bees (and 82% of bees with more than three observations) observed feeding brood at least once. In some instances, there was an interaction between social configuration and task identity. W5 nests had higher brood feeding Shannon indices than QW3 nests (GLMM Tukey contrast: estimate ¼ 0.37 ± 0.123, $t_{47.3}$ ¼ 3.02, P ¼ 0.021). Generally, nectar collection was more specialized in nests with fewer individuals (GLMM pairwise Tukey contrasts: W5 e W3 estimate ¼ 0.77 ± 0.12, $t_{46.8}$ ¼ −6.4, $P <$ 0.0001; W3 e QW3 estimate ¼ −0.39 ± 0.12, $t_{62.6}$ ¼ −3.21, P ¼ 0.011; W3 e QW5 estimate ¼ −0.71 ± 0.12, $t_{59.1}$ ¼ −6.15, $P <$ 0.0001; W5 e QW3 estimate ¼ −0.38 ± 0.12, $t_{47.3}$ ¼ −3.13, P ¼ 0.015; QW5 e QW3 estimate ¼ 0.327 ± 0.12, $t_{57.2}$ ¼ 2.79, P ¼ 0.035), although there was no difference between the two largest group sizes (W5 and QW5) (GLMM pairwise Tukey contrast: P ¼ 0.96). The best-fit model to predict task Shannon in- dex included the frequency of task performance and the interaction between task identity and social configuration as fixed effects.

Individual Task Organization

(1) Degree of task specialization

Of 179 worker bees, 120 met the threshold of at least three raw recorded behavioural observations and were included in the specialization analyses. Shannon indices of individual bees ranged from 0 (i.e. observed carrying out a single task exclusively; N ¼ 20 out of 120) to 1.32 (e.g. observed carrying out all behaviours with similar frequency) (Fig. 2a). Bee Shannon index did not differ based on social configuration (GLMMs: $P >$ 0.1; Fig. 2b), but it could be partially explained by the number of observed behaviours for a given bee. Specifically, bees with fewer observed behaviours were more likely to be perfect specialists (have a Shannon value of 0) than those with more observed behaviours (part 1 GLMM: estimate ¼ −0.12 ± 0.036, z ¼ −3.38, P ¼ 0.0007; not included in part 2 GLMM best-fit model; Fig. 2c). The best-fit model for part 1 included the number of behaviours and social configuration as fixed effects; the best-fit model for part 2 was the null model.

(2) NMDS clustering

The NMDS analysis plotted individual bees across two major axes, with nectar and pollen collection clustering close together, brood feeding clustering near food collection, and egg laying as distinct from the other behaviours (Fig. 3). The NMDS1 axis demonstrated an egg layingefood collection axis, with brood feeding falling between these two other tasks. In NMDS2, egg laying and pollen collection were very similar, whereas brood feeding was more differentiated from the remaining three behav- iours. Individuals fell at all points across the plot and did not display obvious, distinct clustering across these two axes.

(3) K-means categorization

Two behavioural clusters emerged (Appendix, Fig. A1) that separated workers along a pollen collection axis (Fig. 4). Workers who were observed collecting pollen >30 scaled times were assigned to cluster 2 (N ¼ 12 bees from 10 nests), and those who were observed collecting pollen <30 scaled times were assigned to cluster 1 (N ¼ 167 from 44 nests; Appendix, Table A2). Brood feeding and egg laying did not appear to impact clustering (Fig. 4). Significantly more workers were categorized into cluster 1 (gen- eralists) than cluster 2 (frequent resource collectors) (GLMM: estimate ¼ 2.63 ± 0.30, z ¼ 8.81, $P <$ 0.0001). Social configuration did not predict the number of bees assigned to each cluster (GLMM: $P >$ 0.05). The best-fit model predicting the number of individuals per nest assigned to each K-means cluster included behavioural category and social configuration as fixed effects.

(4) Shannon-based categorization

Based on a Shannon threshold of 0.6, we categorized 49 worker bees as specialists (Shannon ∷ 0.6; from 32 nests), 71 as generalists (Shannon > 0.6; from 34 nests) and the remaining 59 bees as other (<3 total observed behaviours; from 28 nests) (Appendix, Table A2). Specialists were either egg layers (N ¼ 7 from 7 nests), brood feeders (N ¼ 25 from 17 nests) or nectar collectors (N ¼ 17 from 15 nests). No pollen collection specialists emerged from this analysis. There were more generalists and other bees in each nest relative to bees specialized on brood feeding, egg laying and nectar collection (all relevant GLMM Tukey contrasts: $P <$ 0.001). Social configura- tion did not significantly predict the number of individuals per behavioural category (GLMM: $P >$ 0.1). The best-fit model

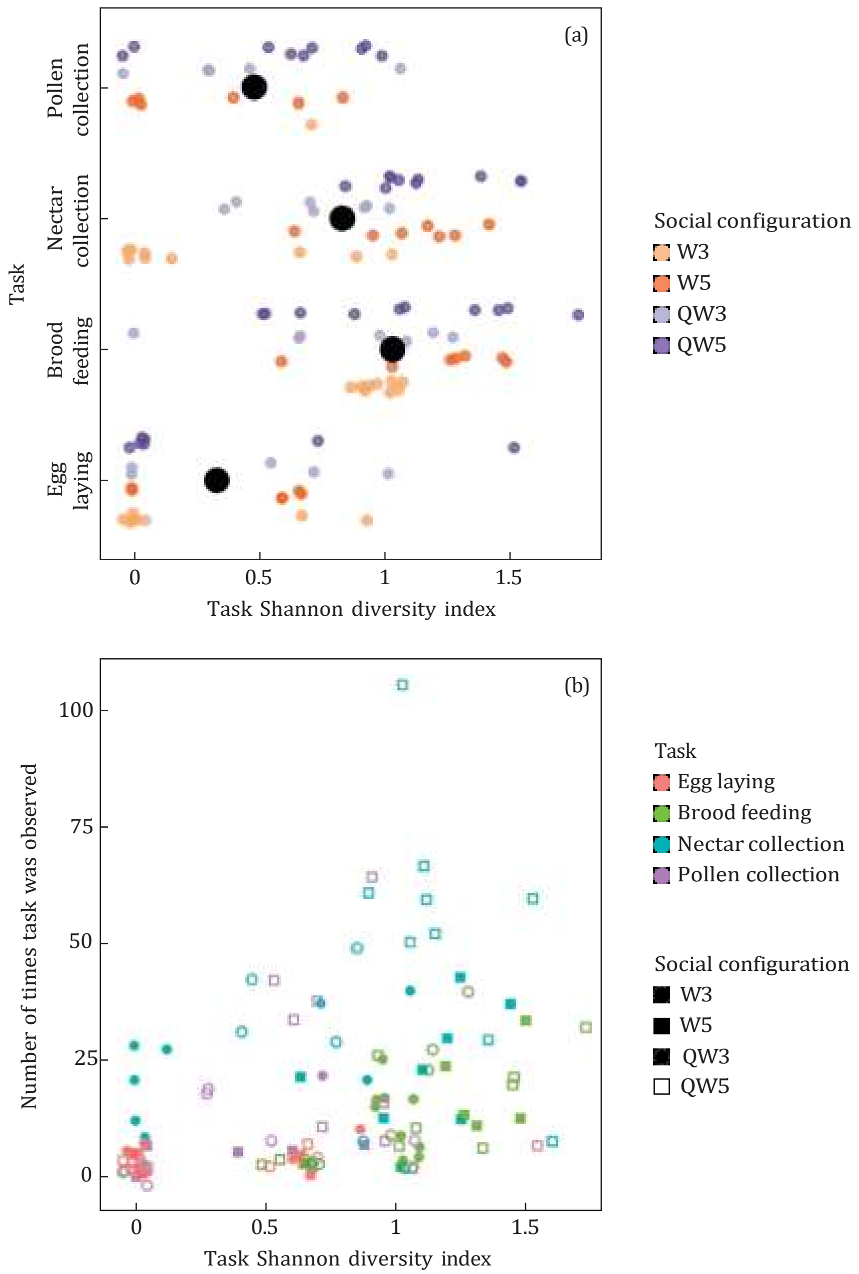

Figure 1. (a) Shannon diversity index for tasks performed by workers in nests of each social configuration (W3 ¼ queenless with three workers; W5 ¼ queenless with five workers; QW3 ¼ queenright with three workers; QW5 ¼ queenright with five workers). Larger points indicate group means. (b) Shannon diversity index for the frequency of observed task performance, where tasks performed more frequently have higher Shannon values than those performed less frequently. Points are jittered for easier visualization of overlapping points (width ± 0.05; height ± 2).

predicting the number of individuals per nest assigned to each behavioural category based on a Shannon threshold of 0.6 included behavioural category and social configuration as fixed effects.

Correlations between Behaviour and Worker Characteristics

In the K-means analysis, bees from cluster 2 (frequent pollen foragers) were, on average, larger-bodied than workers in cluster 1 (infrequent pollen foragers) (GLMM Tukey contrast: estimate ¼ 0.23 ± 0.068, z ¼ 3.43, $P < 0.0001$; Appendix, Fig. A2). Cluster 1 contained a normal distribution of worker body sizes across the full range of body sizes (wing marginal cell length ~1.8e3.2 mm), whereas cluster 2 only contained bees with marginal cell lengths >2.6 mm. The best-fit model to predict the body size of bees in the K-means analysis included behavioural cluster alone as a fixed effect. Behavioural category was not included in the best-fit models for any other relevant analyses, indicating that none of the four behaviours observed could predict body size, ovary stage or ovary resorption status in any of the remaining clustering or categorization analyses (Appendix, Table A2, Fig. A4). The null model was the best fit for all of these analyses.

Repeatability across Time

(1) Task repeatability

Pollen collection was repeatable for individuals, but not for nests or social configurations (RMM: R ¼ 0.28 ± 0.13, P ¼ 0.02; Appendix, Table A3, Fig. A5). No other behaviours were

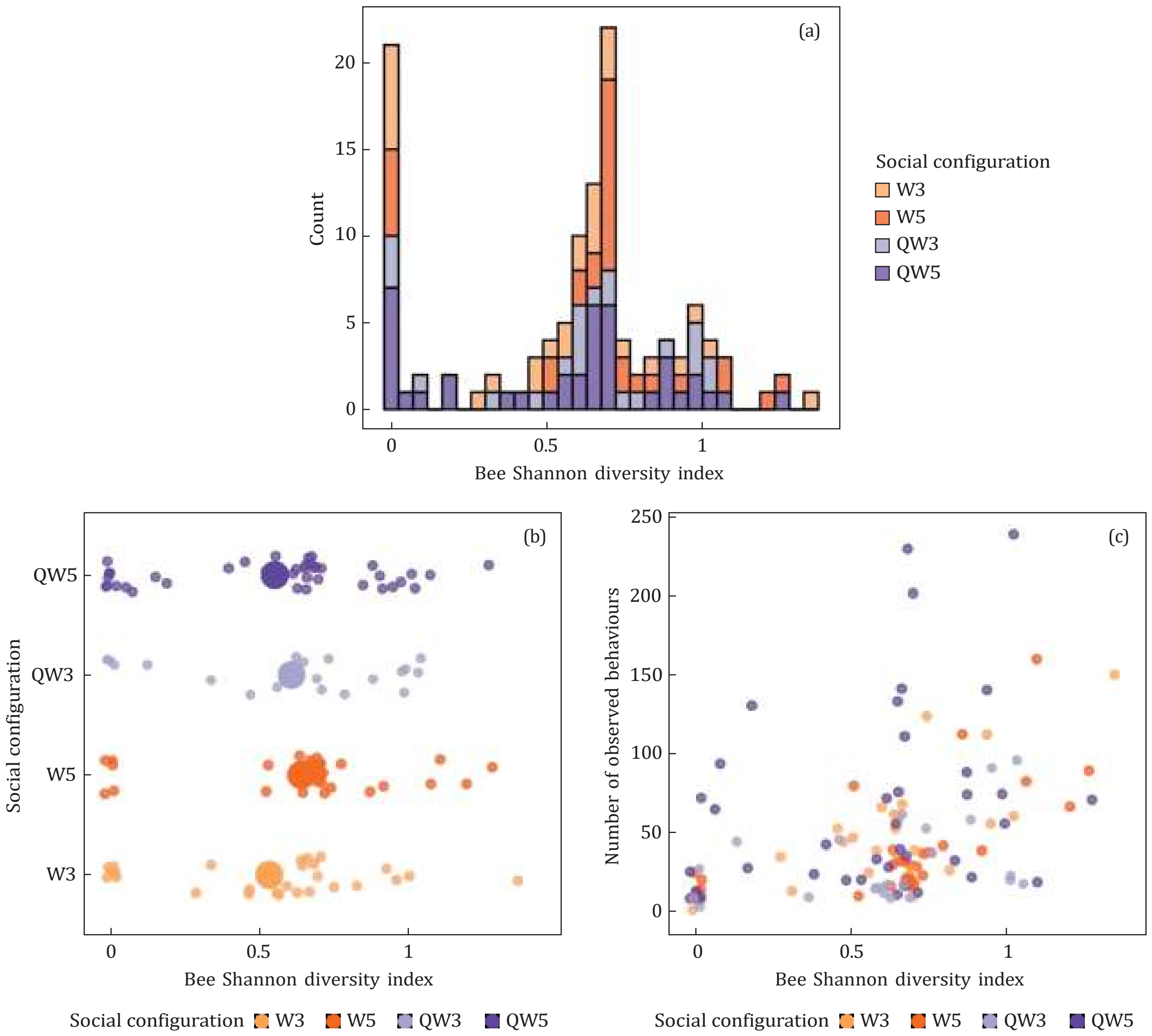

Figure 2. Shannon diversity index of individual bees. (a) Shannon indices for all worker bees, coloured by social configuration. (b) Distribution of Shannon indices for all bees. Larger points indicate group means. (c) Relationship between Shannon index and number of observed behaviours. Bees with fewer observed behaviours were more likely to be perfect specialists (have a Shannon value of 0) than those with more observed behaviours. Individual points (not means) are jittered for easier visualization of overlapping points (width ± 0.02; (b) height ± 0.2; (c) height ± 3).

repeatable for individuals, nests or social configurations. This indicates that individuals change in both the frequency and repertoire of observed behaviours over time, with the exception of those collecting pollen. Nectar collection (RMM: R ¼ 0 ± 0.04, P ¼ 0.5) and brood feeding (RMM: R ¼ 0 ± 0.04, P ¼ 1; Appendix, Fig. A5b) occurred more frequently in nests, on average, during the later time frames.

(2) Average Shannon repeatability

The degree of specialization of individuals did not change over time, as there was no change in mean Shannon index in bees from the early to the late time frame (time frame was not included in best-fit models). The best-fit models to predict time-dependent Shannon index had no fixed effects and included only natal colony and bee identity within nest identity within social configuration as random effects.

(3) ANOSIM

NMDS patterns also did not change with time frame based on our analysis of similarity (R ¼ 0.010; P ¼ 0.16), indicating that similar task repertoires were filled at the early and late time frames (although not necessarily by the same individuals).

DISCUSSION

Identifying how task performance is organized in insect societies is a major goal in sociobiological research (Beshers & Fewell, 2001; Michener, 1969; Oster & Wilson, 1978; West-Eberhard, 1967; Wilson, 1971). We explored task organization in the early nesting phase of bumble bee colonies. Our goal was to investigate patterns of task specialization and flexibility and to explicitly document how food-related tasks are organized among individuals at this stage, which is an understudied phase of the bumble bee life cycle. In our examination of brood-feeding and food collection

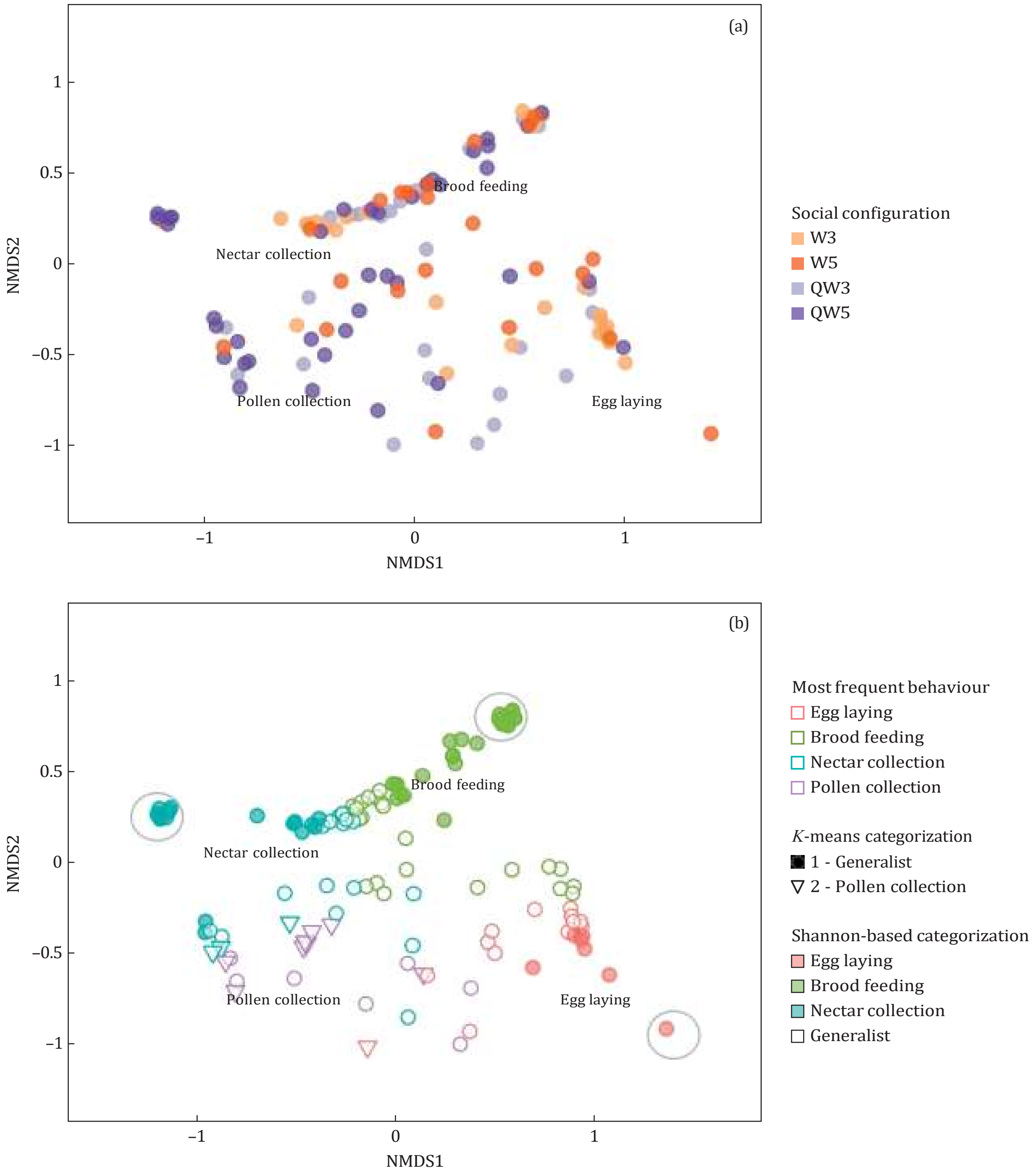

Figure 3. NMDS plots. Points represent individual worker bees. Task names are centred over their respective loci. Points are jittered to more easily visualize overlapping points (width ± 0.05; height ± 0.05). (a) NMDS coordinates of individual bees coded by social configuration. (b) NMDS coordinates of individuals coded by categorization method. Colour and shape refer to the category of each bee from each of the four behavioural categorization methods. Large grey circles surround clusters of perfect specialists. There were no perfect pollen collection specialists. Bees with fewer than three raw behavioural observations are not included in this plot. Among these analyses, there was a high degree of variation: not a single individual bee was sorted into the same category across all four categorization methods employed here.

behaviours, as well as egg laying, we found that pollen collection and egg laying were more exclusive behaviours, in that they were often carried out by fewer individuals in the nest. In contrast, the majority of workers in the nest fed brood. With the exception of pollen collection, individual task performance was not repeatable across time, indicating that the task repertoire of individuals changes over time during the early nesting phase, at least for the approximately week-long period examined here. Workers tended to switch frequently between multiple tasks during the observation periods (two 3-day time frames). Furthermore, bees exhibited a

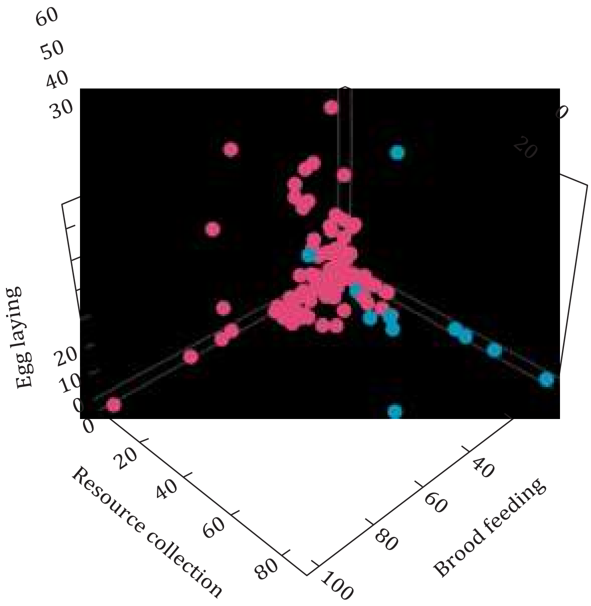

Figure 4. *K*-means clustering analysis. Colours represent clusters: maroon ¼ one (generalists), blue ¼ two (frequent pollen collectors). Resource collection axis is nectar and pollen collection events summed together for easier visualization, but these types of food collection behaviours were assessed separately in the analysis.

broad distribution of task specialization, as some individuals specialized on a single task, but most were more flexible in that they performed multiple tasks.

In many incipiently social and subsocial bees and wasps, there is relatively little task division between helpers (Dew & Michener, 1981; Rehan & Richards, 2010; Wcislo & Gonzalez, 2006). In more socially complex species, however, brood feeding and foraging are generally uncoupled such that a subset of individuals perform brood feeding and another subset collect food resources from outside the nest (Bassindale, 1955; Free, 1955; Robinson, 1987). In our study, we observed a nested pattern of task organization in that most workers fed brood, a subset also collected nectar and a smaller subset also collected pollen. Although our result related to food collection specialization is consistent with studies of mature bumble bee nests, which have found evidence that subsets of workers specialize on pollen and nectar foraging (Goulson et al., 2002; Spaethe et al., 2007), we show that these specialized subgroups likely also feed brood when they are inside the nest, at least in young nests. Brood feeding was not observed in the majority of previous studies, with the exception of Brian (1952), who also found that in mature colonies of *Bombus pascuorum*, workers that forage also feed brood. The food-related behaviours we examined were not correlated with body size in the majority of our analyses, with the exception of pollen foragers being slightly larger-bodied in our *K*-means analysis, which is generally consistent with studies that evaluated size differences between foragers in mature nests (Goulson et al., 2002; but see Smith et al., 2016). These results suggest that bumble bee worker task organization at this stage may be similar to more incipiently social insects, where workers perform tasks more flexibly, in contrast to lineages with more complex eusociality, such as honey bees or ants (Bassindale, 1955; Free, 1955).

The finding that most workers fed brood suggests that performance of this task is shared by most group members at this early stage in nest development. Of the four behaviours we observed, brood feeding was the least exclusive behaviour across all social configurations. Eighty-two per cent of bees with greater than three behavioural observations fed brood at least once, and brood feeders were the most common behavioural category based on the tasks that bees were observed performing most frequently. In incipient nests, it appears that all workers have a relatively low threshold for responding to the signals that elicit brood-feeding behaviour. We attribute this finding to two characteristics of bumble bees. First, brood feeding does not appear to be physiologically constrained in bumble bees as it is in the closely related honey bees (Drapeau et al., 2006; Kupke et al., 2012; Pereboom, 2000; Sadd et al., 2015), where in the latter, nurse bees exclusively produce royal jelly, a key food source fed to honey bee larvae in addition to pollen (Snodgrass, 1925). Second, bumble bee larvae require food continuously (Pereboom et al., 2003; Plowright & Pendrel, 1977), which necessitates that brood feeding occurs frequently and consistently in the nest. Thus, workers that are in close spatial proximity to brood may instead be more likely to perform this behaviour, rather than workers with a unique physiological propensity to do so (Crall et al., 2018; Jandt & Dornhaus, 2009; Nagari et al., 2019).

Pollen collection emerged as a relatively exclusive food-related behaviour, as fewer (21% of bees) individuals performed this behaviour, whereas nectar collection was more common (50% of bees). Of the 108 bees observed collecting food resources, 3% collected pollen only, 62% collected nectar only and 35% collected both. Although pollen collection emerged as a relatively specialized behaviour, all individuals who collected pollen were also observed performing other behaviours. Thus, frequent food collection did not preclude the performance of additional in-nest behaviours. Individuals that collected pollen did so consistently across the observation period, which provides additional evidence that this a more specialized task. These patterns in pollen and nectar collection are generally consistent with other studies in bumble bees (Cartar, 1992), which have demonstrated that a subset of bumble bee workers perform the majority of foraging trips and exhibit long-term specialization on either pollen or nectar collection (Hagbery & Nieh, 2012; O'Donnell et al., 2000; Russell et al., 2017). Foraging is a cognitively demanding task for bees (Menzel, 2012), and unlike brood feeding, there is evidence that foraging is associated with unique behavioural and physiological characteristics, such as positive phototaxicity (Porath et al., 2019) and an increased density of olfactory sensilla (Spaethe et al., 2007). Furthermore, pollen collection specifically may be a more cognitively demanding task than nectar foraging (Heinrich, 2004; Muth et al., 2016). Based on this, we propose that propensity to collect pollen might be an important axis of division of labour in bumble bee nests, at least at the early nesting stage.

Interestingly, we found that the relative performance of food- related tasks was not impacted by the number of workers in the nest or queen presence. The ability of workers at the early stages of nest development to successfully collect food for the colony and feed offspring is pivotal for the nest to advance to a mature stage where reproductive individuals are produced (Malfi et al., 2019). Larger-sized nests in our study produced more offspring, and thus benefited from having small total differences in worker number, but these differences in group size did not strongly impact how tasks were organized. Our findings also suggest that although queens play unique roles in young nests, their presence and contribution to food-related tasks at the early nesting stage (Shpigler et al., 2013; Woodard et al., 2013) does not have a unique influence on how worker behaviour is organized. This is in contrast to the notion that queens in social insect colonies are 'pacemakers' that regulate the behaviour of workers (Kocher & Grozinger, 2011), for which supporting evidence has been detected in mature bumble bee colonies (Orlova et al., 2020). Rather, our findings are more consistent with what has been observed in *Polistes* wasps (Jha et al., 2006), a social insect system with an annually social lifestyle that is relatively similar to bumble bees.

Egg laying was the most exclusive behaviour we measured across all nests: only 19% of workers were observed laying eggs, and these workers were primarily observed in the queenless groups. This finding is consistent with other studies of bumble bee worker reproduction in small groups, which have found that in the absence of the queen, a single worker typically emerges as a dominant egg layer (Amsalem et al., 2013; Cnaani et al., 2007). In the queenright groups, all queens laid the majority of eggs, and workers that did lay eggs in the queenright groups only did so prior to the onset of queen egg laying. Thus, as would be expected based on previous studies in bumble bees (Alaux et al., 2004, 2006; Amsalem et al., 2017; Bloch, 1999; Padilla et al., 2016), queens were reproductive and likely inhibited worker reproduction in the newly formed nests in our study. A novel finding of our study is that nearly all egg- laying workers also performed other, food-related tasks in the nest. Individuals that laid eggs more commonly collected pollen, compared to nectar collection. Individuals are predicted to be less likely to switch from performing a task that has a strong underlying physiological basis, such as those requiring changes in reproductive status (Johnson, 2005). However, our findings suggest that bumble bee workers, at least in young nests, are not constrained by their reproductive status with respect to also performing food-related tasks.

Additional patterns in our data might be explained in light of the biology of bumble bees and other social insects. In naturally- occurring incipient bumble bee nests, which resemble most the QW5 group in our study, there is a transitional period when the first cohort of about five workers emerge and they begin to help the queen with food-related tasks (Shpigler et al., 2013; Woodard et al., 2013). During the period following worker emergence, the queen typically continues to collect food and feed brood for some period of time, before she transitions to primarily egg laying. These dy- namic changes during nest initiation may partially explain why we did not find strong evidence for repeatability in task performance between the two time points, although there was no discernible change in the degree of specialization of workers across time, and the way tasks were organized in relation to one another remained largely consistent. Additionally, we found that one-third of bees in our study performed fewer than three recorded behaviours. This pattern, where a significant proportion of workers are observed carrying out few or no tasks, has also been observed in ants (Charbonneau & Dornhaus, 2015). These observations of inactivity for a subset of workers might be related to the importance of behaviourally plastic ‘replacement workers’ (Hasegawa et al., 2016) for the long-term persistence of social insect colonies, which may even be relevant in small incipient nests.

There has been mixed evidence for foraging specialization in mature *B. impatiens* colonies (Goulson et al., 2002; Russell et al., 2017) and little evidence of in-nest worker specialization; instead, individuals tend to frequently switch between tasks (Crall et al., 2018; Jandt et al., 2009). The majority of bees in our study (56%) were observed performing more than one unique task, including during two relatively short (3-day) time frames. Our re- sults thus build on studies from more mature colonies and suggest that regular task switching is also common in the early stages of the colony, and that bees regularly switch between in-nest and out-of- nest tasks. The framework by which individuals are categorized for behavioural analyses impacts how specialization is interpreted and can underestimate behavioural variation. We demonstrate this in our study, as individuals were grouped into different behavioural categories depending on the framework we employed. Moreover, we found that bees with more observed behaviours were more likely to be categorized as generalists when we included this as a category, suggesting that specialization may be overestimated when there are few observations. Based on this, we propose that individual bumble bee workers cannot be defined by single food- related tasks. Instead, more holistic analyses, which can incorporate multiple behaviours that an individual performs, are more appropriate for this system. These more holistic analyses of behaviour are consistent with a growing shift towards incorpo- rating social complexity in behavioural research, rather than reducing it (Holland & Bloch, 2020).

In reference to all of our results, we caution that bees in our experiment were able to collect pollen without flying or being required to manipulate flowers to access floral rewards, and addi- tional aspects of our experiment, such as the use of commercial colonies, the creation of incipient nests from workers derived from mature colonies and use of unmated queens, might also have influenced our results. In species where colonies grow in size with season or age, like bumble bees, evidence of group size influences on task organization has been mixed and may only emerge when there are external ecological pressures like parasitism or competi- tion for resources (Dornhaus et al., 2012), which were not present in our study. Thus, further studies on more wild species, as well as with mated queens, are needed in order to determine how broadly these results can be extrapolated. Additional studies on incipient nests are also necessary for further elucidating how division of la- bour operates in young nests. Lastly, direct comparisons of food- related behaviours in both incipient and mature colonies are needed to determine how patterns of task organization change as colonies develop.

Conclusions

This is the first contemporary study to explore patterns of food- related division of labour in small incipient bumble bee nests. We found that nearly all bumble bee workers in young nests feed brood, including those that collect food resources, and that pollen collection is the most exclusive food-related task. These novel re- sults could not have been uncovered without quantifying the relative performance of brood feeding and food collection between and within individuals. We found that individual workers in the early nesting stage perform multiple food-related tasks, and vari- ation between workers appears to be based on whether they engage in more complex tasks like food collection, in addition to basic tasks like brood feeding, rather than these tasks being uncoupled between subsets of workers. We further found that or- ganization of these tasks during this early stage is consistent regardless of worker number or presence of the queen. This study demonstrates the importance of tracking individual behaviour and quantifying relative task performance, exclusivity (i.e. how many individuals perform a given task and how evenly it is divided among those individuals) and repeatability, in order to better un- derstand task organization in more flexible social systems like bumble bees.

Data Availability

Data are available through Dryad (https://doi.org/10.6086/ D1ZT2S) and data and code are available through Github (https:// github.com/kalefish52/AnBehEarlyBumbles21).

Author Contributions

K.F. and E.S. led the experimental design, carried out the ex- periments and led authorship of the manuscript. B.M.G. and C.K.M. assisted with experimental design and carrying out experiments.
S.H.W. co-designed the experiment. All co-authors contributed to writing the manuscript. All authors read and approved the final manuscript.

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Declarations of Interest

None.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2021.12.005.

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Appendix

Additional Methods

Quantification of brood-feeding behaviour

To collect behavioural data on brood feeding, a website was developed and used to familiarize individuals with artificial nest components, stages of brood development and certain in-nest behaviours. Annotations and captions accompanied images of nest components, such as bee number tags, wax and nectar cups and brood. The training module also compiled video clips of workers performing in-nest tasks, such as brood feeding, filling nectar cups and egg eating and laying. To ensure that training was successful, following completion of the materials, an additional testing module (Google Forms survey) was used to gauge individual ability to identify brood-feeding events accurately. The module contained 10 questions, each with a 30 s video clip of queenless three-worker colony nests. Individuals were asked to identify the time(s) of brood feeding observed using time stamps and which individual(s) fed brood using their number tags, if visible. Repeated and slowed playback of video was permissible. All observers successfully identified 10 of 10 brood-feeding events in this module. To ensure all observers were identifying behaviours consistently with one another, all observers would occasionally watch the same randomly selected video and compare results. This was done intermittently throughout the data collection period with more than five different videos.

In total, 11 observers went through the training programme and collected data on brood-feeding behaviour for the study. Although the website and training module showcased artificial queenless worker nests, individual task identification skills were transferable to behavioural logging irrespective of bee caste observed. Following training, observers were provided with video data to observe in the BORIS interface. Assigned observations were random with respect to nest, nest type and hour/date of observation.

Single-task frameworks

We quantified task organization among individual workers based on a single, predominant behaviour we observed them carry out, using two approaches previously implemented in social insect research. We also included a framework that categorized individuals to a task if they performed it at least once. Only worker bees with a minimum of three raw behavioural observations (*N* ¼ 120 bees from 40 nests) were included in these categorization analyses.

(1) Most frequently performed task. We categorized worker bees into behavioural groups based on the scaled behaviour they were observed performing most throughout the observation period (Shpigler et al., 2016).

(2) Perfect specialization. Worker bees who were observed performing a single task exclusively were categorized as perfect specialists on the behaviour they carried out.

(3) Tasks performed at least once. Worker bees who were observed performing any task at least once were labelled as performing that task. In this last analysis, individual bees could be assigned to more than one category.

Additional Results

Single-task frameworks

(1) Most frequently performed task. Of the 120 worker bees from 40 nests that were observed performing at least three behaviours, 38 (from 31 unique nests) were classified as nectar collectors, 16 (from 15 nests) as pollen collectors, 48 (from 30 nests) as brood feeders and 18 (from 17 nests) as egg layers, based on the scaled task they were observed performing the most (Table A2). Across all nests, significantly fewer individuals per nest were classified as pollen collectors compared to nectar collectors and brood feeders, regardless of nest social configuration (GLMM pairwise Tukey contrasts: nectar collection e pollen collection estimate ¼ 0.87 ± 0.30, *z* ¼ 2.90, *P* ¼ 0.019; brood feeding e pollen collection estimate ¼ 1.10 ± 0.29, *z* ¼ 3.81, *P* < 0.001; Fig. 3a). The best-fit model, here predicting the number of individuals per nest carrying out a given task most frequently, included behavioural category alone as a fixed effect.

(2) Perfect specialization. Of the 120 worker bees from 40 nests that were observed performing at least three behaviours, 20 (from 15 nests) were perfectly specialized, meaning that all of their observed behaviours were the performance of a single task. Eight (from 6 unique nests) were classified as nectar collectors, 0 as pollen collectors, 11 as brood feeders (from 9 nests) and 1 as an egg layer (from 1 nest; Table A2). Neither social configuration nor behavioural category predicted the number of individuals categorized as specialists according to this method (GLMM behavioural category: *P* > 0.1; social configuration not included in best-fit model; Fig. 3a). The best-fit model predicting the number of individuals per nest perfectly specialized on each task included behavioural category alone as a fixed effect.

(3) Tasks performed at least once. Of the 120 bees from 40 nests that were observed performing at least three behaviours, 89 bees (from

Table A1

Brood development and behavioural counts in each social configuration

Social configuration	Adult offspring size (marginal cell mm)	Total number of brood	Number of			Raw counts of			
			Pupae	Larvae	Eggs	Egg laying	Brood feeding	Nectar collection	Pollen collection
W3	2.82 ± 0.05	49.3 ±5.9	7.9 ± 1.4	21.8 ± 4.5	17.9 ± 3.4	3.3 ± 0.7	9.9 ± 2.4	15.5 ± 3.9	1.8 ± 1.8
W5	2.88 ± 0.04	52.0 ± 6.6	10.3 ± 1.6	23.1 ± 3.7	16.9 ± 6.0	3.0 ± 0.7	11.7 ± 3.5	22.9 ±4.1	3.9 ± 1.1
QW3	2.92 ± 0.05	54.4 ± 4.7	8.5 ± 2.3	27.0 ± 3.7	16.8 ± 3.6	4.5 ± 1.0	11.6 ± 4.0	16.9 ± 6.1	4.7 ± 2.3
QW5	2.84 ± 0.04	68.3 ± 4.8	12.6 ± 1.4	36.8 ± 3.9	17.0 ± 3.2	3.5 ± 0.9	11.4 ± 2.9	43.2 ± 9.0	17.5 ± 6.1

Mean ± SEM.

Table A2

Summary of task organizational framework analyses

	Task organizational framework analyses				
Correlates					
Ovary development	No patterns	No patterns	No patterns	No patterns	No patterns
Body size	No patterns	No patterns	No patterns	Pollen collectors larger than generalists	No patterns
No. of bees in each category					
Other	e	e	e	0	59 (28)
Generalists	e	e	e	167 (44)	71 (34)
Brood feeders	48 (30)	11 (9)	98 (38)	0	25 (17)
Pollen collectors	16 (15)	0	37 (21)	12 (10)	0
Nectar collectors	38 (31)	8 (6)	89 (36)	0	17 (15)
Egg layers	18 (17)	1 (1)	34 (24)	0	7 (7)
No. of bees included in analysis of 179 total	120 (40)	20 (15)	120 (40)	179 (43)	179
Data	Scaled counts of behaviours for workers with ::: 3 raw observations	Scaled counts of behaviours for workers with ::: 3 raw observations	Scaled counts of behaviours for workers with ::: 3 raw observations	Scaled counts of behaviours for all workers	Shannon values based on scaled counts of behaviours for all individuals
Method	(2.1) Task performed most frequently	(2.2) Perfect specialization	(2.3) Task performed at least once	(3.1) K-means clustering	(3.2) Shannon- threshold- based specialization
Question	Are frameworks based on a single behaviour informative to describe task organization?			Are frameworks based on all recorded behaviours informative to describe task organization?	

Values represent number of worker bees assigned to each cluster; numbers in parentheses represent number of nests in which those bees were observed. K-means clusters did not directly correspond to these categories. For the purposes of this table, clusters were subjectively assigned to behavioural categories based on behavioural repertoire of bees in each category.

Table A3

Repeatability results for scaled counts

	Bee ID				Nest ID				Social configuration			
	R	SE	CI	P	R	SE	CI	P	R	SE	CI	P
Brood feeding	0.36	0.1	[0, 0.56]	0.13	0	0.04	[0, 0.15]	1	0.04	0.05	[0, 0.17]	0.06
Nectar foraging	0.06	0.11	[0, 0.35]	0.34	0	0.04	[0, 0.31]	0.5	0.03	0.04	[0, 0.14]	0.13
Pollen foraging	0.28	0.13	[0.004, 0.51]	0.02	0	0.04	[0, 0.16]	0.5	0.04	0.05	[0, 0.17]	0.1

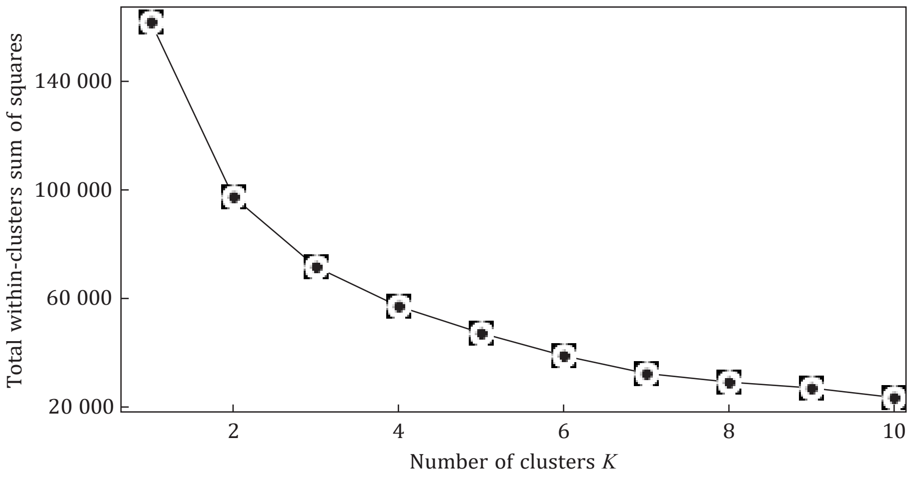

Figure A1. Elbow plot to determine number of clusters in K-means.

36 unique nests) were observed collecting nectar, 37 (from 21 nests) collected pollen, 98 (from 38 nests) fed brood and 34 (from 24 nests) laid eggs (Fig. 3c). Neither social configuration nor behavioural category predicted the number of

individuals catego- rized as specialists according to this method (GLMM behavioural

category: $P > 0.1$; social configuration not included in best-fit model; Fig. 3e). The best-fit model predicting the number of in- dividuals per nest carrying out each task at least once included behavioural category alone as a fixed effect.

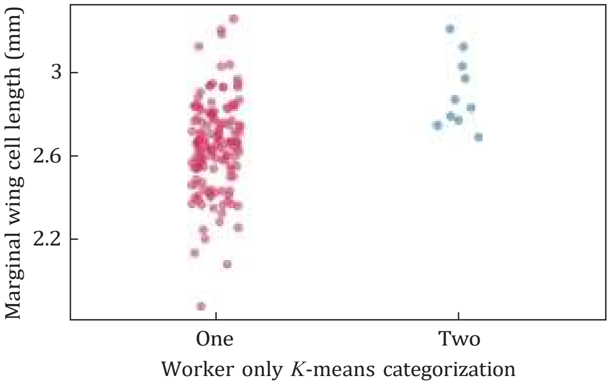

Figure A2. Association between worker body size and *K*-means cluster. Points are jittered to more easily visualize overlapping points (width ± 0.1; height ± 0.05).

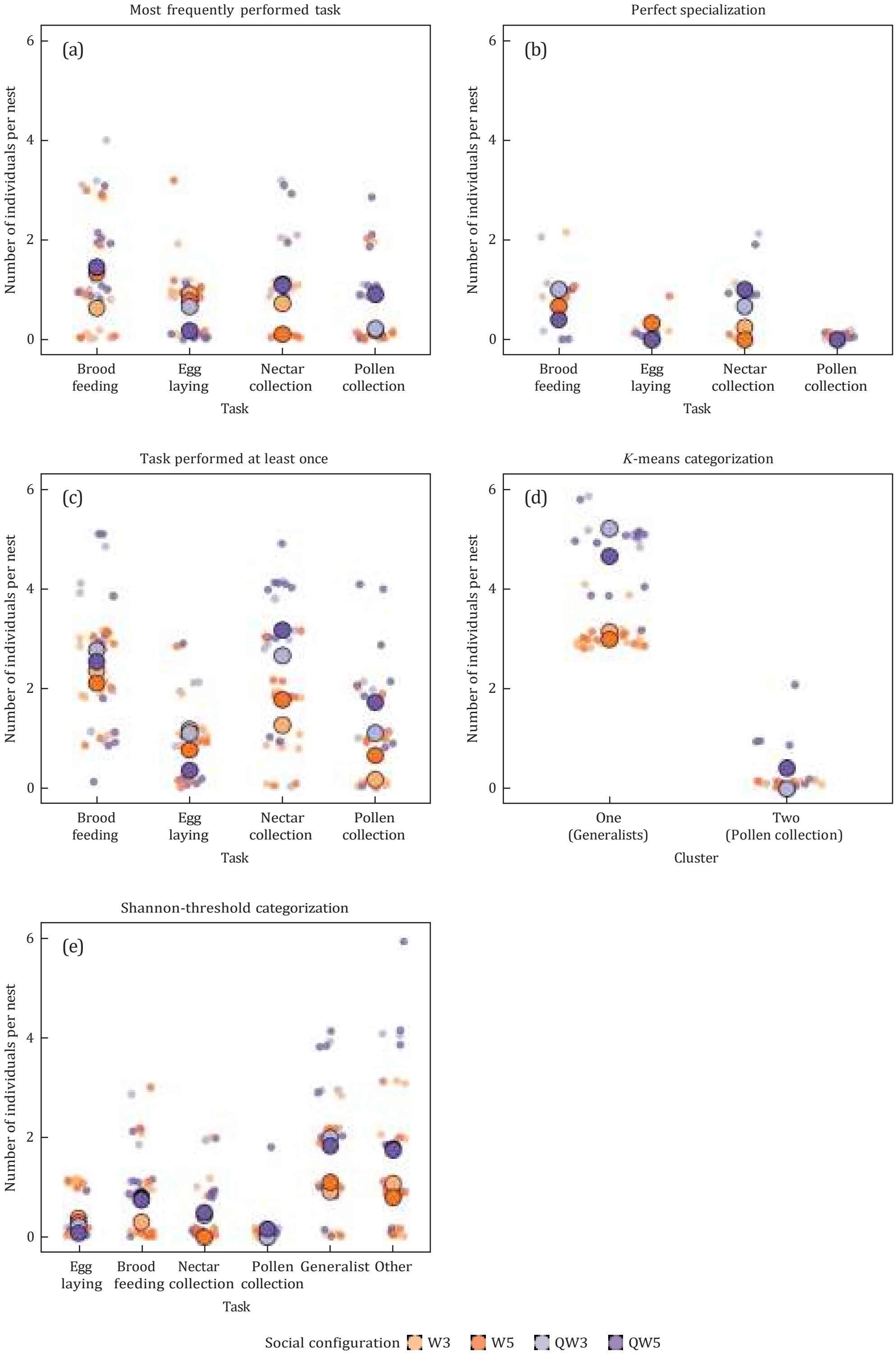

Figure A3. Number of individual workers in a nest assigned to each behavioural category. Smaller points indicate a single nest and larger points indicate means for each social configuration. Individual points (not means) are jittered for easier visualization of overlapping points (width ± 0.02; (b) height ± 0.2; (c) height ± 3).

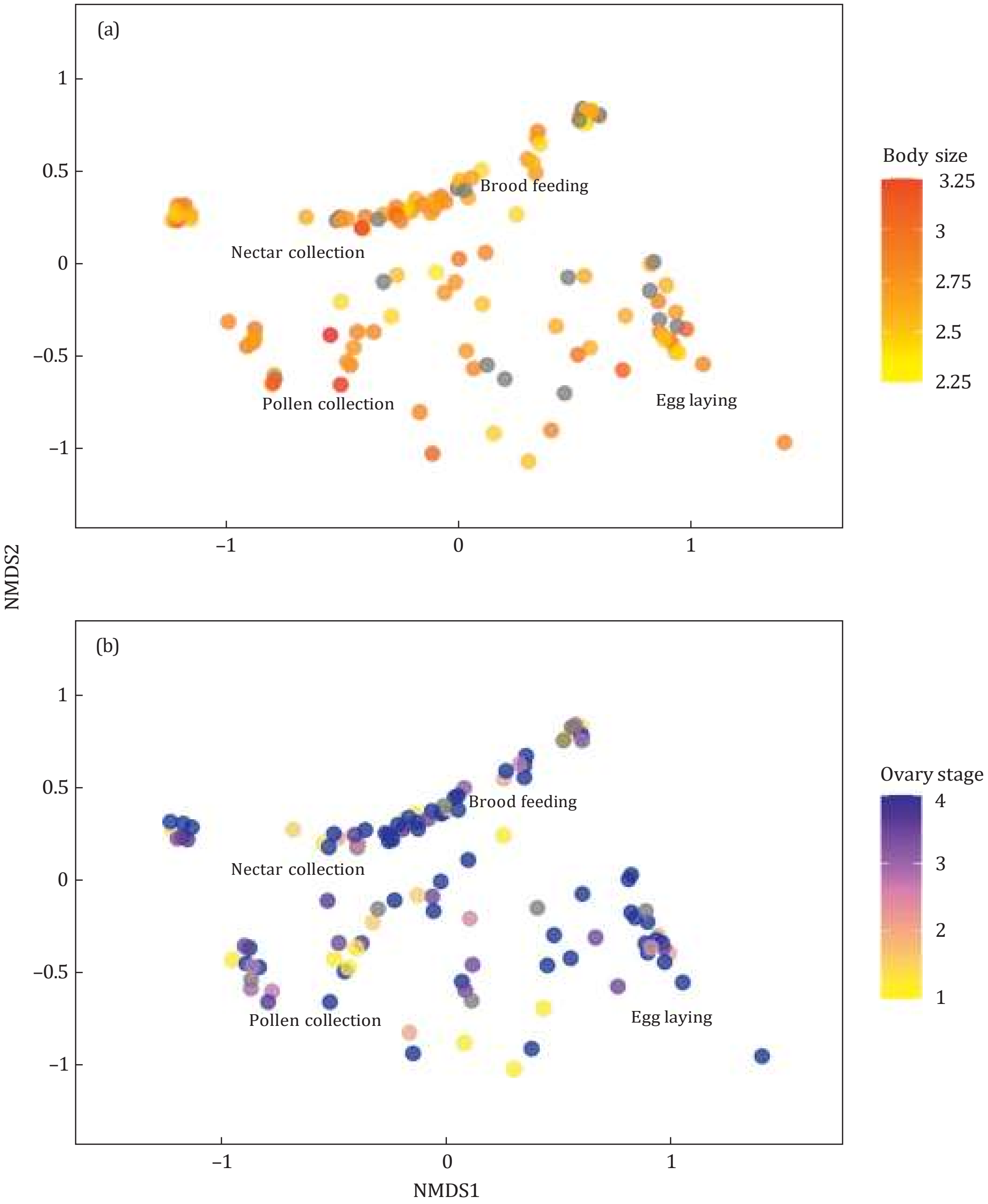

Figure A4. NMDS with worker body size and ovary stage. Grey points are missing data on body size or ovary stage. Task names are centred over their respective loci. Points are jittered to more easily visualize overlapping points (width ± 0.05, height ± 0.05). (a) NMDS coordinates of individual worker bees based on body size. (b) NMDS coordinates of individual worker bees based on ovary stage.

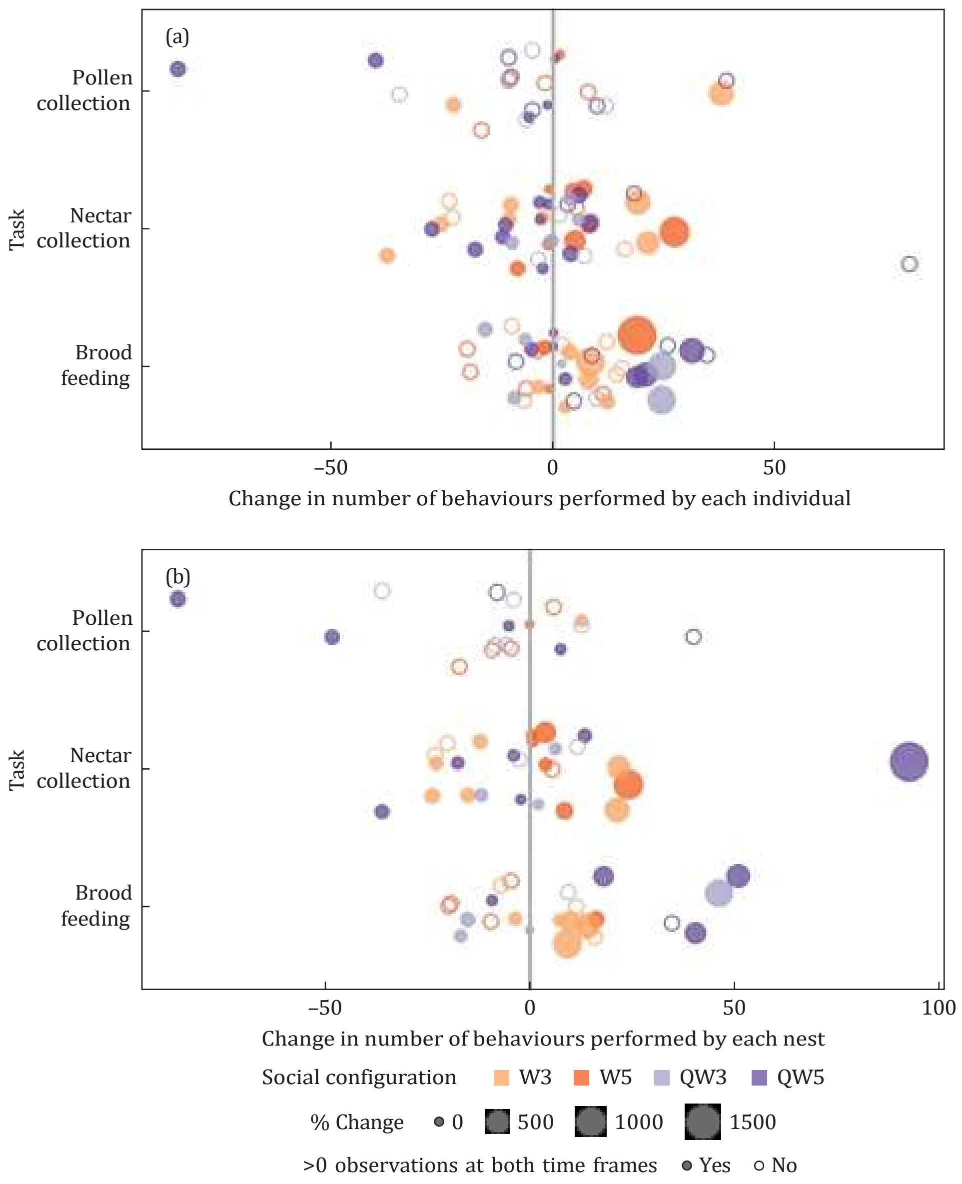

Figure A5. Repeatability analysis. Individual points are jittered to more easily visualize overlapping points (width ± 2; height ± 0.3). (a) Each point represents an individual worker bee and the change in the scaled number of observed behaviours from the early to the late time frame for each individual. (b) Each point represents a nest and the change in the number of observed behaviours from the early to the late time frame within each nest.