Rapid behavioral maturation accelerates failure of stressed honey bee colonies

Clint J. Perry\textsuperscript{a,b,1}, Eirik Sovik\textsuperscript{a,c,1}, Mary R. Myerscough\textsuperscript{d}, and Andrew B. Barron\textsuperscript{a,2}

\textsuperscript{a}Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia; \textsuperscript{b}School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, United Kingdom; \textsuperscript{c}Department of Biology, Washington University in St. Louis, St. Louis, MO 63130; and \textsuperscript{d}School of Mathematics and Statistics, The University of Sydney, Sydney, NSW 2006, Australia

Edited by Gene E. Robinson, University of Illinois at Urbana–Champaign, Urbana, IL, and approved January 21, 2015 (received for review November 18, 2014)

Many complex factors have been linked to the recent marked increase in honey bee colony failure, including pests and pathogens, agrochemicals, and nutritional stressors. It remains unclear, however, why colonies frequently react to stressors by losing almost their entire adult bee population in a short time, resulting in a colony population collapse. Here we examine the social dynamics underlying such dramatic colony failure. Bees respond to many stressors by foraging earlier in life. We manipulated the demography of experimental colonies to induce precocious foraging in bees and used radio tag tracking to examine the consequences of precocious foraging for their performance. Precocious foragers completed far fewer foraging trips in their life, and had a higher risk of death in their first flights. We constructed a demographic model to explore how this individual reaction of bees to stress might impact colony performance. In the model, when forager death rates were chronically elevated, an increasingly younger forager force caused a positive feedback that dramatically accelerated terminal population decline in the colony. This resulted in a breakdown in division of labor and loss of the adult population, leaving only brood, food, and few adults in the hive. This study explains the social processes that drive rapid depopulation of a colony, and we explore possible strategies to prevent colony failure. Understanding the process of colony failure helps identify the most effective strategies to improve colony resilience.

Significance

Honey bee colony death rates are unsustainably high. While many stressors have been identified that contribute to this problem, we do not know why colonies transition so rapidly from a state of apparent health to failure. It is well known that individual bees react to nutritional and pathogen stresses by foraging precociously: our study explains how colony failure arises from the social responses of individual bees to stress. We used radio tracking to monitor performance of bees and found that workers who begin foraging prematurely perform very poorly. This compounds the stresses on the colony and accelerates failure. We suggest how colonies at risk can be identified early, and the most effective interventions to prevent failure.


\textsuperscript{1}C.J.P. and E.S. contributed equally to this work.

\textsuperscript{2}To whom correspondence should be addressed. Email: andrew.barron@mq.edu.au.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1422089112/-/DCSupplemental.
dependent. We then constructed a mathematical model of colony demography inspired by Khoury et al. (34, 35) to consider how this new information might change trajectories of colony growth or decline in chronically stressed colonies. We present an explanation for why colonies appear to change so rapidly from conditions of apparent health to colony failure, propose how to identify colonies at risk for failure, and investigate possible strategies to prevent colony failure.

Results

The RFID data showed when a bee was inside the hive and when outside, and therefore the time spent on orientation and foraging activities (Fig. 1). Foraging is typically preceded by nonforaging orientation flights (36, 37). The average cumulative duration of orientation flights has been estimated at 30.89 min (36); therefore, for the purpose of this study, we classed bees that had spent >30 min outside the hive as foragers.

To experimentally induce precocious foraging, we created three “single-cohort colonies” (SCC) in which all bees were 1-d-old adults at the time of establishment (24, 38, 39), and three colonies that had a normal-worker demography (NDC). SCC (24, 40–42) is an established method for creating a colony with a younger age distribution of foragers. It has been argued that, for many social insects, early foraging by individuals might be a response to individual stress resulting in a shift in the demography of the foraging force (18); SCC provides a social manipulation to reduce the modal age of foragers by reducing social inhibition independent of any pathogen pressure. As expected, in SCC, more bees began foraging when less than 14 d old than in NDC (Fig. 1). A proportion of bees in SCC also start much later than normal. This may be due to the shift in colony needs within the hive once it commences brood production and bees are needed for nursing roles until the first new generation of bees has emerged (Fig. 1).

In both SCC and NDC, foraging performance varied with the age at which bees began foraging. Bees’ survival per hour of time spent outside the hive (Fig. 2A and B) and number of completed trips per bee (Fig. 2C and D) were greatest for bees that began foraging at about 14 d old. Polynomial survival regression (Fig. 2A and B) accounting for colony type and replicate (colony type was treated as a fixed factor, whereas replicate was included as a random factor) showed a significant relationship between age at onset of foraging and total time spent outside the hive ($\chi^2 = 34.04, P < 0.001, n = 749$).

The consequences of precocious foraging were severe. Bees that began foraging when <14 d old spent less time outside the hive, completed fewer flights, and performed longer foraging trips than bees that began foraging at ≥14 d (Fig. 2E–G and Table S1). In both SCC and NDC, the likelihood of surviving past 30 min of flight activity (and becoming a forager) increased as age of first flight increased (Fig. 3 and Table S2).

Modeling Consequences of Precocious Foraging for Colony Function.

Precocious foraging is a reaction to various acute stressors (5, 16–18, 23, 24), which may be adaptive, as it rapidly replaces any losses to the foraging force to enable more food gathering. There is a risk, however, that chronic stress could lead to a progressively younger and less effective foraging force. To examine the consequences of chronic stress on the colony, we developed a compartment model of honey bee population dynamics, implemented as a set of delay differential equations, where survival and food collection per forager varied with age (Fig. 4).

Modeling forager performance and survival as age dependent caused a dramatic change in trajectories of colony growth and
Fig. 3. Proportion of bees that successfully accumulate more than 30 min of time outside the hive plotted against age of first flight for SCC (A) and NDC (B). Summary of logistic regression analysis of these data in Table S2. Overlaid line is fit from logistic regression analysis of mortality during the first 30 min outside colony and age of first flight, green area is 5E of fit (Table S2).

Fig. 4. Schematic representation of the model. Workers develop as brood, emerge as adult hive bees, and mature to foragers. Existing foragers inhibit hive bees from becoming foragers by social inhibition (20, 49). Purple lines represent consumption of food. Food also influences brood numbers indirectly because, in periods of low food influx, workers will cannibalize larvae (purple dotted line). Death rates during the transition from hive bee to forager, forager death rate, and the rate of food collection by foragers (indicated by red arrows) were functions of the age at onset of foraging. “Capped brood” appears in a box with a dashed border because it is not modeled explicitly, but the period that brood spends as capped pupae is modeled by a delay in the model between the time a larva enters pupation and emergence as an adult.
honey (carbohydrate) into a single category of food because too few field data exist on the fluxes of these in hives to consider them separately. A colony with abundant residual honey stores could still be nutrient-limited and fail to produce brood if it had no pollen. Better field data on the consequences of protein and carbohydrate limitation on colonies would certainly further improve our understanding of how nutritional factors contribute to colony growth and failure.

Effective interventions to stop colony failure hinge on early identification of vulnerable colonies. Beekeepers usually rely on snapshot assessments of brood production or colony honey stores to assess colony health, but our modeling suggests these parameters would be slow to react to a change in forager mortality (Fig. 5 A–D). Rather, our modeling suggests that forager mortality rate and age at onset of foraging may provide the best information on colony condition (Fig. 5 A–D).

Understanding the social dynamics that drive colony population decline is essential if effective preventative measures are to be developed. Our model framework allows exploration of the impacts of possible treatments. Here we focused on three (Figs. 7 and 8): supplemental feeding (either in the initial condition or progressive food supplementation throughout the simulation), adding brood, and blocking the precocious foraging response to stress. The latter might be achieved by supplementing colonies with pheromones that inhibit foraging in young bees (20). Of these possible treatments, progressive supplemental feeding was clearly the most effective in delaying or preventing colony population collapse (Fig. 8B). Adding brood also had a positive effect when a hive was marginal, but too much brood accelerated colony failure, because demands of the brood increased nutritional stress on the colony (Fig. 7A). Slowing worker behavioral development to prevent precocious foraging (20) was not as effective, because this strategy also compromised food collection (Fig. 8 C and D).

Our modeling framework clarifies why honey bee colonies might collapse and refines hypotheses for how it might be prevented, but experimental studies are urgently needed to test the validity of these hypotheses. We recognize that there are many different stressors impacting bee colonies, and each has specific modes of action and symptoms. We do not try to capture this detail in our simple modeling approach, but our objective is to provide an overview of how colonies’ internal demographic processes might generally react to stressors.

Given the difficulties beekeepers have in identifying stressed colonies while there is still time to intervene, better strategies to identify vulnerable colonies and reduce colony losses are urgently needed. Our findings propose an explanation for the enigmatic phenomenon of rapid colony population collapse. In situations of chronically elevated forager loss, the social mechanisms that normally stabilize colony function instead accelerate colony failure by causing a destructive positive feedback response that increases forager mortality and decreases forager age, resulting in colony population collapse. Monitoring rate of forager mortality may give a more immediate assessment of colony health than assessments of brood and stored food.

Materials and Methods

Experiments were carried out between February 2012 and May 2013. Honey bees (Apis mellifera) were obtained from research apiaries maintained at Macquarie University (Sydney, Australia). Bees were housed in four-frame nucleus hives each containing ~3,000 bees located inside a laboratory building but connected to the outside via a custom-designed entrance that separated entering and exiting bees into different channels, each with their own entrance. Single SCC and NDC colonies were constructed simultaneously and observed for 40 d. The experiment was replicated three times. Bees and brood to establish SCC and NDC colonies were sourced from the same seven research colonies. Each paired SCC and NDC was provided with new young sister queens. For NDC, ~3,000 bees (estimated by weight) were shaken off brood and honey combs from donor colonies into the nucleus hive box, which was then sealed with mesh and placed in a dark room at 24 °C for 2 d before connecting to the entrance tunnel and adding a new queen. SCCs were constructed from ~3,000 bees that were within 24 h of emergence from brood frames. To obtain newly emerged bees, brood combs were removed from research colonies and placed in an incubator maintained at 32 °C and 37% humidity overnight. Newly emerged bees were brushed from frames the following morning.

RFID System. Between 500 and 1,000 Radio Frequency Identification (RFID; Invengo Technology) tagged bees were introduced to each colony. RFID were glued to the dorsal thoraxes of newly emerged bees within 12 h of emergence. Each nucleus colony was equipped with two RFID antennae placed within a modified entrance to monitor each of the entering and exiting channels.

Fig. 5. The effects of stress on the model colony shown as plots of populations of uncapped brood (dotted line), hive bees (dash-dot black line), and foragers (dashed line); food (solid line) and age at onset of foraging (dashed red line) against time for different values of the underlying death rate. Death rate is expressed as the ratio between death rate of the simulated hive and a healthy hive (m). All hives start with 1 kg of food, 16,000 hive bees, 8,000 foragers, and no brood. (A) Plot for a healthy hive (m = 1); (B) m = 1.6; (C) m = 1.9. At this value, the hive will collapse eventually but not when t < 150 days; (D) m = 2.0. For this death rate, the forager population reaches zero at about t = 99 d. At about 3 wk before collapse, the colony in D had 8,250 uncapped brood items, 6,780 hive bees, and 1,780 foragers. The mathematics are such that modeling could not continue beyond the point of zero foragers. Note that A and B are on a different vertical scale to C and D.

Fig. 6. (A) Plot of the age at onset of foraging as a function of the death rate of foragers as a function of time for different values of m (that is, the ratio between death rate of the simulated hive and a healthy hive). Each hive started with 1 kg of food, 16,000 hive bees, 8,000 foragers, and no brood, and the simulation was run until t = 300 d or the hive collapsed, whichever occurred sooner. The same values of m were used as in Fig. 5 A–C: solid line, m = 1; dotted line, m = 1.6; dashed line, m = 1.9. Each red cross is a time point at an integer value of t. The black cross is the initial value, and the black dot is the median value over all integer values of t. (B) Plot of the median age at onset of foraging (illustrated for three different values in A) as a function of the ratio of death rate of the simulated colony to the death rate of the healthy hive (m). The median age of onset of foraging drops rapidly for values of m, that produce colonies that are close to collapse. When the colony collapses, the median age of onset of foraging is 2 d, which is the minimum allowed in the model.
Contour plots that show time to collapse as a function of $m$, beyond 2 kg made a negligible difference to the results. 

$\gamma$ is the quantity of food collected on a single trip (expressed in grams) and $M(a)$ is the number of trips that a forager makes each day as a function of age at onset of foraging, $a$; $\gamma_1$ and $\gamma_2$ are the average weight of food consumed per day by an individual adult bee or brood item, respectively. 

The rate of change of the population of uncapped brood is denoted by $B$, and the weight of stored food by $F$. 

The rate of change of stored food is given by the rate that food is collected minus the rate at which it is consumed by adult bees and brood, 

$$ \frac{dB}{dt} = \frac{H}{C_2} \frac{a}{\alpha + \beta} \left( F + \frac{b}{c + F} + 1 \right) - \gamma_2 B. $$ 

where $\alpha = 2$ is the minimum age at which hive bees can become foragers in this model.

The first term on the right-hand side models the production and survival of uncapped brood, which depends on the queen’s laying rate $L$ and the amount of stored food and the number of hive bees. Here $b$ and $c$ are constants. Brood leaves the uncapped brood class at a rate of $\gamma$ when it pupates.

The rate of change of the hive bee population is given by 

$$ \frac{dH}{dt} = \frac{H}{C_1} \frac{a}{\alpha + \beta} \left( F + \frac{b}{c + F} + 1 \right) - \gamma_1 H. $$ 

The first term models the emergence of adult bees that entered pupation $r$ days earlier. The second term models the recruitment of hive bees to foraging where 

$$ R(H,F,f) = r_{\text{max}} - r_{\text{cen}} \left( \frac{b}{c + F} + 1 \right) - \frac{1}{F + R}. $$ 

The first two terms in the function $R(H,F,f)$ represent the intrinsic rate of transition from hive bee to forager and the enhanced transition rate when stored food is scarce. The last term represents social inhibition.

The rate of change of the forager population is modeled as 

$$ \frac{dF}{dt} = \frac{1}{T(a) R(H,F,f) H - m_f M(a) F} $$

where $T(a)$ is the transition survival, represented as the proportion of bees that leave the hive bee class that successfully become foragers. $M(a)$ is the death rate of foragers in a healthy hive as a function of age at onset of foraging, $a$, and $m$ is the ratio of the death rate in a hive under stress to the death rate of a healthy hive. 

The expressions for the functions $N(a)$, $T(a)$ and $M(a)$ that depend on the age at onset of foraging are 

$$ N(a) = \begin{cases} 
-0.02(a - 10)^2 + 3.5 & \text{for } a \leq 10 \\
0.015(a - 10)^2 - 3.5 & \text{for } a > 10 
\end{cases} $$ 

$$ T(a) = \begin{cases} 
-0.06(a - 5) + 0.5 & \text{for } a \leq 13.3 \\
1 & \text{for } a > 13.3 
\end{cases} $$ 

$$ M(a) = \begin{cases} 
(a - a_0)^3 + 3 & \text{for } a \leq 13.3 \\
(4.94 + 0.8a)(a - a_0) & \text{for } a > 13.3 
\end{cases} $$ 

RFID tags contained a unique 12-byte hexadecimal identifier that allowed us to individually track the life history of each bee. Data were collected on a PC (Windows) into a .csv file containing data for each successful trip for each bee, including the date and time the bee left the hive, the date and time the bee returned to the hive, and the RFID number for that bee. The RFID entrance tunnel was customized to detect >99% of all tagged bee entries and exits from the colony from adult emergence to the time of last detection. Trips that were filtered from the data included trips that lasted over 8 h (some bees spent one or more nights outside the hive, and these data skewed the comparisons) and those that lasted less than 10 s (trips less than 10 s long were considered misreads by the hardware and occurred infrequently and usually in addition to successfully logged trips). MATLAB (version 8.0) was used to analyze data and create figures.

**Model Formulation.** The population of uncapped brood is denoted by $B$, hive bees by $H$, foragers by $F$, and the weight of stored food by $F$. 

The rate of change of stored food is given by the rate that food is collected by foragers minus the rate at which it is consumed by adult bees and brood, 

$$ \frac{dB}{dt} = \frac{H}{C_2} \frac{a}{\alpha + \beta} \left( F + \frac{b}{c + F} + 1 \right) - \gamma_2 B. $$ 

The population of uncapped brood is denoted by $B$, which depends on the queen’s laying rate $L$ and the amount of stored food and the number of hive bees. Here $b$ and $c$ are constants.
Most parameter values are taken from ref. 34, where they replicate the field data of Harbo (47); that is, \( L = 2000, F = 1.19, s = 5000, \alpha = 1.3, \text{min} = 0.25, \text{max} = 0.3, \gamma = 0.007, \) and \( \gamma = 0.018. \) We also use \( c_r = 12 \) as worker bees puate for 12 d, and \( c_f = 0.033. \) Estimating \( c_f \) is not easy from available data. Harbo (47) does not measure this directly, but from his data, it can be inferred that foragers returned 0.1 g food each day to the colony (47, 48). From our flight data, the mean number of trips per day for all bees (including those that did not complete any foraging trips) was approximately three. Therefore, for our model, we set \( c_f = 0.033. \)

In all of the simulations shown in Fig. 6, the age of onset of foraging changed over time and was a function of the actual death rate of foragers, \( m, M, (A). \) We tracked these changes for \( m_0 = 1, 1.6, \) and 1.9 and recorded the age of onset of foraging for each day, that is, for each integer value of \( t. \) Although the age of onset of foraging did change with time, there were long periods when it changed very little and so we took the median value of age of onset of foraging over the simulations. The mean duration of the typical age of onset of foraging for each value of \( m, \) which is the ratio of the death rate in the simulated hive to the death rate in a healthy hive. Fig. 6 shows three simulations, each corresponding to a different value of \( m, \) and a plot of median age of onset of foraging vs. \( m. \) The age of onset of foraging drops steadily as \( m \) increases and, as \( m \) approaches the critical value where col-lapse occurs before \( t = 300, \) the median age of onset of foraging plummets. Once collapse occurs within 300 d, the median age of onset reduces to 2 d, which is the youngest age that bees are able to commence foraging in this model. The small kink in the curve of Fig. 6B at median age 13.3 is caused by the slope discontinuity of the function \( T(a) \) when \( a = 13.3. \)

We included the effect of extra, in-hive feeding in the model by adding an extra constant term \( C_m \) to the equation for the rate of change of food so that the equation became

\[
\frac{df}{dt} = c_f(N_f(F - g_f(F + H) - \gamma)B + C_f).
\]

The effect of pheromone treatment to mimic the presence of extra foragers and thus inhibit hive to forager transition was modeled by adding a constant \( Q \) in the numerator of the ratio of foragers to total adult bee in the transition function so that

\[
R(H, F, F') = \frac{\alpha}{\alpha + Q + F}(\beta)^{-\frac{1}{\alpha}}(Q + F \div H).
\]

Here \( Q \) effectively represents that number of foragers that the hive bees perceive to have been added due to the increased concentration of the social inhibition pheromone (Fig. B C and D).