

Altruistic self-removal of health-compromised honey bee workers from their hive

By: Olav Rueppell, M.K Hayworth and N.P. Ross

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Abstract:

Social insect colonies represent distinct units of selection. Most individuals evolve by kin selection and forgo individual reproduction. Instead, they display altruistic food sharing, nest maintenance and self-sacrificial colony defence. Recently, altruistic self-removal of diseased worker ants from their colony was described as another important kin-selected behaviour. Here, we report corroborating experimental evidence from honey bee foragers and theoretical analyses. We challenged honey bee foragers with prolonged CO₂ narcosis or by feeding with the cytostatic drug hydroxyurea. Both treatments resulted in increased mortality but also caused the surviving foragers to abandon their social function and remove themselves from their colony, resulting in altruistic suicide. A simple model suggests that altruistic self-removal by sick social insect workers to prevent disease transmission is expected under most biologically plausible conditions. The combined theoretical and empirical support for altruistic self-removal suggests that it may be another important kin-selected behaviour and a potentially widespread mechanism of social immunity.

Keywords: altruism | *apis mellifera* | colony collapse disorder | host–pathogen interactions | hygienic behavior | immune function | kin selection | longevity | mortality | social evolution | suicide | honey bees | biology

Article:

Introduction

Kin selection theory predicts altruistic behaviour to evolve when its inclusive fitness benefits outweigh its costs (Hamilton, 1964). Kin selection has led repeatedly to the evolution of complex insect societies characterized by various forms of altruistic behaviour. Most fundamentally,

nonreproductive workers support their reproductive kin by altruistic food sharing, nest maintenance and allo-parental brood care, benefitting colony productivity and survival (Oster & Wilson, 1978). However, more extreme forms of altruistic behaviour exist, in the form of adaptive suicide, which has been documented in many social insects in the context of colony defence. For example, honey bee workers sting vertebrates to defend their colony (Hermann, 1984) and specialized ant workers and termite soldiers release defensive secretions by rupturing their own body (Maschwitz & Maschwitz, 1974; Bordereau et al., 1997). Even pre-emptive suicidal behaviour has been described in the ant *Forelius pusillus* (Tofilski et al., 2008).

Another form of altruistic suicide that may be expected based on kin selection is altruistic self-removal of individuals whose presence is harmful to the colony. Accordingly, *Temnothorax* ant workers cease social contact and leave their colony without ever returning when challenged with prolonged narcosis or infection with an entomopathogenic fungus (Heinze & Walter, 2010). Other, circumstantial evidence for self-removal exists in honey bees: diploid males may signal their infertile status at an early developmental stage to be cannibalized and preserve colony resources (Santomauro et al., 2004). Honey bee workers infected with parasitic *Varroa* mites frequently do not return to their hive (Kralj & Fuchs, 2006), and developmentally deformed workers crawl out of the hive (Shimanuki et al., 1994).

The most significant context for altruistic self-removal is presumably the prevention of disease transmission by infected workers because many pathogens can quickly spread through and devastate a colony once they are established (Schmid-Hempel, 1998; Cremer & Sixt, 2009; Wilson-Rich et al., 2009). Diseases transmission in colonies is facilitated by the close physical contact and social feeding of nestmates (trophallaxis). Self-removal and suicide of diseased or parasitized individuals may thus decrease the infection risk for surrounding kin (Smith-Trail, 1980). However, demonstrations of workers merely leaving (Woyciechowski & Kozłowski, 1998; Woyciechowski & Moron, 2009) and not returning to their hive (Kralj & Fuchs, 2006; Heinze & Walter, 2010) when compromised provide incomplete evidence for altruistic self-destruction because diseased workers could simply show stress-induced foraging (Schulz et al., 1998) and loss of orientation abilities (Kralj & Fuchs, 2006).

Another complication for testing whether diseased individuals adaptively respond by abandoning their colony is added by potential host manipulations of the pathogen. Parasites frequently manipulate the behaviour of their hosts to increase transmission chances to the next host (Moore, 1995; Adamo, 2002). For example, ants do not return to their colony but expose themselves in the environment if infected by larval flatworms (Carney, 1969) or parasitic fungi (Andersen et al., 2009), enhancing transmission of the pathogen. Therefore, behavioural changes of social

insects that result from an infection can reflect either a successful host manipulation by the pathogen or an adaptive response by the host (Moore, 1995; Poulin, 1995), and the outcome of an experimental infection is unpredictable. Surrogate treatments that realistically simulate serious disease but do not affect the animal in other ways are needed to provide evidence for adaptive self-removal as an immune defence strategy of social insects at the colony level (Cremer & Sixt, 2009). Studies that combine surrogate treatments with real pathogen infections have been successfully performed (Heinze & Walter, 2010) but ultimately rely on the effect of the surrogate treatment to exclude the possibility of host manipulation (Chapuisat, 2010).

Novel pathogens most likely enter social insect colonies via returning foragers (Schmid-Hempel, 1998). To extend the empirical support for adaptive self-removal of compromised members of social insect societies, we therefore specifically tested the prediction that artificially compromised honey bee foragers abandon their normal social function and remove themselves promptly from their colony, preventing the intra-colonial spread of a potentially acquired pathogen. We found that two experimental manipulations, CO₂ narcosis and hydroxyurea feeding, led to elevated forager mortality, foragers permanently leaving their colony, and multiple, more specific behavioural changes that were consistent with each other and the notion of altruistic self-removal. The measured behavioural response variables explicitly distinguished a specific cessation of the foragers' social role from a general loss of functionality. We corroborate these experimental results with a brief theoretical analysis, demonstrating widespread selection for altruistic self-removal of social insect workers from their colony when perceiving disease.

Materials and methods

A set of pilot tests was performed to test the efficacy of two experimental treatments to elicit mortality and behavioural changes in honey bee (*Apis mellifera* L.) workers. These two treatments were feeding of the cytostatic drug hydroxyurea (Timson, 1975) and prolonged narcosis with CO₂ (Woyciechowski & Moron, 2009). The methodological details of the first pilot test are described elsewhere (Ward et al., 2008): essentially, 7-day-old workers were fed sucrose solution with hydroxyurea (HU) or a sucrose control, and mortality in the two treatment groups was monitored with or without access to the outside of their hive.

For the pilot study of CO₂ narcosis, 125 seven-day-old workers of mixed European origin were subjected to a previously determined maximal sub-lethal dose of > 95% CO₂ for 120 min. This resulted in the death of 25 bees, and the remainder were individually tagged with coloured,

numbered plastic discs, used for queen identification (BeeWorks, Canada). As control, 75 workers from the same cohort were briefly (< 1 min) anaesthetized and individually tagged. Both groups were introduced simultaneously into a standard, four-frame observation hive. At the time of introduction, the entrance was blocked off. After 16 h, the entrance of the observation hive was opened and connected to an outside, five-metre-long walkway that was covered with wire mesh. The behaviour and survival of all tagged bees leaving the observation hive were monitored for 2.5 h. In a second CO₂ pilot experiment, we retained 26 CO₂-treated bees in a large cage within their colony and monitored survival for 3 days before releasing them and recording their subsequent survival.

After concluding that CO₂- and HU-treated bees promptly leave their colony and do not return (see results), a more complex main experiment was designed to specifically distinguish bee self-removal from other possible reasons of disappearance from the hive, such as a loss of orientation abilities (Kralj & Fuchs, 2006). This main experiment was conducted four times (trials) using separate cohorts of workers of mixed European descent. The four trials were performed at 2-week intervals during May–July 2008. For each trial, frames with mature worker brood were removed from 8–10 standard hives in the UNCG bee yard. These hives were not inbred or artificially selected, thus representing a random, genetically heterogeneous sample. The brood frames were transferred to an incubator (34 °C, 60% R.H.). All workers that emerged overnight ($n > 500$ for each trial) were paint marked with a specific colour and introduced into an unrelated observation hive that provided the subsequent testing environment. The native workers of this observation hive were not observed in the experiment.

The observation hive was one-sided with three frames and populated throughout the experiment with at least 1000 nonmarked workers and one reproducing queen of mixed European descent. The experiment was started only after a regular brood pattern and foraging activity had been established, allowing individuals unambiguous orientation inside the hive towards the hive exit or centre. The hive had a gated entrance on the top to introduce bees without disturbance to the hive, and it had a single exit on the bottom with access to the outside. The exit runway was split into eight parallel plastic tubes that could each individually be disconnected to facilitate the collection of exiting bees.

For each experimental trial, one marked cohort of > 500 workers matured for 16 days in the hive before all actively foraging bees of that cohort were collected from the hive entrance and randomly assigned to a control, CO₂, or hydroxyurea (HU) treatment group. The control group received a sham treatment. Bees were briefly (< 1 min) anaesthetized with carbon dioxide, tagged with individual numbered plastic tags (BeeWorks, Canada) and placed overnight in a

plastic storage container with air holes, a moist paper towel, and ad libitum sucrose. Powdered sucrose was mixed with water until it attained a dough-like consistency, known as queen candy (Laidlaw & Page, 1997).

A similar procedure was used for the two experimental groups. However, in the first group, hydroxyurea (Sigma, MO) was mixed with the queen candy provided to the bees (30 mg hydroxyurea/gram of queen candy). In the CO₂ group, bees were anaesthetized in > 95% carbon dioxide for 105 min, and subsequently maintained under control conditions. Treated bees were also marked with individual numbered plastic tags and reintroduced together with the control bees into the hive during the next day. Before introduction of the bees, the hive exit was blocked to prevent any immediate hive exits of the experimental bees. It remained blocked overnight to allow 12 h for the treatments to take effect and for any potential effects of reintroducing the experimental bees into their hive to subside.

Any dead, marked bees were recorded and removed before the hive exit was opened and the experimental observations were started. These recordings were used to compute initial mortality rates by dividing the number of recorded dead bees by the total number of bees in each treatment group. After opening the hive exit, the first recorded variable was the time until individual bees attempted to leave the hive (Departure time; Fig. 1). Each exiting, tagged bee was collected and transferred into a preweighed vial to determine its exit weight on a Mettler Toledo AX105 analytical scale. Each weighed forager was then placed individually onto an artificial feeder, containing 30% sucrose solution. It was allowed to feed there ad libitum until it started to flap its wings or crawled away. When it showed these signs of departure, the bee was collected and weighed again. For each bee, Feeding time 1 was quantified as the total time spent on the feeder and Intake 1 by subtracting the weight when exiting the hive from the weight when departing from the feeder. After the second weighing, each bee was immediately placed back into the hive through the top opening. The subsequent time that each bee spent in the hive before its next exit attempt was recorded as Hive time 1. When exiting, the bee was again collected and weighed to measure any in-hive food transfers (Transfer 1). Subsequently, the whole observation cycle as described above was repeated to determine a second Feeding time 2, Intake 2, Hive time 2 and Transfer 2, until the bee left the hive for the third time. This third exit was recorded and the hive exit weight determined, but the bee was placed back into the hive exit from where it could directly leave the hive to the outside.

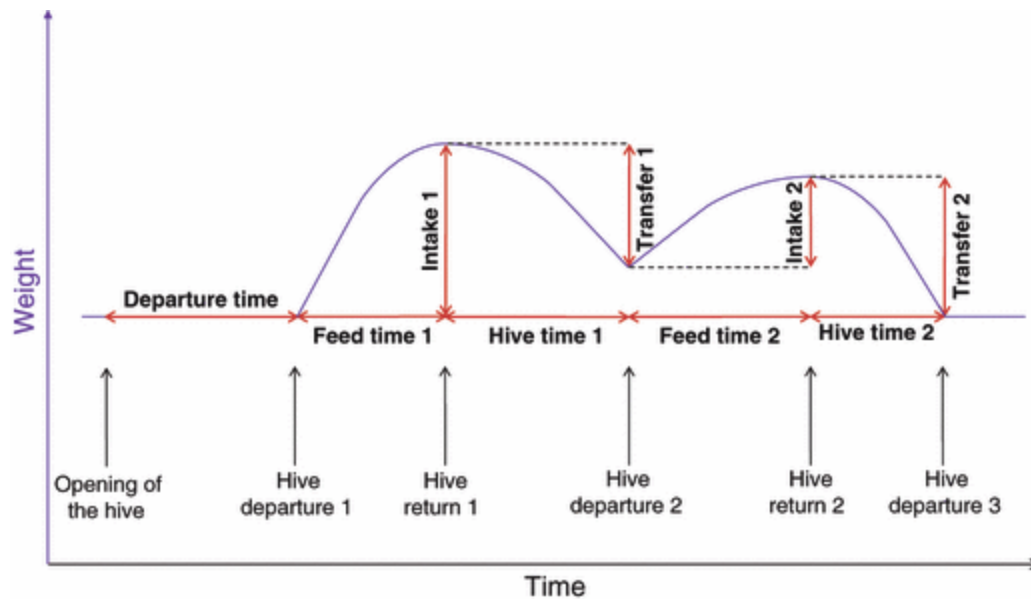


Figure 1. Overview of the design of one trial of the main experiment to measure behavioral responses in honey bee workers of compromised health. Four trials with separate cohorts of workers were conducted. All behavioral response variables are boldfaced and indicated by brackets [in print]/red arrows [online]. The ordinate describes the weight of a studied worker and the abscissa the experimental time. The dotted curve [in print]/blue line [online] represents data of one hypothetical bee for illustration purposes.

All behavioural observations for each of the four trials were made during 1 day. The data for all nine variables (Fig. 1) conformed to parametric assumptions and were subsequently analysed with mixed-effect anovas (trial as random factor, treatment as fixed factor), or single, fixed-effect anovas (treatment as fixed factor) with Dunnett's T3 post hoc tests in spss™ (version 14.0, SPSS Inc., Chicago, IL, USA). In addition, we evaluated whether in-hive food transfers relative to the previous food uptake (transfer 1/intake 1 and transfer 2/intake 2) were significantly affected by treatment with a Kruskal–Wallis anova. We also analysed the number of hive departure attempts per individual for bees that survived at least until the end of the observation day with a Kruskal–Wallis anova. Survival of bees at different time points of the experiment was compared among the three treatment groups with χ^2 -test, including data from a final census of all remaining tagged bees 6 days after the observations.

Experimental results

The pilot studies showed that hydroxyurea-treated (HU) bees died significantly earlier than control bees, both with and without access to the outside (Ward et al., 2008). However, with access to the outside, 90% of the HU bees were missing from the hive within 2 days, whereas only 50% of the caged HU bees were missing after 2 days, and 90% mortality of caged HU bees was only reached on the 4th day. The initial CO₂ manipulation experiment showed that significantly more treated workers (36/100 individuals) than control workers (15/75) left the hive (Fisher's exact $P = 0.029$). Mortality was significantly ($P = 0.005$) higher in treated workers (14/100) than in control workers (1/75). The treated workers also left significantly faster ($Z(15,36) = 5.3, P < 0.001$) after the hive was opened. The second, caged CO₂ cohort experienced 23.1% mortality (6/26) over 2 days directly after treatment. In contrast, 95% of the bees (19/20) disappeared from the hive on the 3rd day, directly after their release from their cage within the hive, which represents a significant difference in mortality (Fisher's exact $P < 0.001$).

In the main experiment, the CO₂ and HU treatments also decreased survival until the end of the experiment relative to the control group (Fig. 2, overall $\chi^2 = 52.3, d.f. = 2, P < 0.001$; Bonferroni-corrected Fisher's exact tests of between-group differences: $P(\text{CO}_2 \text{ vs. control}) < 0.001, P(\text{HU vs. control}) < 0.001, P(\text{CO}_2 \text{ vs. HU}) = 0.714$). This effect persisted until 6 days after the observations ($\chi^2 = 34.9, d.f. = 2, P < 0.001$; between-group differences: $P(\text{CO}_2 \text{ vs. control}) < 0.001, P(\text{HU vs. control}) < 0.001, P(\text{CO}_2 \text{ vs. HU}) = 1.0$). Before the opening of the hive for the experimental observations, mortality was significantly higher in the CO₂ treatment group than in both other groups (Fig. 2; $\chi^2 = 27.1, d.f. = 2, P < 0.001$; between-group differences: $P(\text{CO}_2 \text{ vs. control}) < 0.001, P(\text{HU vs. control}) < 0.001, P(\text{CO}_2 \text{ vs. HU}) = 1.0$). Thus, there was a significant increase in overall mortality rate in the experimental groups relative to the control group after giving individuals the opportunity to leave the hive (CO₂ vs. control: $\chi^2 = 46.8, P < 0.001$; HU vs. control: $\chi^2 = 44.3, P < 0.001$), with no significant difference existing between treatments (HU vs. CO₂: $\chi^2 = 1.1, P = 0.295$). Among individuals that survived until the end of the experiment, the foragers from the control group attempted to leave the hive significantly less often than the foragers from the CO₂ (Mann-Whitney test: $Z = 2.3, n_{\text{control}} = 81, n_{\text{CO}_2} = 35, P = 0.024$) and the HU ($Z = 2.8, n_{\text{control}} = 81, n_{\text{HU}} = 43, P = 0.006$) treatment groups (overall Kruskal-Wallis anova: $\chi^2 = 10.4, d.f. = 2, P = 0.006$).

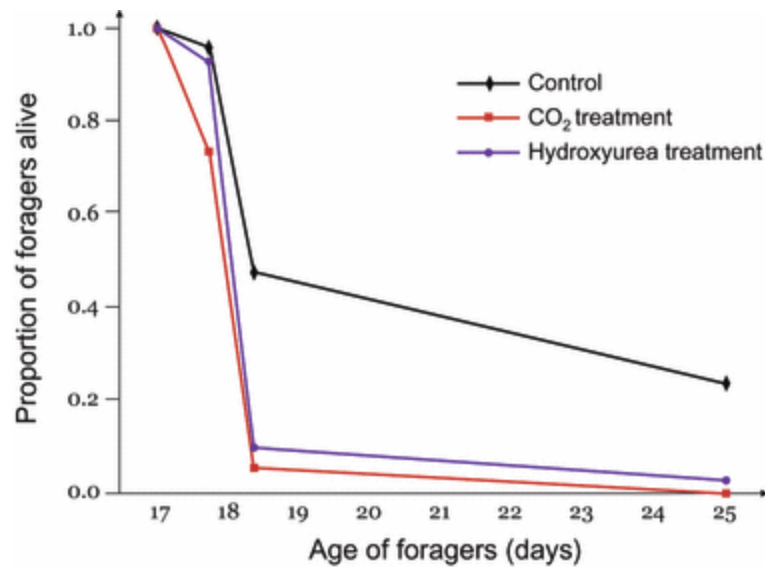


Figure 2. Survival of experimental bees after treatments were administered on day 17. All behavioural observations were made on day 18 after opening the hive exit. Thus, the deaths recorded after that date mostly include foragers that leave the hive repeatedly after being fed and placed back into the hive.

Treatments significantly affected most of the behavioural response variables, but no significant trial or treatment-by-trial interaction effects were detected after Bonferroni correction (Table 1). Therefore, the pooled data from all four trials were subsequently analysed to assess treatment effects. Both groups of compromised workers attempted to depart their hive significantly earlier than control workers (Fig. 3a; $F(2,199) = 24.8$, $P < 0.001$, post hoc $P(\text{CO}_2\text{vs. control}) < 0.001$, $P(\text{HU vs. control}) < 0.001$), with no significant difference between the CO₂ and HU treatments ($P = 0.699$). When placed on a feeder, food intake by compromised foragers was significantly less than that of control foragers during the first and second interval (Fig. 3b; intake 1: $F(2,206) = 25.7$, $P < 0.001$, post hoc $P(\text{CO}_2\text{vs. control}) < 0.001$, $P(\text{HU vs. control}) < 0.001$; intake 2: $F(2,133) = 27.7$, $P < 0.001$, post hoc $P(\text{CO}_2\text{vs. control}) < 0.001$, $P(\text{HU vs. control}) < 0.001$), with no significant differences between the experimental treatments in either interval (intake 1: $P = 0.850$; intake 2: $P = 0.763$). Similarly, the time that an individual took to leave the hive again after being placed back was significantly decreased relative to the control by both treatments in both intervals (Fig. 3c; hive time 1: $F(2,108) = 27.6$, $P < 0.001$, post hoc $P(\text{CO}_2\text{vs. control}) < 0.001$, $P(\text{HU vs. control}) < 0.001$; hive time 2: $F(2,74) = 15.3$, $P < 0.001$, post hoc $P(\text{CO}_2\text{vs. control}) < 0.001$, $P(\text{HU vs. control}) < 0.001$), but CO₂ and HU treatments did not significantly differ in either interval (hive time 1: $P = 0.198$; hive time 2: $P = 0.934$). Concomitantly, the amount of food transferred to nestmates while in the hive was significantly lowered by both experimental treatments in both intervals (Fig. 3d; transfer 1: $F(2,108) = 51.8$, $P < 0.001$, post hoc $P(\text{CO}_2\text{vs. control}) < 0.001$, $P(\text{HU vs. control}) < 0.001$; transfer 2: $F(2,73) = 6.0$, $P = 0.004$, post hoc $P(\text{CO}_2\text{vs. control}) = 0.004$, $P(\text{HU vs. control}) = 0.050$). The effect of the experimental treatments in both intervals was statistically indistinguishable (transfer 1: $P = 0.401$; transfer 2: $P = 0.401$).

= 1.0). The amount of food transferred was not significantly different from zero for the CO₂ treatment group in the first interval and for both treatment groups in the second interval.

Treatment even affected the transfer amounts relative to the previous uptake as predicted (control > HU > CO₂), although this trend was not significant in the second interval (transfer 1/intake 1: $\chi^2 = 31.9$, d.f. = 2, $P < 0.001$; transfer 2/intake 2: $\chi^2 = 5.5$, d.f. = 2, $P = 0.064$).

Table 1. Results of mixed two-factorial ANOVAs, testing treatment and trial effects* on behavioural response variables.

Variable	Treatment effect	Trial effect	Treatment x Trial effect
Departure time	$F(2,7.0) = 21.1, P = 0.001$	$F_{(3,6.5)} = 1.0, P = 0.433$	$F_{(6190)} = 1.1, P = 0.365$
Feed time 1	$F_{(2,6.8)} = 1.0, P = 0.415$	$F_{(3,6.2)} = 9.1, P = 0.011$	$F_{(6202)} = 2.2, P = 0.048$
Feed time 2	$F_{(2,6.6)} = 1.3, P = 0.332$	$F_{(3,6.2)} = 8.9, P = 0.012$	$F_{(6135)} = 2.0, P = 0.069$
Intake 1	$F(2,6.8) = 19.3, P = 0.002$	$F_{(3,6.4)} = 4.1, P = 0.063$	$F_{(6197)} = 1.3, P = 0.237$
Intake 2	$F(2,10.0) = 58.8, P < 0.001$	$F_{(3,7.7)} = 3.5, P = 0.070$	$F_{(6124)} = 0.4, P = 0.902$
Hive time 1	$F(2,6.9) = 30.3, P < 0.001$	$F_{(3,7.2)} = 2.4, P = 0.152$	$F_{(6,99)} = 0.8, P = 0.540$
Hive time 2	$F(2,7.6) = 14.9, P = 0.002$	$F_{(3,7.2)} = 1.5, P = 0.303$	$F_{(6,65)} = 0.8, P = 0.603$
Transfer 1	$F(2,15.7) = 151.7, P < 0.001$	$F_{(3,11.6)} = 2.0, P = 0.177$	$F_{(6101)} = 0.2, P = 0.981$
Transfer 2	$F(2,17.3) = 8.8, P = 0.002$	$F_{(3,9.1)} = 3.0, P = 0.086$	$F_{(6,64)} = 0.5, P = 0.822$

*Uncorrected probabilities are listed, significant effects after Bonferroni correction are printed in bold.

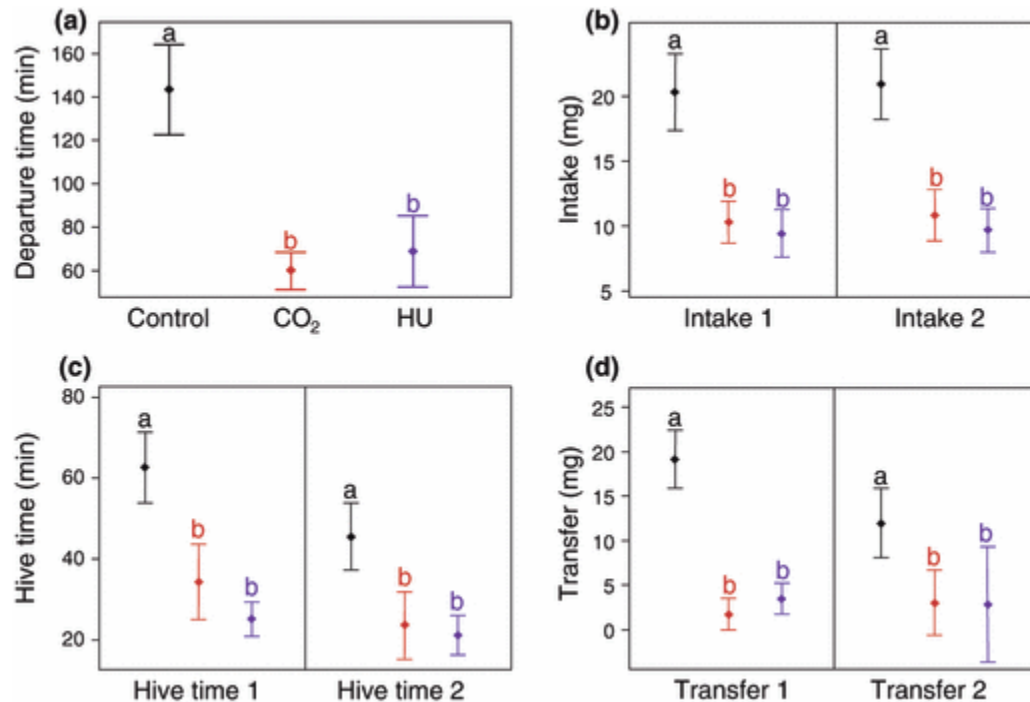


Figure 3. Behavioral differences among experimental treatment groups. Mean values (\pm 95% confidence intervals) are shown for the seven behavioral response variables that showed consistent differences between experimentally treated honey bee foragers (CO₂ narcosis [in red online], hydroxyurea diet [in blue online]) and control [in black online]. Included are the time spent in the hive before (a) and between (c) hive departure attempts, food intake when placed on a feeder (b), and weight lost due to food transfers in their hive (d)

Feeding times were the only variables that did not show a consistent effect of the experimental treatments because only the HU group spend significantly less time feeding during the first interval (feed time 1: $F(2,211) = 4.4$, $P < 0.013$, post hoc $P(\text{CO}_2\text{vs. control}) = 0.993$; $P(\text{HU vs. control}) = 0.016$, $P(\text{CO}_2\text{vs. HU}) = 0.026$; feed time 2: $F(2144) = 0.9$, $P = 0.429$, post hoc $P(\text{CO}_2\text{vs. control}) = 0.705$; $P(\text{HU vs. control}) = 0.946$, $P(\text{CO}_2\text{vs. HU}) = 0.562$).

Epidemiological model

We consider the behavioural choice of a general social insect worker that perceives itself as ill in a simple, linear model. Upon feeling ill, the worker either continues to fulfil its current social function or it removes itself from the colony to prevent spreading its disease, resulting in altruistic suicide. Altruistic self-removal is expected when the inclusive fitness of an individual that removes itself from the colony is on average larger than the fitness of an individual that remains in the colony once it perceives itself as diseased:

$$R + I < P \times T \times F \times N$$

where R is a general measure of the residual contribution of an average individual to colony performance, I is the possible additional fitness gain through subsequent individual reproduction, P is the probability that an individual correctly determines itself as infected, T is the disease transmission probability between two interacting individuals, F is the reduction in colony performance when one colony member is infected, and N is the number of individuals that interact with the focal individual. For most advanced social insects, I is negligible (Bourke, 1988), particularly for a worker that is feeling ill. I can therefore be ignored. The residual contribution of an individual to colony performance (R) depends on whether this individual is healthy or diseased. Thus, the term R needs to be replaced by the residual contribution of a healthy (R_h) and a diseased individual (R_d), weighted by their respective probability:

$$(1 - P) \times R_h + P \times R_d < P \times T \times F \times N$$

The effect of decreasing the residual contribution from a healthy individual (R_h) to that of a diseased individual (R_d) equals the reduction in colony performance when one colony member is being infected. Thus, $F = R_h - R_d$, which leads with a basic transformation from Eqn 2 to:

$$\frac{(1 - P) \times R_h + P \times R_d}{P \times (R_h - R_d)} < T \times N$$

A reasonable approximation of Eqn 3 for P close to 1 is

$$\frac{R_d}{R_h - R_d} < T \times N$$

and if P is small, Eqn 3 can be approximated by

$$\frac{R_h}{P \times (R_h - R_d)} < T \times N$$

Sufficient data on the considered variables do not exist to the best of our knowledge, but plausible value ranges can be estimated as follows. For most insect societies, N will depend on colony size but for all advanced social insect colonies $N > 10$ holds, and for honey bees $1000 > N > 100$ is most likely (Naug, 2008). $R_h > R_d$ because disease is by definition detrimental. If the considered disease is serious, a conservative assumption may be $\frac{R_h}{2} > R_d > \frac{R_h}{10}$ although lower values of R_d , including negative ones because of the cost of corpse removal, are conceivable (Schmid-Hempel, 1998). Equation 4 holds for these assumptions when $T > \frac{1}{N}$ allowing for relatively small transmission probabilities.

R_h and R_d have an upper bound of $\frac{1}{N}$ because N is always smaller than the total colony size (S), and each individual's lifetime contribution to colony performance is $\frac{1}{S}$ on average. The range of P and T is $[0..1]$. It follows that Eqn 5 also holds for most of the plausible parameter space. For example, it would be true for mature honey bee colonies even when the transmission probability T and recognition probability P are both as low as 0.5%. Any values of P , T , N and $(R_h - R_d)$ that are higher than our conservative estimates further strengthen our prediction of altruistic self-removal (Eqn 1). This includes intermediate values of P , which are not covered by Eqn 4 or Eqn 5.

One of the model's simplifications is the assumption of linear relationships. This simplification is justified even when colony performance may be distinctly nonlinear (Oster & Wilson, 1978), because one individual's contribution is sufficiently small for the local performance function to be approached by a linear function. In addition, the model assumes that pairs of individuals interact only once, with a specific T . In reality, individual workers have a high probability of repeated interactions, which increases the transmission probability for each pair of interacting individuals. This effect is trivial for T close to one, but it significantly increases the costs of self-

continuation by increasing the chance of disease transmission when the transmission probability during a single interaction is low. Thus, relaxing this assumption also strengthens our conclusions.

Most importantly, our model neglects the indirect effects of further disease spread through the colony from individuals that have been infected by the focal individual. This nonlinear reinforcement of the disease transmission effect is expected to amplify the negative effect of each initially infected nestmate because it becomes the source of further infections. Further disease spread beyond the primary contacts of the focal individual can only increase F because

R_d will diminish to R'_d by subtracting $\sum_{i=0}^n R_{hi} - R_{di}$, with n = number of indirectly infected individuals. R'_d will become negative for $R_d < n \times (R_h - R_d)$, even without considering the effect of further infections by the indirectly infected individuals. Thus, the biologically plausible potential for indirect disease transmission further strengthens our theoretical conclusions.

Discussion

Altruistic self-removal of moribund workers from their colony has been reported in ants of the genus *Temnothorax* (Heinze & Walter, 2010). We present extensive experimental data from honey bees and theoretical analyses suggesting that this specific, kin-selected behaviour is more common in social insects and not restricted to species with small colonies (Chapuisat, 2010; Heinze & Walter, 2010). Altruistic self-removal presents a new form of altruistic suicide that differs from direct suicidal colony defence because it serves a pre-emptive function, similar to the suicidal entrance closure by *Forelius* ants (Tofilski et al., 2008). However, it may also be interpreted as a special form of hygienic behaviour (Cremer & Sixt, 2009; Wilson-Rich et al., 2009).

Our preliminary experiments showed that experimentally compromised bees promptly exited their colony when given the opportunity without returning. This significantly increased their mortality over bees that were forced to stay in their colony, a finding that mirrors results from *Temnothorax* workers (Heinze & Walter, 2010). In contrast to ants, honey bees fly out of the colony and thereafter observations are limited in a natural context. Thus, we could not exclude the possibility that our preliminary observations were because of stress-induced foraging and loss of orientation abilities (Kralj & Fuchs, 2006). Specifically, CO₂ treatment can severely compromise insect memory functions that are required for orientation (Nicolas & Sillans, 1989). Even though unlikely, this interpretation could also potentially explain the results of

Temnothorax workers predominantly dying outside the nest, far away in their foraging arena (Heinze & Walter, 2010).

Thus, we employed simulated foraging and repeated hive reintroduction of foragers as a more sophisticated experimental design in our main experiment to specifically distinguish the predicted behavioural response of altruistic self-removal from stress-induced foraging and a general loss of functionality. Hydroxyurea and CO₂ resulted in striking behavioural changes that were consistent with each other, the preliminary observations, and our prediction of altruistic self-removal. Relative to controls, the compromised bees left their hive environment more than twice as fast (departure time) and remained only about half as long in their hive after repeatedly feeding and being reintroduced into the hive top (Hive time 1 and Hive time 2). Furthermore, CO₂- and HU-treated bees attempted to leave the hive more often than control bees during the observation period. Antagonistic behaviour by other bees towards the experimental individuals was not observed, and our modified hive exit allowed bees only to crawl out of the hive on their own accord. Thus, the treated workers efficiently oriented towards the hive exit and actively sought out this exit on their own, despite being satiated with sucrose.

Treated and control bees were left on an ad libitum sucrose feeder until they showed no further signs of voluntary food uptake. Sucrose uptake is mediated by the bees' proboscis extension reflex (Bitterman et al., 1983), and responsiveness to sucrose is increased by stress (Pankiw & Page, 2003). Thus, the significantly smaller food uptake by CO₂- and HU-treated bees relative to control bees cannot be explained as a general stress response. In contrast, the results support the notion that treated bees were less inclined to forage than the control bees when leaving the hive. Moreover, treated bees did not transfer significant amounts of food to their nestmates after reintroduction to their hive, in contrast to the normal forager behaviour exhibited by control bees (Seeley, 1989). We cannot exclude the possibility that nectar processors avoided the CO₂- or HU-treated bees (Richard et al., 2008) and prevented them from unloading their food. However, normal foragers that are denied unloading seek out additional nectar processors and do not immediately leave the hive again (Huang & Seeley, 2003). Consequently, failure to unload should delay foragers from exiting the hive. In contrast, the CO₂- or HU-treated bees were exiting the hive much quicker than control bees, which suggests that they had abandoned their social role.

The CO₂ or HU treatments of foragers resulted in remarkably similar changes in behaviour, although the two experimental treatments act very differently. Hydroxyurea is a cytostatic drug that is designed to inhibit ribonucleotide reductase, an essential enzyme for deoxyribonucleotides and DNA synthesis (Yarbo, 1992). Prolonged CO₂ narcosis may also cause high mortality but

many potential mechanisms affecting many tissues are conceivable, because direct effects of CO₂ are generally accompanied by hypoxia and changes in the haemolymph pH (Nicolas & Sillans, 1989). The similarity of the two treatment groups suggests that the behavioural changes represent an unspecific response to poor vitality and declining health. This remains to be further tested in comparative experiments including specific disease agents, for example with *Nosema ceranae* (Chen et al., 2009).

Our experimental studies revealed a suite of behavioural changes in honey bee foragers that are consistent with the hypothesis that workers that feel ill or otherwise compromised actively abandon their social role as foragers and remove themselves from their colony. Our observations are more complex and thus more conclusive than previous studies that demonstrate increasing hive departures of compromised workers and their death outside the colony (Kralj & Fuchs, 2006; Woyciechowski & Moron, 2009; Heinze & Walter, 2010). The putative adaptive value of the behaviour of altruistic self-removal is further supported by our theoretical analyses. The evaluated model is simple but consistently predicts that altruistic self-removal of ill-feeling workers is adaptive if illness has a contagious cause. This conclusion is robust across the parameter space and possible model extensions only strengthened our conclusion. One potential limitation for our model prediction would be a very low value of P , the probability of correct self-diagnosis. Pathogens are selected for disguising themselves until after their infectious stage if their recognition triggers self-removal by social insect workers. The ensuing evolutionary arms race over detection between pathogen and host (Deitsch et al., 2009) could lead to low P .

Altruistic self-removal can only be understood as an adaptation at the colony level because isolated colony members do not normally survive away from their nest. This process shows a functional similarity to programmed cell death of compromised cells in multi-cellular organisms. In both cases, selection at the higher level of biological organization has resulted in altruistic suicide of lower level units (Maynard Smith & Szathmáry, 1995). Thus, altruistic self-removal strengthens the view of a colony as an integrated superorganism (Amdam & Seehuus, 2006; Hölldobler & Wilson, 2008; Cremer & Sixt, 2009). However, the mechanisms of the fundamental behavioural changes involved in altruistic self-removal remain to be studied.

Preventing the spread of diseases that are transmitted by direct contact among nestmates (Schmid-Hempel, 1998; Otterstatter & Thomson, 2007; Naug, 2008; Chen et al., 2009), the benefits of altruistic self-removal outweigh the potential cost of its erroneous execution in individual cases. However, error accumulation at the colony level may result in a large portion of adult workers leaving their colony, resembling the phenomenology of the recently reported colony collapse disorder in honey bees (vanEngelsdorp et al., 2009). Currently, it is not clear

whether a single factor is responsible for colony collapse disorder, although several possible causes have been identified (Cox-Foster et al., 2007; Cornman et al., 2009; Johnson et al., 2009). Novel pathogens, pesticide exposure, or malnutrition could lead to aberrant self-removal behaviour of honey bee foragers, potentially explaining one of the symptoms of the colony collapse.

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