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Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels

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Workers in many eusocial insect species show a phenomenon sometimes referred to as 'elitism', in which a small proportion of individual workers engaged in a task perform a disproportionately large fraction of the work achieved by the colony as a whole. This phenomenon has not been well studied for foraging behaviour in honeybees (*Apis mellifera*) because detailed observational studies of foraging activity have been limited by the difficulty of successfully tracking large numbers of individual workers. Here, we used radio frequency identification technology to monitor honeybee flight behaviour automatically and generate lifetime flight activity records for large numbers of individuals from multiple colonies. We observed a consistent skew in activity levels of honeybee foragers, similar to that reported in many other social insects. However, this skew was a consequence of modulation of foraging activity by environmental and social factors rather than the existence of a distinct group or subcaste of elite foragers. Individual responses to experimental manipulation of the foraging workforce confirmed that activity level was flexibly adjusted according to colony needs. These results demonstrate that elitism in insect societies can arise as the extreme of a stable spectrum of individual behavioural activity that allows the colony to respond easily to unexpected needs rather than relying on responses of a rigidly defined subgroup of workers.

Chaucer's *Canterbury Tales* quotation 'busy as a bee' mirrors an assumption made in early models of eusocial insect behaviour: that workers specializing on a given behavioural task display similar or identical rates of task performance. Because eusocial insect colonies are composed largely of genetically related workers and because reproductive fitness is determined at the level of the colony, variation in performance among workers within a colony had no obvious origin or possible advantage. In reality, for a wide range of species and behaviours, individual workers show great variation in activity levels (Beverly, McLendon, Nacu, Holmes, & Gordon, 2009; Dornhaus, 2008; Hurd, Nordheim, & Jeanne, 2003; Möglich & Hölldobler, 1974; O'Donnell & Jeanne, 1990; Oster & Wilson, 1979; Pendrel & Plowright, 1981; Plowright & Plowright, 1988; Robson & Traniello, 1999). The term elitism has been used to describe the phenomenon in which a subset of the individuals performing a given task show a higher activity level than the others (Oster & Wilson, 1979).

Several explanations have been proposed for the adaptive value of variation in activity level within the set of individuals performing a task. Individuals with lower activity may represent a reserve workforce, enabling the colony to respond rapidly to sudden and unpredictable demands or opportunities (Plowright & Plowright, 1988). Alternatively, individuals within a workforce may have behavioural specializations that make them more or less able to perform a task efficiently (Oster & Wilson, 1979). It is likely that the factors promoting variation in activity level are different for different species, or even for different tasks performed by a single species.

Despite a vast body of literature devoted to foraging behaviour of honeybees (*Apis mellifera*), reports of systematic studies of elitism in honeybee foragers are absent. Only a few studies in the last half century have alluded to elite forager honeybees. Sekiguchi and Sakagami (1966) described the presence of a few individual honeybee foragers with unusually high flight activity relative to

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other foragers from the same colony. Ribbands (1953) also reported a maximum of 150 trips in a single day made by individual nectar foragers to an artificial feeder. These findings indicate the potential for great variation in activity level within a colony's foraging population. To our knowledge, no prior study has investigated whether elite honeybee foragers represent a distinct subgroup, how the tendency to extreme foraging activity might change over the individual life span, or how foraging elitism relates to colony-level regulation of foraging activity.

One likely reason that these types of questions have not been previously explored in honeybees is the difficulty of monitoring flight activity. The type of continuous manual observation and recording of individual behaviour that would be required is a significant challenge. The recent appearance of technologies that enable automated detection of individual insects and other small animals has presented the means to pursue research that previously faced these obstacles.

In this study, we adapted existing radio frequency identification (RFID) tagging technology, along with custom-written recording software and analysis algorithms to track the lifetime flight activity of several hundred individuals in several different colonies of honeybees. RFID microtransponder tags have been used several times over the last 10 years (Molet, Chittka, Stelzer, Streit, & Raine, 2008; Robinson, Richardson, Sendova-Franks, Feinerman, & Franks, 2009; Robinson, Smith, Sullivan, & Franks, 2009; Streit, Bock, Pirk, & Tautz, 2003; Sumner, Lucas, Barker, & Isaac, 2007) to track the entry and exit of bees, wasps or ants from the nest. RFID tags have also been used to monitor the long-term exploratory behaviour and locomotor activity of mice in a seminaturalistic environment (Freund et al., 2013; Lewejohann et al., 2009). RFID tags are small, light weight and robust, they can be coded with many unique IDs and they are economical enough to allow for many individuals to be monitored simultaneously. These characteristics make them a particularly good choice for studies with honeybees; RFID tags already have been used successfully in studies of honeybee navigation and homing behaviour (Core et al., 2012; Decourtye et al., 2011; He et al., 2013; Henry et al., 2012; Pahl, Zhu, Tautz, & Zhang, 2011).

Our use of RFID technology allowed us to analyse the flight activity of a large sample of individually identified bees over an extended period. This large data set allowed us to confirm the existence of a spectrum of activity levels among honeybee foragers, from relatively inactive to highly active. We investigated potential differences between high-activity foragers and other foragers within the same colony. We also examined the effect of the removal of high-activity foragers on the activity levels of remaining tagged individuals in the colony's foraging population. Our results demonstrate that individuals with high ('elite') foraging activity represent the extreme of a continuum of activity levels. However, we also found that honeybee foragers can adjust their foraging activity in response to changes in the foraging workforce, including a rapid assumption of high-foraging activity status.

METHODS

Monitoring Technology

Bees were tagged with laser light-activated 'p-chip' microtransponders (tags) (PharmaSeq, Princeton, NJ, U.S.A.). The tags were detected by laser readers (PharmaSeq) connected via a USB cable to a computer. Each tag carried a unique identification number; the tag's upper surface contains photocells that, when lit by a reader's red laser beam, activate the chip to transmit its ID for a distance of up to 10 mm to a pickup coil in the head of the reader. Processing and decoding of the ID were performed with firmware and p-Chip Reader software provided by PharmaSeq.

Figure 1. Typical positioning of two PharmaSeq p-chip microtransponders on the thorax of an adult worker honeybee.

Because of the small 1.5 mm diameter of the laser beam, two tags were attached to a bee to increase the likelihood of detection (Fig. 1). Each tag was 500 × 500 × 100 μm with a weight of 90 μg; two tags fit easily on the thorax of the bee, and their combined weight was only 0.56% of the average load carried by a nectar forager (Winston, 1987). This means that it is unlikely that the presence of the tags impaired natural foraging behaviour.

To read tagged bees, a 10 × 10 mm plastic tube walkway was attached to the hive entrance, with two laser readers projecting into the top of the tube (Fig. 2). Bees passed sequentially under each reader as they entered and exited the hive, so that the order of detection by each reader could be used to infer the direction of travel. The top and sides of the walkway were coated with Fluon (Bioquip Products, Rancho Dominguez, CA, U.S.A.) while the floor of the walkway had numerous small drilled holes to provide grip; this encouraged most bees to walk on the floor of the walkway, maximizing the probability that their tags would be detected. Upon

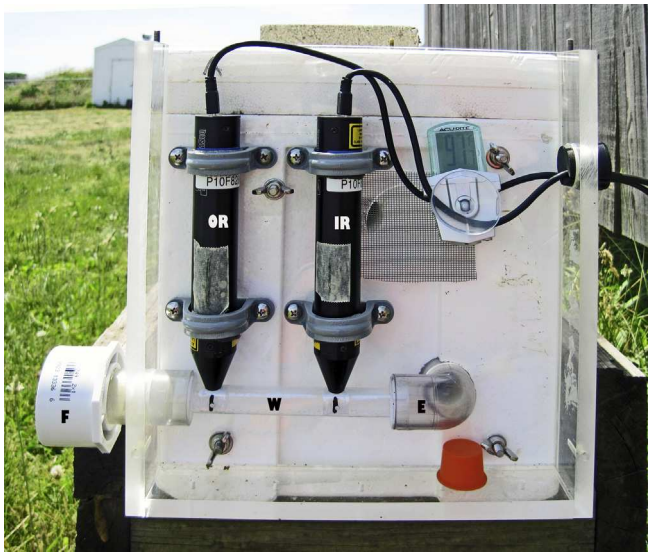

Figure 2. Recording apparatus attached to the front of a six-frame nucleus hive. Bees leave the hive through a 90° entrance elbow (E), then pass through a 10 mm square walkway (W) exiting to the outside at the funnel (F). Outer (OR) and inner (IR) readers record bees passing in the walkway underneath. A ventilated Plexiglas cover fits over the apparatus for rain protection.

detecting a passing bee, the reader passed the detected ID and reader number to an attached PC, where a time stamp was associated.

Tagging Procedure

Bees were tagged when they were 6–24 h old. Each bee was anaesthetized on ice and kept on a container of shaved ice covered with aluminium foil. A vacuum pickup tool (Hakko Model 394 with 0.26 nozzle, Osaka, Japan) was used to select and manipulate upright two tags into a small dish. The tags' serial numbers were read and recorded. A small dollop of Loctite Super Glue Gel Control (Henkel, Düsseldorf, Germany) was applied to the bee's thorax. Tags were picked up with the vacuum tool, a slightly wet toothpick, or a pair of microdissection forceps and positioned on the bee's thorax. The bee was then placed in a cradle to stay upright for the minute or so required for glue drying and recovery. The entire tagging process for a single bee lasted 3–5 min.

Colony Set-up

Single-cohort colonies, composed of approximately 2000 1-day-old bees, were established as in Robinson, Page, Strambi, and Strambi (1989). In the absence of older bees, some younger bees start to forage precociously, which meant we were able to begin the monitoring more quickly. The small populations allowed us to tag and thus monitor a proportionally high number of the colony's foragers. Frames of honeycomb containing pupae were collected from 5 to 10 typical colonies maintained according to standard procedures at the University of Illinois Bee Research Facility, Urbana, Illinois, with naturally mated queens and stored in a dark, humid incubator at 34 °C. Adults were removed from the comb within 24 h of emergence, marked on the thorax with paint (Testors) or provided with tags as described above, and introduced to an experimental colony. Each colony was created by placing a laying queen, about 1000–1500 paint-marked 1-day-old adult workers bees, and about 100–500 tagged 1-day-old adult worker bees into a small styrofoam hive box. The colony was given three honeycomb frames containing nectar and pollen, but no eggs, larvae or pupae.

Experiments

All activity-tracking experiments were conducted in Urbana, Illinois, in the summers (May–October) of 2011 and 2012. Specifics of each experimental colony are detailed in Table 1. Each experimental colony was maintained for about 5–7 weeks, during which time the flight activity of tagged bees was continuously recorded.

Three colonies (O1, O2, O3) were located outside, with unrestricted access to the surrounding landscape. No experimental manipulations were performed on these colonies; their activity provided a baseline of foraging activity on naturally occurring resources.

Table 1
Information on the set-up, reader performance and skew in activity level for each colony

Colony	Set-up	No. of tagged/untagged bees	Reader % detection	% Bees=50% activity
O1	15 Jun 2011	102/958	46.4±2.5	20.2%
O2	26 Aug 2011	119/932	37.4±1.8	20.5%
O3	4 Jun 2012	369/979	45.7±1.0	21.4%
E1	10 Sep 2011	163/921	49.0±2.1	20.6%
E2	22 Aug 2012	391/1051	31.2±1.2	16.2%

Two colonies (E1, E2) were located inside an outdoor mesh enclosure (6 m wide × 20 m long × 3 m high) and provided ad libitum pollen, water and 50% sucrose solution, placed in feeders positioned near the colony and replaced daily. In the fourth week after colony set-up, hand-held laser readers were used to detect and record the presence of tagged bees continuously at both the sucrose and pollen feeder over a 5 h period, 0900–1400 hours for 3 consecutive days. On the second day of recording, we collected and killed all bees (both tagged and paint-marked) observed foraging at the feeders during an hour-long interval during peak foraging activity, 1100–1200 hours. These removal experiments were performed to monitor the response of remaining tagged bees to the loss of a portion of the colony's foraging workforce.

Data Analyses

Software

Activity data were recorded and basic analyses performed using custom-written software developed in TenCORE. Further analyses were performed using Python.

Inference of round-trip flight activity

Because the walkway attached to the colony entrance required bees to walk successively under two tag readers (the inner reader 'I', and the outer reader 'O'), we were able to infer an individual's direction of travel. In an ideal system where the rate of successful detection of a passing tagged bee is 100%, round-trip events (in which a bee first exits the hive and then returns) could be detected simply by looking for instances where an individual bee was detected by I followed by O, then O followed by I, with an interval of several minutes in between the two sets of reads. To accommodate instances in which one or both readers failed to detect a passing bee, or in which a bee passed under one reader and then reversed direction, we used several further criteria to detect possible trips.

Reader success

From our inference of round-trip flights, it was possible to estimate a mean detection success rate for each individual bee. The success rate was computed using the number of instances where only a single reader (S) detected a bee passing through the walkway during its path in or out of the hive and where both readers (D) detected a bee passing through the walkway (with time separation of at most 40 s). Suppose that a given bee passes both detectors n times while making foraging trips. Then, if p is the probability that a reader will successfully read a passing bee, then in n trials where the bee passes both readers, we can estimate the fraction of events where both detectors would successfully detect the bee as approximately $p^2 = D/n$, while the probability that only a single reader will detect the bee would be approximately $2p(1-p) = S/n$. Eliminating n from these two equations yields $p = 2D/(2D+S)$, and p is interpreted as the estimated average success rate of detection for that bee.

When we averaged the success rate of all tagged bees in each colony, the colony mean detection success rate was about 30–50% (Fig. 3, Table 1). When we compared this inferred entryway reader success rate against confirmed trip data (generated on days when visits to artificial feeders were recorded using hand-held readers), the inferred and actual entryway reader success rates were closely correlated (Pearson correlation: $r_{71} = 0.634$, $P < 0.0001$). This success rate suggests that, on a given round-trip flight, a bee has approximately a 76–94% chance of being detected on at least one of its four passes under readers: this estimate can be obtained by calculating the probability that a bee will not be detected in any of those four passes; that is, $(0.6)^4 = 0.1296$, and subtracting that value from 1 to yield a probability of, in this case, 87.4%.

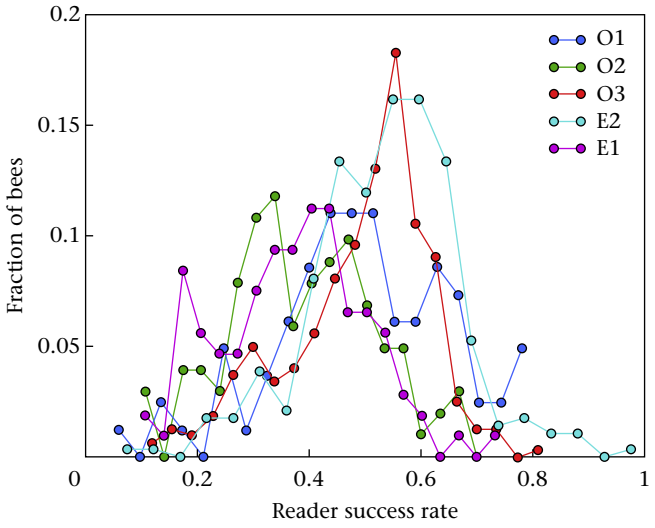

Figure 3. Estimated reader success rates for all tagged bees in the study. Each curve shows the distribution of reader success rates for each bee in the five study colonies, 01-3 and E1-2.

Definition of daily activity level

Because of the greater probability of detecting a bee at some point during a round-trip flight than of detecting a complete trip, we used detection events (reads) rather than trips as a metric for activity level. To account for variation in the success rate for individual bees due to small variation in tag placement or locomotor behaviour, we corrected each bee's number of reads (per day) by dividing by the calculated average reader success rate for that same bee. Bees with less than 20% average success rate were discarded from subsequent analyses.

Furthermore, to correct for colony-wide variation in activity by day, we normalized the number of reads for a bee on a given day by the total number of reads on that day. This allowed us to compare activity across different days of each experiment, despite variation in a colony's overall flight activity caused by weather, availability of forage or other factors. Thus, the activity of a bee on a given day was effectively the fraction of reads that it contributed to the total reads on that day.

The data showed almost no correlation between the reader success rate of an individual and the activity level of that bee. For example, the correlation between estimated reader success and average daily activity for tagged bees in colony O3 was not significant (Pearson correlation: $r_{161} = 0.046$, $P = 0.56$; Supplementary Fig. S1). This result indicates that even when uncorrected, an individual bee's activity level was not an artefact of tag placement or other factors affecting reader success.

A bee's day of death was defined as the day after which no reads were detected for her tags until the end of the experiment; reads before the age at which foraging behaviour began (see next section) and after death were not included in the analyses of activity. Days with no reads that occurred before the day of death were included in the analyses.

Detection of orientation flights

To focus our analyses on foraging activity, it was necessary to define and omit flight activity likely to be related to orientation flights, which occur for several days prior to the onset of foraging (Capaldi et al., 2000). Because in our locality most orientation flights occur in the afternoon (C. Lutz, personal observation), we defined each bee's first day of foraging as the first day on which it had at least six reads and when more than 25% of its reads occurred before 1200 hours. This heuristic generated estimates of age at first foraging that agreed closely with those produced by a human observer. Any flight activity on or after this day was defined as foraging activity; the daily flight activity defined above was used as a proxy measure of foraging activity.

Ethical Note

In some studies involving the automated tracking of animal subjects, it may be desirable or necessary to recover the tracking equipment by recapturing or sacrificing the subjects at the end of the tracking period. At the time of this study, the cost of a single tag was $1.35 (U.S.), low enough that we were able to treat the tags as disposable. It was therefore not necessary to sacrifice tagged bees at the end of each experiment.

RESULTS

RFID Tracking Detected Normal Onset of Foraging Behaviour

We first confirmed that the flight behaviour detected by the PharmaSeq system fitted with established norms of honeybee foraging behaviour by examining the age at onset of foraging in each experimental colony (Fig. 4). Because our colonies were initially composed of 1-day-old worker bees, we expected to observe precocious foraging behaviour (Huang & Robinson, 1996). Considering only the first 10 days after colony establishment, the average percentage of tagged bees that had begun to forage was 27.2%. This was consistent with previous reports of levels of precocious foraging in single-cohort colonies (Huang & Robinson, 1992).

Unlike most behavioural maturation experiments in bees, which rely on human observation of flight activity, we were able to use the monitoring technology to collect data from tagged bees for 1 month or more after colony establishment. When we considered the first 5 weeks of flight activity for all colonies, the average age at onset of foraging was 20.4 days. This age is consistent with previous reports of the average age at first foraging (Winston, 1987). These results indicate that our automated monitoring system did not produce observable disruption of normal fight behaviour and that the

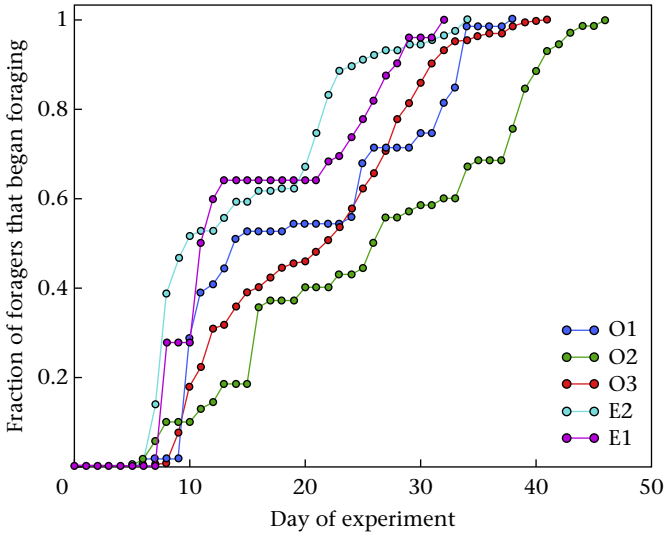

Figure 4. Age at onset of foraging for tagged bees. Each curve shows the cumulative proportion of bees with observed foraging activity in each colony. Results were typical for bees in single-cohort colonies, indicating that the tags did not affect normal behaviour.

recordings produced by the system could be used to infer flight behaviour accurately.

Daily Activity Level Varied Greatly within the Colony Foraging Workforce

In examining the records of daily flight activity from our initial experimental colonies, we observed that a few individuals appeared to be much more active than the rest of the foraging population. These individuals often began to make trips as soon as the colony became active each morning, and they made regular, closely spaced trips throughout the day until the cessation of colony-wide flight activity in the evening. Approximately 20% of the foraging workforce accounted for 50% of the total flight activity over the course of the experiment in each colony (Table 1). This occurred in all five colonies studied: three with natural outdoor foraging and two that foraged in a large screen enclosure.

To quantify the degree of inequality in flight activity among the foragers, we plotted the cumulative distribution of total flight activity for each colony in the form of a Lorenz curve (Fig. 5, Supplementary Fig. S2). Such a curve displays the share of foraging activity (Y axis) accounted for by the bottom x% of foragers in the colony. A perfectly equitable distribution of foraging activity would correspond to the line $Y =$ on such a curve. The ratio of the area between the Lorenz curve and the line $Y = X$ to the triangular area under the line $Y = X$, is known as the Gini coefficient. This value provides a measure of the degree of inequality in the distribution of foraging activity, ranging from 0 (perfect equality) to 1 (perfect inequality). Our colonies showed an average Gini coefficient ± SD of 0.493 ± 0.032 (Table 2), establishing a more formal description of the 20–50% relationship that we first observed. The appearance of continuously increasing concavity of the Lorenz curve fitted to the results suggests that the 'elite' foragers were actually the extreme of a unimodal continuum of activity levels within the colony

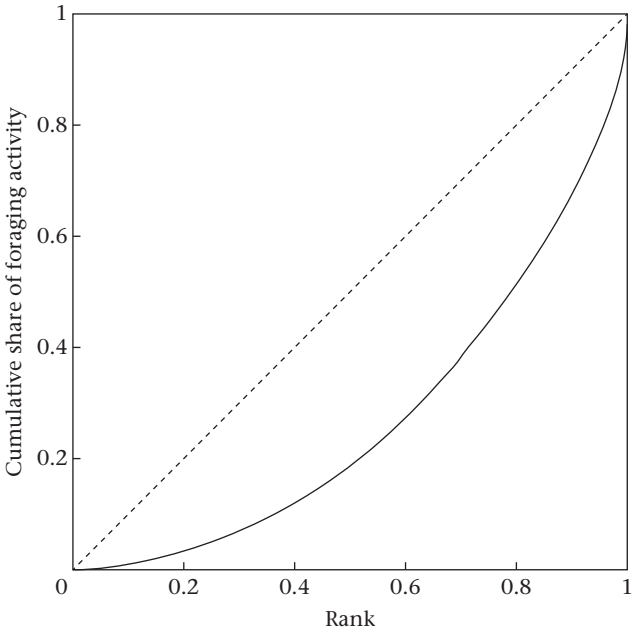

Figure 5. Example plot of a typical Lorenz curve of cumulative share of foraging activity for one of five study colonies (colony O3). Individual bees were ranked by their contribution to total colony activity in ascending order, and the fraction of each bee's contribution to the total activity of the colony was cumulatively plotted to produce the curve. The dotted line represents the plot that would result if the contributions of all individuals were perfectly equal. The Gini coefficient is proportional to the area between the curve and the dotted line.

Table 2
Calculated Gini coefficients representing the degree of skew in foraging activity level for each colony

Colony	Gini coefficient
O1	0.501
O2	0.479
O3	0.454
E1	0.488
E2	0.541

(Krause, 2013); this can also be seen in histograms of forager contributions to colony activity (Supplementary Fig. S3).

This analysis, unlike those presented below, examined cumulative foraging activity through the duration of the experiment. Therefore, some of the observed skew can be attributed to variation among bees in the number of days spent foraging. Although, as expected, number of days spent foraging was significantly associated with total foraging activity (Pearson correlation: $r_{161} = 0.286$, $P < 0.0005$), the low correlation coefficient indicates that the majority of the variation underlying the skew in colony activity cannot be explained by variation in foraging life span.

Because of this consistent inequality, for some of the following analyses, we used the individual bees with the top 20% of bees ranked by activity level within a given time period as a working definition for bees showing high activity 'elite' behaviour.

High-activity Foragers in a Controlled Environment

Two of our experimental colonies, E1 and E2, were housed in a screened enclosure and provided with artificial feeders of sucrose solution (as artificial nectar) and pollen. During a 3-day observation period for each colony, we recorded visits to each feeder type using hand-held tag readers, allowing us to confirm what resource was being gathered on individual foraging trips. For the high-activity bees (defined as those in the top 20% of bees foraging on each of these days), there were individuals that foraged exclusively on either pollen or nectar, as well as a few individuals that collected both pollen and nectar on separate trips or on the same trip (Table 3). When we compared the resource preference of high-activity bees with that of all foragers detected at the feeders, there was no significant difference in the distribution of preference between the high-activity bees and the larger foraging population (chi-square test: $\chi^2_2 = 0.486$, $P = 0.784$).

Deviation in Individual Foraging Activity Patterns across Time

Use of the automated tracking system enabled us to collect data from each colony for more than 1 month; for many bees, this period encompassed their entire life span. Behavioural parameters such as foraging efficiency and foraging strategy of honeybee workers have been found to change over a forager's life span (Biesmeijer & Seeley, 2005; Dukas, 2008; Schippers et al., 2006). We asked how the foraging activity level of an individual bee changes over time.

To look more closely at the dynamics of activity level, we generated heat maps of deviations in daily activity level from each

Table 3
Observed forage preferences of all tagged bees and of high-activity bees during the removal experiment

Feeder type	Percentage of tagged bees observed at feeders	Percentage of high-activity bees observed at feeders
Nectar	51	50
Pollen	36	33
Nectar and pollen	13	17

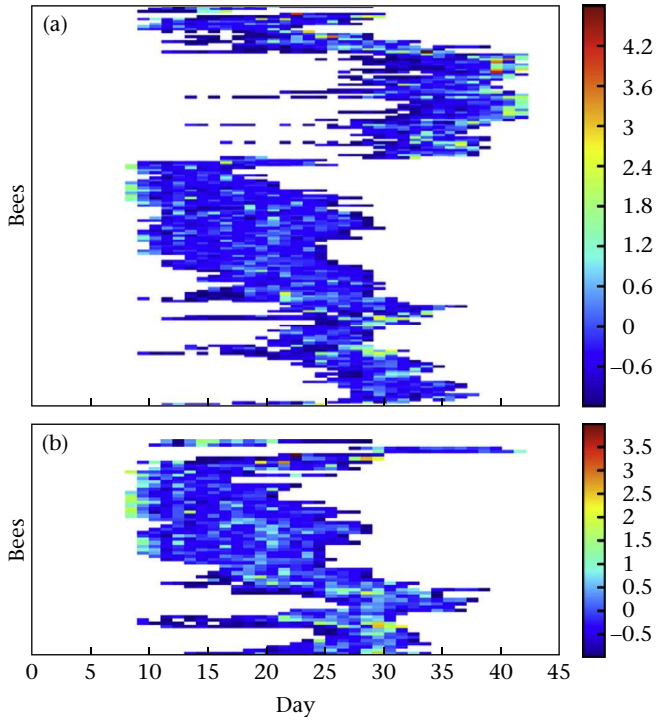

Figure 6. (a) Example heat map of deviations in daily activity level for individual bees in colony O3. Each row represents data for an individual bee and each column corresponds to 1 day of the experiment. Rows are ordered by similarity of the bees' daily activity levels. (b) Plot for colony O3 with content identical to that in panel (a), but for only the individuals in the top 20% of bees ranked by activity level on days 10, 20 and 30.

individual's lifetime mean activity level (normalized by the mean; Fig. 6a, Supplementary Fig. S4). That is, if $a(b, d)$ is the foraging activity of bee b on day d, Fig. 6a shows $(a(b, d) - A(b))/A(b)$, where $A(b)$ is the average of $a(b, d)$ over all days d. The heat maps suggested that rather than one apparent shared pattern of activity level over the life span, individual bees showed a diversity of lifetime patterns of activity level. For some individuals, activity level remained fairly constant. For others, activity increased or decreased at various times throughout their foraging career. Note that these deviations were observed relative to the average activity level of that individual, after normalization to daily total activity level of the colony; the deviations therefore represent the isolated behavioural pattern of that individual, rather than reflecting communal changes in activity of the whole colony.

We wondered whether lifetime activity level dynamics might be more uniform in individuals with the highest activity levels. To look more closely at the lifetime patterns of these bees, we selected the top 20% of individuals (ranked by the sum of their daily activity levels on days 10, 20 and 30) of each colony. We selected these time points to yield a small, easily observed sample of high-activity individuals from early, middle and late phases of a single-cohort colony's development. We examined the deviations about the mean activity level throughout each bee's life span (Fig. 6b, Supplementary Fig. S4). Again, there was a great deal of variation in lifetime activity levels among these bees: for some, activity level remained fairly constant, while for others, activity increased or decreased at various times throughout the bees' lifetime.

Individual Flexibility in Foraging Activity in Response to Changes in the Colony Workforce

Our observation that activity level did not remain constant across a forager's life span suggests that 'elite' behaviour is at least partially environmentally determined. In other words, individuals may be able to adapt their activity level in response to external environmental factors, such as an increase or decrease in the profitability of a food source to which an individual forager is loyal (Townsend-Mehler, Dyer, & Maida, 2011), or in response to the appearance or disappearance of a resource exploited by a subset of the foraging population (Visscher & Seeley, 1982). Internal factors such as changes in colony demography might also affect individual activity levels; for example, the loss of a few high-activity individuals might trigger the onset of foraging or an increase in foraging in a few individuals rather than a more generalized response by the whole colony. One way to test for this type of responsiveness is to remove the most active individuals and compare the activity level of the remaining workers before and after the removal (Beverly et al., 2009; Pendrel & Plowright, 1981; Robinson & Page, 1995; Robson & Traniello, 1999). If individuals are able to adjust their activity levels according to environmental factors, removal of the most active individuals is expected to promote increased activity in those that remain.

Table 4
Details of removal experiments

Colony	No. of tagged bees	No. of tagged/untagged bees removed	Colony age (days)
E1	163	24/67	26
E2	391	23/118	22

We conducted removal experiments with each of our enclosure-housed colonies, E1 and E2; details of these experiments are included in Table 4. In these experiments, we captured and killed all the bees, both tagged and paint-marked, that arrived at either the pollen or the nectar feeder during a 1 h period of high foraging activity. Although we did not specifically target foragers with high activity for removal, the very fact of their greater number of foraging trips increased their probability of being captured during this limited interval. In fact, when we reviewed the activity records from the previous day of the removed tagged bees, we found that the majority of the tagged bees removed (80%) were in the top 20% of activity levels. We cannot confirm that this was also the case for paint-marked bees, but their distribution of activity level was expected to be similar to that of the tagged bees. It was essential to target both tagged and paint-marked bees to ensure that we removed enough highly active bees to affect the colony workforce noticeably.

From the time of removal to the end of that day's observation period, the number of visits to both the pollen and nectar feeders was negligible (<10 visits total). However, by the next morning, foraging activity and visits to the feeders had recovered to previous levels. The number of new foragers observed the day after the removal ($N = 4$ in E1, $N = 6$ in E2) was not significantly higher than on the day before the removal ($N = 1$ in E1, $N = 15$ in E2; paired t test: $t_1 = 0.5$, $P = 0.705$), suggesting that existing foragers adjusted their activity levels to compensate for the loss of those removed. Supporting this interpretation, among bees that were observed at the feeder both before and after the removal, daily activity levels were significantly higher on the day after removal (paired t test: $t_{15} = 5.94$, $P < 0.0001$), increasing 488% on average (Fig. 7).

DISCUSSION

We used RFID tagging technology to detect lifetime flight activity automatically for a large number of individuals in five colonies. The extensive data set generated by automatic monitoring revealed a large skew in foraging activity across individuals; the activity of a few individuals in each colony accounted for a large

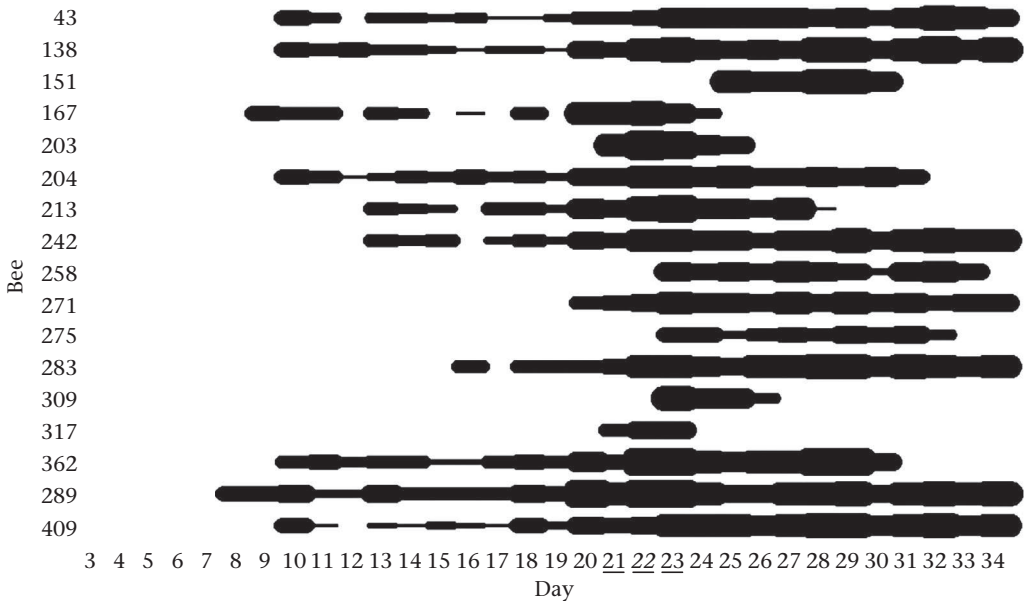

Figure 7. Daily activity levels of tagged foragers in colony E2 that were detected at a feeder on the day after the removal experiment, in which many tagged and untagged high-activity foragers were removed. Each row contains a lifeline that represents the activity of a single bee; the thickness of the line is proportional to the log-transformed activity level. The day of the removal experiment (day 22) is indicated in italics. Days on which foraging activity was monitored at artificial feeders (days 21–23) are underlined.

proportion of that colony's foraging activity. These bees resembled 'elite workers' reported in a number of other species. However, our results also show that honeybee foraging activity level is flexibly adjusted during a bee's lifetime, suggesting that in honeybees, elitism does not involve a distinct subcaste of foragers but rather stems from an extreme of a range of individual activity levels that are continuously adjusted and may be influenced by environmental cues.

Three aspects of our results support this idea of flexibility. First, we found that individual lifetime flight activity levels formed a continuous distribution. Second, bees in our study showed a variety of activity level patterns throughout their lives, indicating that there is no set developmental progression of foraging activity level and suggesting that individuals may adjust their foraging activity level according to individual experience of social and environmental conditions. Third, as described in the final section of the results, removal of a fraction of the foraging population was associated with an almost five-fold increase in activity level in previously low-activity foragers. Taken together, these results support the view that individual workers continuously adjust their activity level to ensure that the colony's nutritional needs are being adequately and efficiently met, and that the net activity of the whole foraging population is likely to be one of the factors that influences this decision.

We cannot rule out the possibility that genetically based differences in neural or neuroendocrine function contribute to the striking differences in individual activity levels observed in this study. It is also possible that a small subset of high-activity bees do maintain those high levels consistently throughout their foraging career, and even more extensive data collection would reveal the existence of such a group. However, our observations contrast with the description of elitism offered by Oster and Wilson (1979) and with results of studies of elitism in other species showing a lesser degree of plasticity in individual activity level. In several ant species, elite workers represent a subcaste that is distinguishable from non-elite workers engaged in the same task; in some cases, elite workers are not replaced by individuals in the non-elite population after their removal, resulting in the cessation of the task (Oster & Wilson, 1979; Robson & Traniello, 1999). Activity levels of elite and non-elite *Temnothorax* ants are consistent for a variety of tasks across an individual's life span (Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012), and elite *Vespula* foragers show a lifetime progression of activity level that is stereotyped and distinct from that shown by most non-elites (Hurd et al., 2003), suggesting that activity levels in these species are less dependent on environmental factors than those of honeybee foragers. However, Pinter-Wollman et al. also found that non-elite workers that were engaged in brood transport during emigration were able to increase their activity level in response to removal of elite workers, as were harvester ant foragers in another study (Beverly et al., 2009); in these species, as in honeybees, individual worker activity level is plastic. Hurd et al. (2003) were able to identify elite wasp foragers as a distinct group via clustering methods, and they attributed the unimodal distribution to the small number of elite individuals they were able to observe. In contrast, the relatively large number of individuals and colonies that we were able to track using RFID technology makes the finding of continuous distributions in activity levels here much more robust. It remains to be seen whether some previous reports of elite individuals in other species also could be interpreted similarly if there were lifetime records of activity available for analysis.

The findings reported here emphasize the value of modelling elitism as an extreme of a range of individual activity levels that are more or less plastic depending on the species and context, rather than assuming the existence of a distinct subcaste of workers. Beverly et al. (2009) demonstrated that much of the variation in foraging activity level in harvester ants could be explained by individual fidelity to food patches that varied in quality. Honeybee colonies similarly exploit floral resources that may vary widely in quality as well as in distance from the hive and in temporal availability (Visscher & Seeley, 1982). Some of the intra- and interindividual foraging activity level variation we observed may be due to bees adjusting their effort to the availability and profitability of particular foraging sites. However, this type of environmental factor cannot fully explain the skew in activity that we observed in our enclosure hives; some foragers in these hives may have been loyal to

the provided feeders while others searched (fruitlessly) for alternative resources, and the overall activity distributions were remarkably similar to the outdoor hives. Honeybee foragers may modulate their activity level to maintain a typical distribution of levels as well as a net activity level appropriate for the colony's needs at that moment. Future investigations that manipulate aspects of colony demography and foraging environment simultaneously could help to clarify the relative importance of these factors.

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Supplementary Material

Supplementary material for this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2014.06.006.

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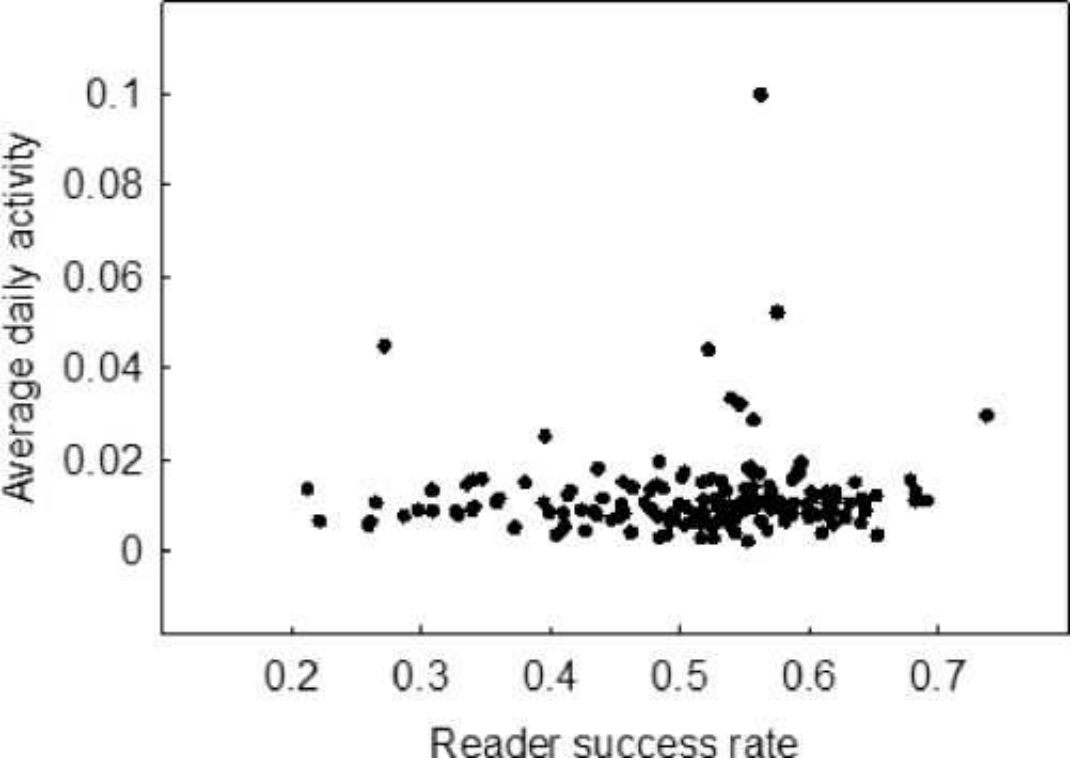

Figure S1. Scatterplot of the estimated reader success rates and the average daily activity levels for individual bees in colony O3. Reader success was not significantly correlated with activity level.

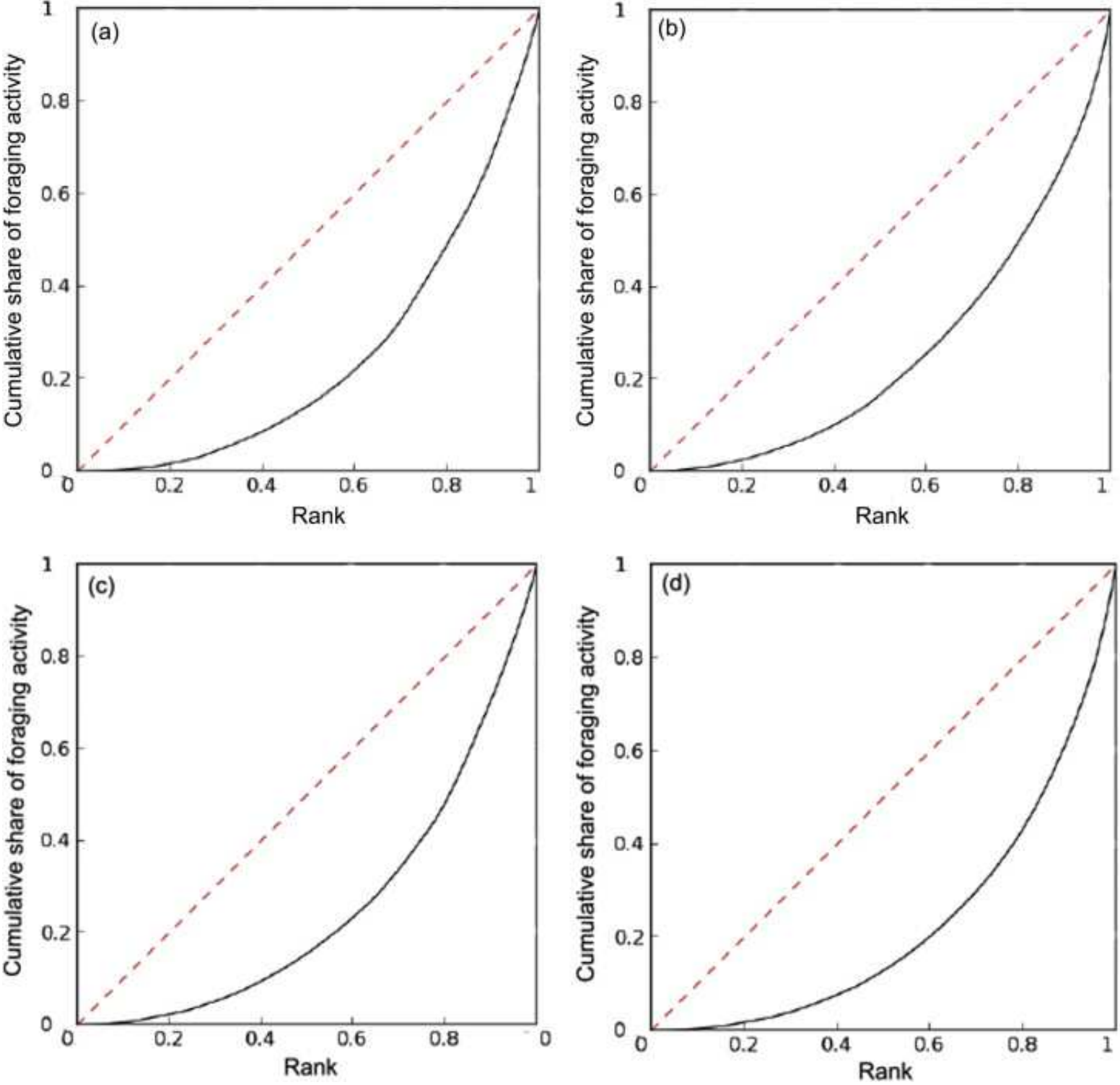

Figure S2. Lorenz curve plots of cumulative share of foraging activity for colonies O1, O2, E1 and E2 (a, b, c and d, respectively). Individual bees were ranked by their contribution to total colony activity in ascending order, and the fraction of each bee's contribution to the total activity of the colony was cumulatively plotted to produce the curve. The red dotted line represents the plot that would result if the contributions of all individuals were perfectly equal. The Gini coefficient is proportional to the area between the curve and the red dotted line.

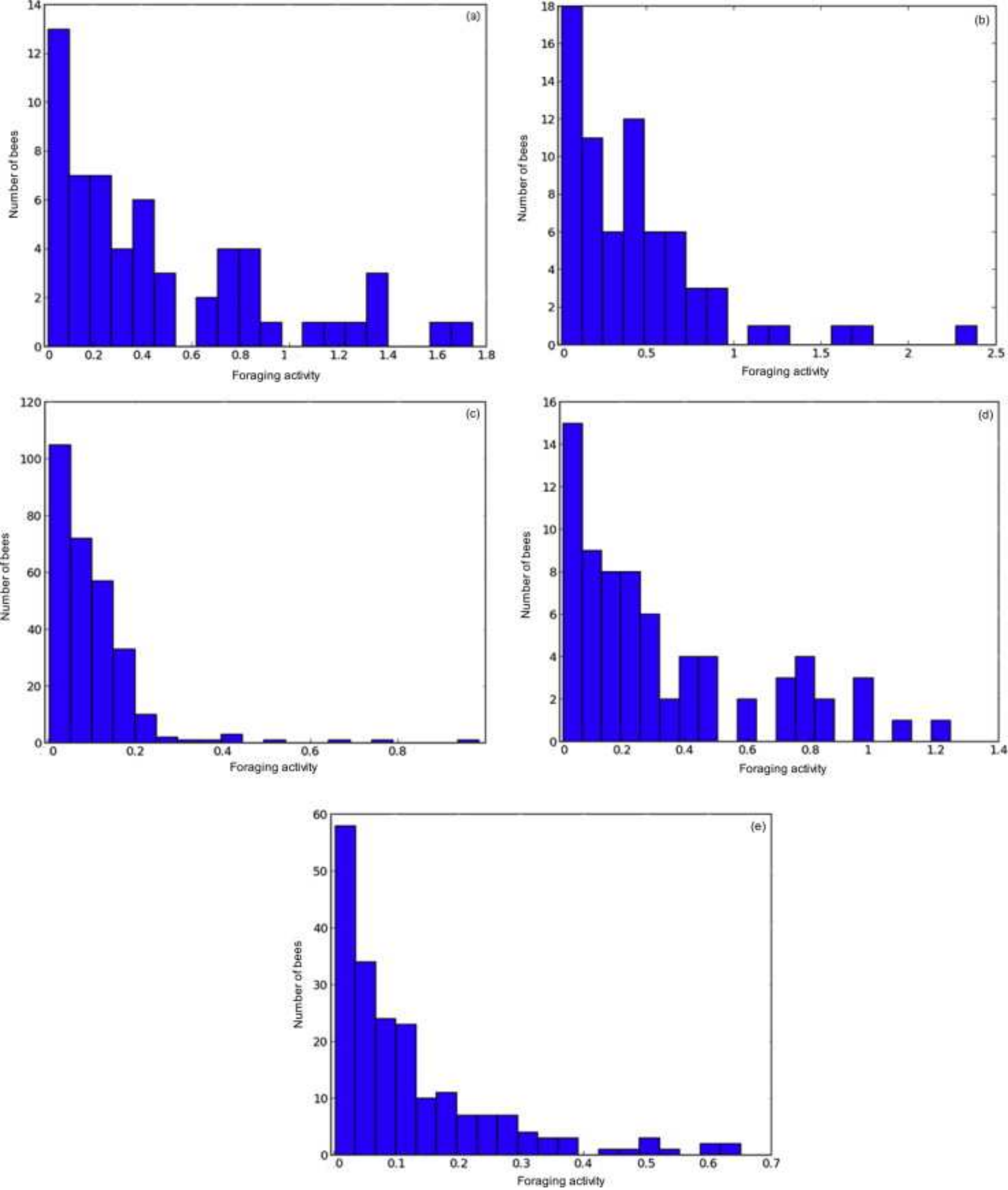

Figure S3. Contributions of individual bees to the total foraging activity in colonies O1, O2, O3, E1 and E2 (a, b, c, d and e, respectively). Values on the X axis represent normalized relative contributions to total colony foraging activity, not numbers of counts or trips.

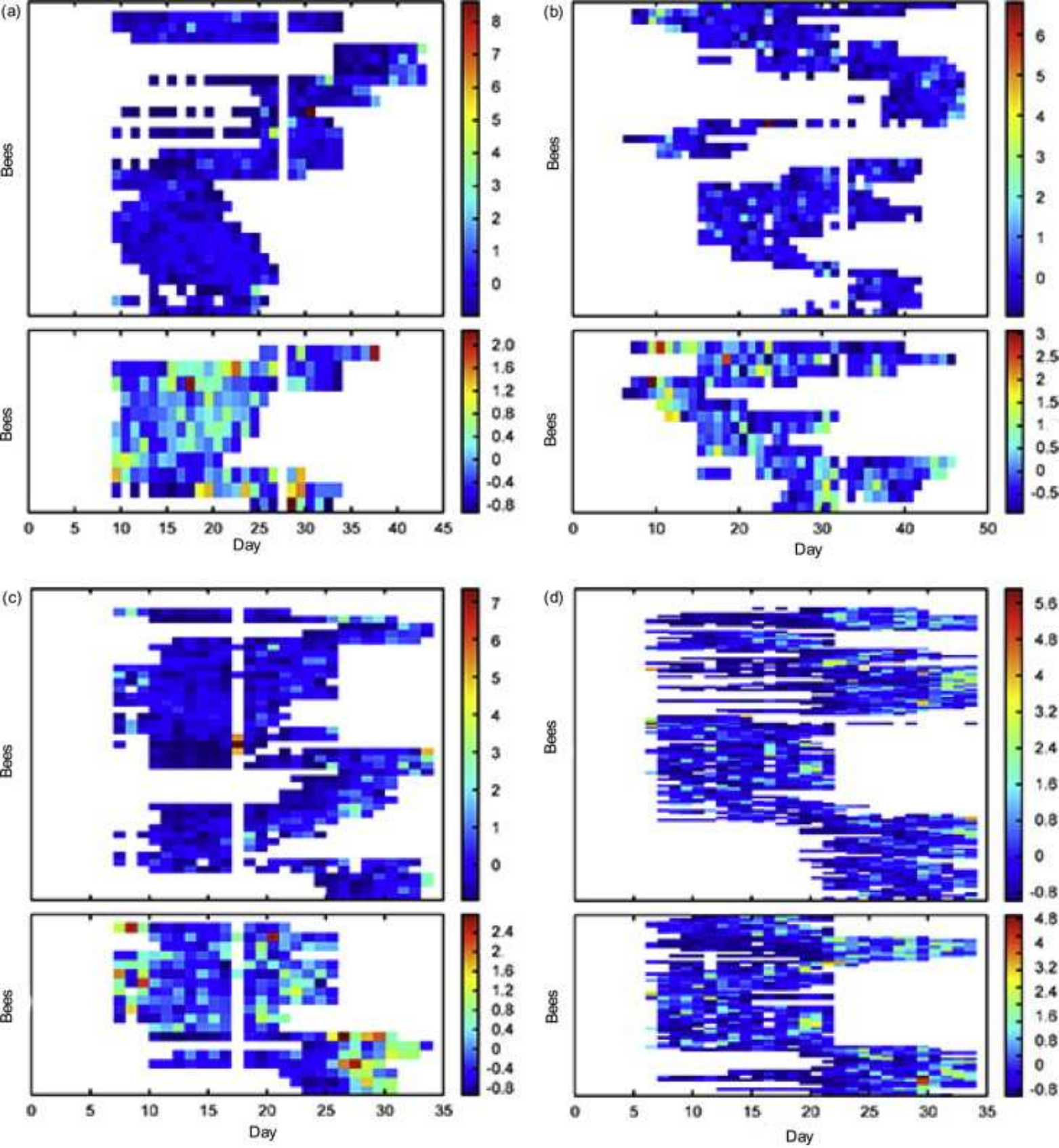

Figure S4. Heat maps of deviations in daily activity level for individual bees in colonies O1, O2, E1 and E2 (a, b, c and d, respectively). Each row represents data for an individual bee and each column corresponds to 1 day of the experiment. Rows are ordered by similarity of the bees' daily activity levels. The upper plots in (a)–(d) show all bees in the analysis, while the lower plots show only the individuals in the top 20% of bees ranked by activity level on days 10, 20 and 30.