

Soldier-based defences dynamically track resource availability and quality in ants

Scott Powell*, Anna Dornhaus

University of Arizona, Department of Ecology & Evolutionary Biology, Tucson, AZ, U.S.A.

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Specialized defence traits and strategies are crucial in surviving enemy attacks and in resource acquisition. In numerous social insect lineages, soldiers function as specialized defence traits of the colony, but associated defence strategies are poorly known. The turtle ant *Cephalotes rohweri* is an obligate cavity-nesting ant with highly specialized soldiers. To maximize growth and reproduction, colonies must use their limited availability of soldiers to defend multiple cavities. Using laboratory experiments informed by field data, we addressed how soldier 'deployment' across cavities adjusts to changes in cavity availability and quality. From initial field-like conditions, soldier deployment to newly available cavities was rapid, stabilized quickly, and at least doubled the number of cavities defended by each colony. New cavities were defended by fewer soldiers than original cavities still in use. Nevertheless, when new cavities differed in size, an important quality metric, large cavities were used more often and defended by more soldiers than small cavities. Despite these dynamic responses, total soldier deployment to new cavities was limited to an approximately constant proportion (0.4) of overall soldier availability across colonies and resource contexts. Moreover, there was a significant positive relationship between total soldier deployment to new cavities (greater for larger colonies) and both the number of newly defended cavities and their average level of defence. These results demonstrate that colony-wide soldier deployment is dynamic, predictable and context sensitive but ultimately constrained by the availability of soldiers in the colony. Furthermore, the consistently lower number of soldiers in new cavities, which always limits the potential losses to enemies, is concordant with a 'conservative bet-hedging' life history strategy. Broadly, our findings show that a specialized soldier caste can be associated with a far more sophisticated defence strategy than previously recognized. This provides a more complete perspective on the evolution of soldier-based defences in insect societies.

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Specialized defence traits can be key axes of adaptive evolution, because they play a crucial role in surviving enemy attacks and in securing resources (e.g. insects: Gentry & Dyer 2002; vertebrates: Vamosi & Schluter 2004; Marchinko 2009; plants: Agrawal 2007). For any organism, specialized defence traits yield fitness gains through the combination of functional characteristics and associated defence strategies. In some social insects, soldier morphotypes, or 'castes', function as specialized defence traits of colonies that have attained a level of functional integration and conflict resolution comparable to unitary organisms (Pepper & Herron 2008; Powell 2008; Hou et al. 2010; Strassmann & Queller 2010). In these cases, the colony is the adaptive unit, and selection for colony defence can drive the morphology and behaviour of soldiers to such extremes that they can no longer feed themselves (reviewed in: Hölldobler & Wilson 1990; Dornhaus & Powell 2010).

While the basic functional morphology of soldiers is well understood, the defence strategies with which they are associated are not (Dornhaus & Powell 2010).

Soldier defence strategies are defined here as the processes that determine the number of soldiers that meet a given threat. They are critical for fitness because threats faced by a colony can be distributed unpredictably in space and time (Oster & Wilson 1978). Studies of soldier defence strategies are thus needed to fill a significant gap in our understanding of adaptive evolution in derived social organisms. Moreover, the problem of having to meet distributed threats effectively with a limited supply of specialized defence traits has intriguing parallels with animal immune defences (reviewed in: Jean 2001; Schmid-Hempel 2005, 2011) and plant defences (reviewed in: Kaspari & Byrne 1995; Agrawal 2011). Deepened understanding of soldier-based defences in derived social taxa may therefore facilitate comparisons of the evolution of defence across levels of biological organization.

Soldiers have evolved in at least six major social insect lineages (ants: Dornhaus & Powell 2010; aphids: Stern 1994; polyembryonic wasps: Cruz 1981; stingless bees: Grüter et al. 2012; termites:

* Correspondence and present address: S. Powell, George Washington University, Department of Biological Sciences, 2023 G Street NW, Lisner Hall Room 340, Washington, D.C. 20052, U.S.A.

E-mail address: scottpowell@gwu.edu (S. Powell).

Thorne et al. 2003; thrips: McLeish & Chapman 2007). Moreover, they have evolved many times independently in ants, including in some of the most diverse and ecologically important genera (Baroni Urbani 1998; Dornhaus & Powell 2010). Ant soldiers typically develop from totipotent eggs, with the developmental pathways turned on in the larval stage by worker-controlled feeding regimes (Wheeler 1991). Once mature, the specialized soldier morphology is always coupled with specialized behavioural responses to threats (reviewed in Dornhaus & Powell 2010). Soldier defence strategies thus have two main components that may be adaptive: availability and deployment. Availability is defined here as the total number of soldiers in the colony at any given moment, which is the net balance between soldier production and death rates. Deployment is defined as how the available soldiers are dynamically used in space and time.

Examples of soldier availability responding to environmental change are rare in ants (but see Passera et al. 1996; McGlynn & Owen 2002), and this process is always bound by egg-to-adult development times of one to a few months (e.g. 1 month in *Eciton*: Schneirla 1971; 3 months in *Cephalotes*: Creighton 1963; Wilson 1976a). Changes in soldier availability are thus likely to lag behind the rate of change of important environmental contingencies. For instance, more soldiers may be needed to defend a burst of new resources on a timescale of days, and threats from resource competitors can escalate in minutes. Indeed, the likely discrepancy between the rate of change in environmental threats and soldier availability is thought to explain why many social insects have stable, nonplastic soldier production rates (Oster & Wilson 1978). Put simply, if threats change faster than a colony can change soldier availability, selection should favour a stable production rate that provides an adequate number of soldiers for a range of threats. Moreover, stable soldier production at levels that could cover all conceivable threats is likely to be prohibitively expensive in terms of resources needed to produce them and the opportunity cost of reduced worker production (Oster & Wilson 1978). All considered, it is clear that soldier availability and the underlying production rates lack flexibility in ants, even if the degree to which they are adaptive remains an open question (Schmid-Hempel 1992; Dornhaus et al. 2012).

With soldier availability fixed over the timescale of one to a few months, natural selection should shape the evolution of dynamic soldier deployment. More specifically, the number of soldiers deployed to certain locations, and how colony-wide deployment patterns adjust to changing demands for defence, are likely to have significant fitness consequences. This may be particularly true in the acquisition of new resources for future growth, and under

elevated enemy threat. For instance, soldiers are deployed rapidly to newly available nesting cavities in wild *Cephalotes persimilis* colonies, which obligately use multiple cavities (Powell 2009). But how is this deployment to new locations balanced against defence of existing locations? Similarly, ant soldiers are known to be deployed rapidly to escalated threat at a specific location (e.g. Wilson 1976b; Powell & Clark 2004), but does this come at the cost of increased vulnerability elsewhere? Studies that address how soldiers defend specific locations are a critical component of understanding colony defence. However, they do not provide a complete picture of the colony-wide, potentially adaptive strategies that are being used. Studies that address colony-wide dynamics of organization are exceptionally rare and, to our knowledge, there are no studies on the colony-wide dynamics of soldier deployment.

Ants in the genus *Cephalotes*, known commonly as ‘turtle ants’, represent an ideal group for advancing our understanding of the colony-wide dynamics of soldier deployment. First, most species have a highly specialized and morphologically discrete soldier caste (de Andrade & Baroni Urbani 1999; Fig. 1) with well-understood functional morphology: soldiers have heavily armoured heads that they use to physically block nest entrances of arboreal nesting cavities (Creighton & Gregg 1954; Powell 2008; Fig. 1, inset). *Cephalotes* do not make the cavities they inhabit. Instead, they use abandoned tunnels of wood-boring beetles (Creighton 1963), and typically show strong preferences for particular cavity properties (Powell 2008, 2009). Second, colonies obligately expand into additional cavities as they grow (Powell 2008, 2009). Each new cavity provides additional shelter for adults and brood, as space in existing cavities becomes limited. Third, cavity acquisition and defence is dangerous, because nesting cavities are a limited and aggressively contested resource used by most arboreal ants (e.g. Carroll 1979; Philpott & Foster 2005; Powell 2009; Powell et al. 2011). Cavity availability is probably influenced by seasonal shifts in production by beetles, cavity decay rates, competitor abundance and predation rates on occupied cavities. These contingencies suggest that selection for dynamic deployment strategies may be particularly strong in this group. Finally, recent studies have demonstrated that soldiers are critical in cavity defence, and that growth and reproductive output are strongly related to the number of cavities a colony defends (Powell 2008, 2009). Moreover, the number of soldiers per cavity reflects the level of defence, because soldiers cooperate in blocking larger entrances and in forming additional lines of defence behind the front-most individual (Powell 2008, 2009, unpublished data; present study). The number of cavities that a colony secures through the process of soldier



Figure 1. Worker and soldier (right) of the turtle ant *Cephalotes rohweri*. Note the highly specialized and armoured head-disc (dorsal surface) of the soldier. The inset shows how a soldier uses its specialized head-disc to block the entrance of a nesting cavity in the wild (worker and soldier photo, Alex Wild; blocking photo, Scott Powell).

deployment, and the number of soldiers per cavity, can therefore be interpreted reliably as having real fitness consequences for the colony.

Here we address how soldier deployment strategies in the turtle ant *Cephalotes rohweri* track changes in the availability and quality of nesting resources. We do this by first evaluating evidence for variation in soldier deployment in wild colonies, using colony collections. Laboratory-based experiments, informed by colony-collection data, are then used to address how different aspects of soldier deployment track changing resource conditions. More specifically, we use the experiments to ask the following questions. (1) To what extent do colonies defend newly available nesting cavities, and do colonies discriminate between cavities of different quality? This question addresses the capacity of colonies to dynamically track resources and change soldier deployment accordingly. (2) Do colonies consistently deploy a lesser, equal or greater number of soldiers to newly defended cavities, compared to original cavities? This question addresses how colonies manage the risks associated with expanding into potentially dangerous nesting locations while necessarily reducing the level of defence in original cavities. (3) Are any components of the deployment process constrained by the overall number of soldiers in a colony? Soldier availability may limit total deployment to new cavities, the number of new nests defended and the level of defence in cavities, or any combination of these components.

METHODS

Colony Collection

Colony collections were used to provide a snapshot of natural patterns of soldier deployment in wild colonies, and to provide the complete colonies for laboratory experiment. The *C. rohweri* colonies used in this study were collected from the Tucson Mountain Park, Tucson AZ, U.S.A. Colonies were initially located by placing nitrogen-rich bait (urine) on all shrubs and trees on $5 \times 50 \text{ m}^2$ transects and visually scanning for foragers. On trees with *C. rohweri*, high-density baiting was then used to identify all occupied cavities of the colony, following the methods of Powell (2009). Colonies were collected by sealing cavity entrance holes when colonies were inactive, removing the branches containing the cavities and counting their contents in the laboratory. Cavity entrance holes were also measured using calibrated digital images (following the methods of Powell 2008) to identify an appropriate entrance size for experimental cavities (below). Twenty-one complete colonies were collected. Three were in the 'incipient' stage of colony ontogeny (recognizable by having distinctively smaller workers and soldiers) and occupied a single cavity. The remaining 18 colonies, each occupying two or more cavities, were used to examine natural patterns of soldier deployment. Twelve of the colonies, spanning a broad range of nonincipient colony sizes, were then used in the laboratory-based experimental studies.

Experimental Set-up

Each of the 12 experimental colonies was housed in a 16-box experimental arena, with the boxes in a 4×4 grid arrangement (box dimensions: $11 \times 11 \times 3.75 \text{ cm}$ high). Each box was connected to each of its immediate neighbours with an arched paper bridge, which went from floor to floor over their adjacent walls. The bridges allowed ants to move freely among boxes, while Fluon[®] on all the walls prevented them from escaping the 16-box arena. This set-up provided 16 discrete locations for experimental cavities. The presence of a cavity in any box, as well as the cavity's properties, varied depending on the experiment (below). Each experimental cavity

was made by sandwiching a piece of card between two standard microscope slides. The card had an internal space cut out of it for the ants to use. The standard internal space measured $40 \times 5 \times 3.2 \text{ mm}$ high and was open at one end. Length was varied in some cavities in the cavity quality experiment (below). The open end of each cavity was closed with a Plexiglas block that had a laser-cut entrance hole of 3.0 mm^2 . Collection data (above) showed that 50% of natural cavity entrances are between 2 and 4 mm^2 . Entrances in this size range are just large enough to fit the head of one soldier (Fig. 1, inset). All experimental cavities were covered with a blacked-out slide to mimic the dark conditions inside natural cavities.

Cavity Availability Experiment

This experiment tested how soldier deployment changes under increased cavity availability. The experiment had a low availability stage followed by a high availability stage. The duration of each stage was 10 days. Preliminary studies suggested that following the introduction of new cavities, soldier deployment and overall occupation patterns stabilized before 10 days. This was verified with six of the experimental colonies by censusing soldier deployment and cavity occupation every 2 days during both stages (colony number limited to six for logistical reasons; see Results). For all colonies, the full contents (all adults and brood) of each cavity was censused at the end of each 10-day stage. The number of cavities given to each colony in the low availability stage was determined by standardizing to an approximate average density of 50 ants per cavity (i.e. cavities given = colony size/50). Using this standardized approach, the number of cavities available to each colony was not significantly different from the number of cavities the colonies used originally in the wild (paired *t* test: $t_{11} = -1.17$, $P = 0.3$), which is likely to be representative of some degree of cavity limitation (Powell 2009). The location of each cavity within the 16-box arena was chosen randomly, to control for differences in distance between cavities. In the high availability stage, new cavities were added to every remaining box, making a total of 16 cavities available to each colony. This mimicked the sudden availability of new beetle-produced cavities, which colonies are likely to face seasonally in nature. All cavities had the standard dimensions and entrance size (above).

Cavity Quality Experiment

This experiment tested how resource quality affects soldier deployment. The experiment had a 10-day low availability stage followed by a 10-day cavity choice stage. The low availability stage was the same as the cavity availability experiment (above). In the cavity choice stage, four small cavities (20 mm long, standard width, depth and entrance) and four large cavities (60 mm long, standard width, depth and entrance) were added to the 16-box arena occupied by each colony. This manipulation mimicked the availability of cavities produced by different beetle species, which is a choice that colonies face in nature. The new cavities were distributed randomly across the remaining cavity-free boxes, to control for distance effects. Cavity size was used as an important metric of quality because it limits colony growth. The limited ability of turtle ants to excavate natural cavities (Powell 2008) means that cavity size, like cavity number, has significant consequences for growth and reproduction (Powell 2009). The full contents of each cavity was censused at the end of each 10-day stage.

Metrics of Deployment and Statistical Analyses

We used the mean absolute deviation from the mean (MAD), a standard metric of dispersion expressed in the original units of

the data (Zar 1996), to summarize the pattern of numerical and proportional soldier deployment across nests in wild colonies. Numerical deployment used the raw soldier counts in each cavity, while proportional soldier deployment in each cavity was calculated by dividing the number of soldiers by the total ants in the cavity (thus standardizing deployment for total number of cavity occupants). Linear least squared regression was used to describe the relationship between soldier availability in colonies and the variation in deployment (captured in the MAD metric). Data were normalized with log transformations.

The experiments were designed to allow simple but powerful paired comparisons between metrics of soldier deployment before and after cavity manipulation treatments, and between cavity types within treatments. Key metrics of deployment were the number of soldier-defended cavities, the level of defence (number of soldiers) in soldier-defended cavities, and total soldier deployment. Paired *t* tests were used for these comparisons, with the data normalized using log transformations. Wilcoxon signed-ranks tests were used whenever the assumptions of the paired *t* test were not met. Linear least squared regressions were also used to describe the relationship between soldier availability and total deployment to new cavities, and between total deployment to new cavities and cavity-level metrics of deployment (e.g. number of defended cavities). Data were again normalized using log transformations. All statistical analyses were run using a standard installation of R v.2.14.

RESULTS

Natural Patterns of Soldier Deployment

These results address the presence of variable soldier deployment in nature, and the extent to which any variation may be explained simply by soldier availability within colonies. Colony size (workers plus soldiers in 18 nonincipient colonies) ranged from 58 to 324 ants, and the total number of soldiers (i.e. soldier availability) ranged from 8 to 63 individuals. These colonies occupied between two and seven cavities. The number of soldiers deployed to each cavity of a colony was typically highly variable (Fig. 2a). This variation in colony-wide deployment (captured by the MAD of deployment for each colony) increased significantly with soldier availability, but it explained a relatively small proportion of the total variance among colonies (Fig. 2b). The variation in colony-wide soldier deployment after standardizing against the number of workers (captured by the MAD proportional soldier deployment) was also sizable (range 2–13%; mean = 6%), indicating that soldier number does not simply track the number of workers in cavities. There was no significant relationship between soldier availability and variation (MAD) in proportional soldier deployment ($F_{1,16} = 0.3$, $R^2 = -0.04$, $P = 0.6$). Therefore, notable variation in colony-wide soldier deployment was present in wild colonies, with only a small proportion of the variation in numerical soldier deployment explained by soldier availability. Queen location also appeared to have no significant influence on soldier number, because the cavity with the most soldiers contained a queen in only seven of 18 colonies, and four of these seven were colonies that occupied only two cavities. Similarly, the cavity with the highest number of soldiers also contained the most brood in only six of 18 colonies.

New Soldier-defended Cavities

These results address how colonies adjust the number of cavities they defend when new resources become available. In the cavity availability experiment, each colony defended all of the cavities made available to them in the low cavity availability stage (i.e. no

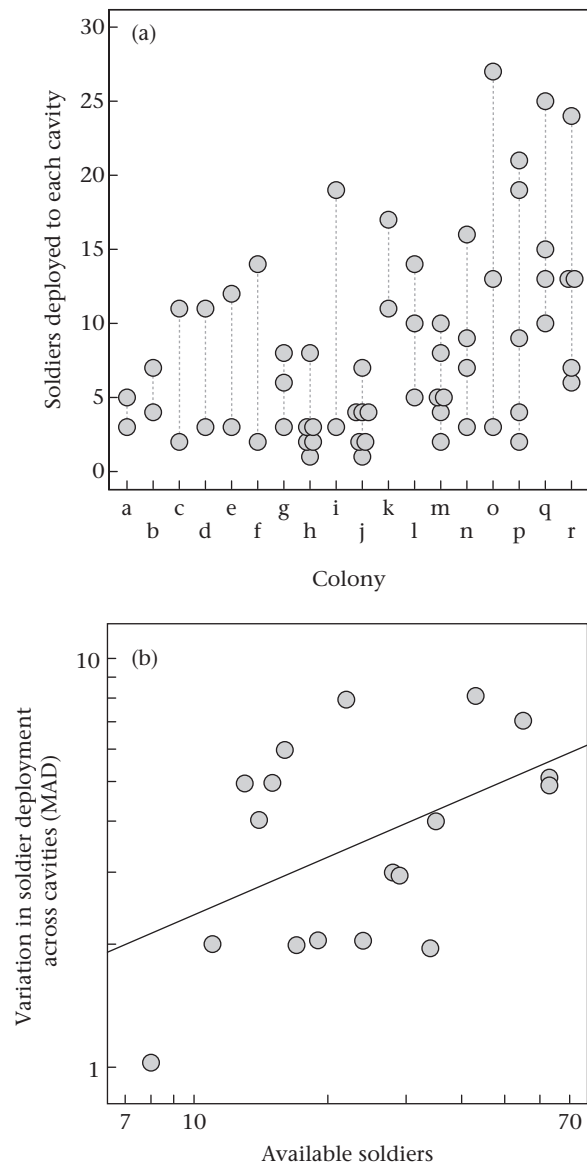


Figure 2. Natural patterns of soldier deployment in field-collected colonies of the turtle ant *Cephalotes rohweri*. (a) Number of soldiers deployed to each cavity occupied by the collected colonies (each point is the soldier count for an individual cavity; each set of connected points represents all the cavities of a colony). Colonies are in rank order, according to the total number of soldiers (soldier availability) that they had. (b) Relationship between soldier availability (total number of soldiers in the colony) and variation in soldier deployment across cavities (described by the dispersion metric mean absolute deviation from the mean, MAD) on a log–log scale. The line represents the linear least squared fit to the logged data ($F_{1,16} = 4.9$, $R^2 = 0.19$, $P = 0.04$, $\eta^2 = 0.24$; slope = 0.11, intercept = -3.23). Note that a MAD of zero is indicative of perfectly even soldier deployment across cavities.

empty or worker-only cavities). In the high cavity availability stage, all colonies significantly increased the number of cavities they defended with soldiers (paired *t* test: $t_{11} = -10.91$, $P < 0.00001$), at least doubling their original number in all cases (Fig. 3a). All original cavities continued to be defended after expansion into new cavities. The expansion into the new cavities was rapid and stabilized quickly, because significantly more soldier-defended cavities were being used after just 2 days (data from 6 of 12 colonies; paired *t* test: $t_5 = -5.97$, $P = 0.004$), but any subsequent 2-day changes were not significant (negative statistical results not shown). Each colony also defended all of the cavities made available to them in the low availability stage of the cavity quality experiment.

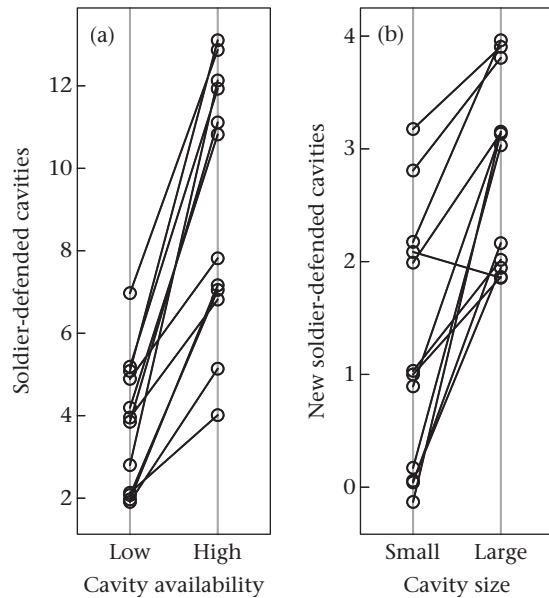


Figure 3. Cavity defence by colonies of the turtle ant *Cephalotes rohweri* under different resource contexts. (a) Number of cavities defended by soldiers under low and high cavity availability (cavity availability experiment). Lines join the paired data points for each colony. Low cavity availability offered a number that would allow a minimum average density of 50 ants per cavity, while high availability provided all colonies with access to 16 cavities. (b) Number of new soldier-defended cavities with small and large cavity volume (cavity quality experiment). Each colony had access to four small and four large cavities, and lines join the paired data points.

Nevertheless, when presented with a choice of new small cavities and higher-quality large cavities, colonies deployed soldiers to significantly more of the large cavities (Wilcoxon signed-ranks test: $V = 0$, $N = 12$, $P = 0.003$; Fig. 3b).

In both experiments, new soldier-defended cavities always contained workers and most contained brood (60% and 74% of cavities, respectively, for the two experiments). Brood items were always eggs or early-instar larvae, and colonies moved an average of 22% of their brood into new cavities in both experiments. All colonies occupied at least one new cavity with workers only, but there were always fewer worker-only cavities than soldier-defended cavities. Moreover, worker-only cavities rarely contained brood, with brood lacking entirely from such cavities in seven of 12 colonies in the cavity availability experiment, and all worker-only cavities in the cavity quality experiment. Queens always remained in one of the original cavities. Expansion into new cavities was therefore generally characterized by colonies rapidly establishing a number of new soldier-defended cavities with workers and brood, plus a small number of worker-only cavities.

Level of Defence in New Cavities

These results address how the level of defence (number of soldiers) in all cavities is adjusted when colonies expand into new cavities. In the high cavity availability stage of the cavity availability experiment, the mean number of soldiers in newly defended cavities was significantly less than that in the original cavities the colony continued to use (paired t test: $t_{11} = 5.67$, $P = 0.0001$; Fig. 4a). When colonies defended at least one new small and large cavity in the cavity quality experiment (eight of 12 colonies), larger cavities had more soldiers, on average, than smaller ones (paired t test: $t_7 = -2.51$, $P = 0.04$; Fig. 4b). Nevertheless, combining data on the number of soldiers in new small and large cavities, newly defended cavities again had a significantly lower average number of

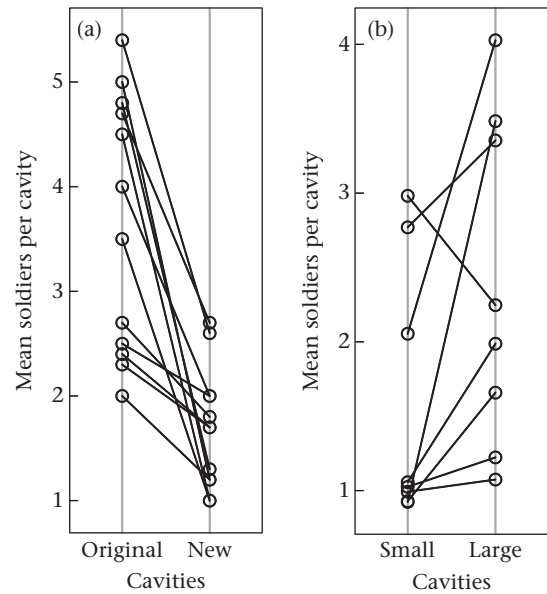


Figure 4. Level of defence in different types of cavities in the turtle ant *Cephalotes rohweri*. (a) Mean number of soldiers in original and newly defended cavities (cavity availability experiment). Lines join paired data points for each colony. (b) Average number of soldiers in new small and large cavities (cavity quality experiment). Lines join paired data points for each colony.

soldiers than the original cavities the colonies continued to use (paired t test: $t_{11} = 4.55$, $P = 0.0008$). Even when considering only the new large cavities, which were larger than the original cavities, the average number of soldiers in original cavities was still higher (paired t test: $t_{11} = 3.72$, $P = 0.003$).

Much like the smaller soldier numbers in newly defended cavities, the associated worker numbers were also significantly smaller than those maintained in original cavities (paired t test: cavity availability experiment: $t_{11} = 7.12$, $P = 0.00002$; cavity quality experiment: $t_{11} = 3.59$, $P = 0.004$). Comparisons of proportional soldier representation in original and newly defended cavities were not valid because of the significantly smaller number of individuals in new cavities. Nevertheless, these analyses demonstrate that soldiers and workers rapidly deployed to new cavities, but in numbers that were well below those maintained in the original cavities, and, therefore, considerably below the maximum capacity of the new cavities.

Total Soldier Deployment and New Cavity Defence

These results address how total soldier deployment is related to overall soldier availability in colonies, and how the relationship influences the defence of new cavities. In both experiments, total soldier deployment to new cavities was an approximately constant percentage of overall soldier availability, at around 40% (Fig. 5). The scaling relationship between the number of available soldiers and the number of soldiers deployed to new cavities was isometric (proportional) for both the cavity availability experiment and the cavity quality experiment (Fig. 5). The relationships for the two resource contexts were also not significantly different from each other (Wald test comparing slope and intercept: $F_{2,16} = 0.57$, $P = 0.6$). Soldier deployment to new cavities therefore appeared to be constrained by soldier availability in a consistent manner across colonies and resource contexts.

In the cavity availability experiment, the total number of soldiers deployed to new cavities (greater in colonies with more soldiers available; Fig. 5) was a significant predictor of the total

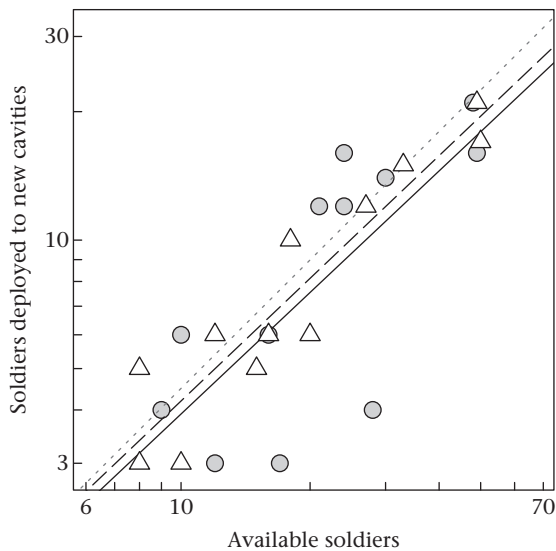


Figure 5. Relationship between soldier availability and total soldier deployment to newly available cavities in colonies of *Cephalotes rohweri*, plotted on a log–log scale. Grey circles: cavity availability experiment, with solid line of best fit (linear least squared fit: $F_{1,10} = 11.6$, $R^2 = 0.49$, $P = 0.007$; slope = 0.95, intercept = -0.81); white triangles: cavity quality experiment, with dashed line of best fit (linear least squared fit: $F_{1,10} = 66.8$, $R^2 = 0.86$, $P < 0.00001$; slope = 0.95, intercept = -0.76); grey dotted line: perfectly isometric (proportional) scaling of the data (general line relationship of the form $\log(y) = a \times \log(x) + \log(k)$, where y is soldiers deployed to new cavities, x is available soldiers, k is the intercept, and a is the scaling exponent equal to 1; Huxley 1993). In both cases, the fitted lines did not differ significantly from isometric scaling (cavity availability versus slope of 1: $t = -0.19$, $P = 0.8$; cavity quality versus slope of 1: $t = -0.39$, $P > 0.7$).

number of new cavities that were defended by soldiers ($F_{1,10} = 47.8$, $R^2 = 0.81$, $P = 0.00004$; Fig. 6a). A similar significant relationship was seen in the cavity quality experiment, after summing newly defended small and large cavities ($F_{1,10} = 19.9$, $R^2 = 0.63$, $P = 0.001$). The total number of soldiers deployed to new cavities was also a significant predictor of the average number of soldiers in new cavities in the cavity availability experiment ($F_{1,10} = 19.0$, $R^2 = 0.62$, $P = 0.001$; Fig. 6b) and the cavity quality experiment (combined data for small and large cavities; $F_{1,10} = 13.7$, $R^2 = 0.54$, $P = 0.004$). Total soldier deployment, a constant proportion of overall availability, therefore appeared to limit both the number of new cavities that a colony defended and the level to which each was defended.

DISCUSSION

Here we have addressed whether colony-wide soldier deployment in the turtle ant *C. rohweri* adjusts dynamically to resource-related environmental contingencies. Consistent with dynamic soldier deployment, wild colonies showed considerable variation in numerical and proportional soldier deployment across cavities. From the initial field-like conditions established in laboratory experiments, the number of soldier-defended cavities increased significantly after just 2 days and was stable thereafter, with all colonies more than doubling the number of cavities they defended. Within this rapid expansion, they also discriminated between cavities of different quality, by defending more large cavities. The number of soldiers in new cavities, representing the level of defence, was significantly lower than that in the original cavities that colonies continued to defend. Nevertheless, colonies again discriminated between cavities of different quality, by deploying more soldiers to larger cavities. Overall soldier deployment to new cavities was limited to an approximately constant proportion of overall soldier availability across colonies and resource contexts,

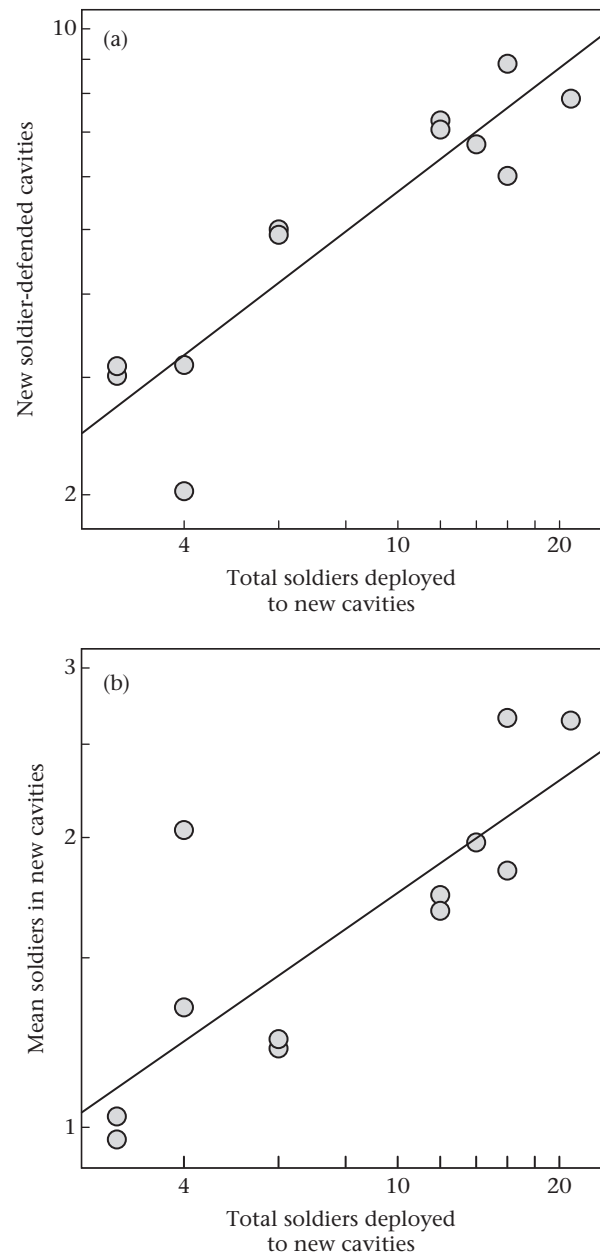


Figure 6. Relationship between total soldier deployment to new cavities and (a) number of newly defended cavities, and (b) average level of defence (mean soldier number) in the turtle ant *Cephalotes rohweri*.

suggesting a consistent limit on the deployment processes. Concordantly, there was a significant positive relationship between the total number of soldiers deployed to new cavities, a product of total availability, and both the number of new soldier-defended cavities and the level of defence in each. To our knowledge, this is the first study to demonstrate that soldier-based colony defences can be governed by dynamic, context-sensitive and predictable deployment strategies, with these processes ultimately constrained by soldier availability.

The rapid use of new cavities by *C. rohweri* is typical of species that face frequent cavity limitation (e.g. Herbers 1986; Philpott & Foster 2005; Powell et al. 2011). However, of greatest interest here is that new cavities were not treated equally. Other ants can quickly establish well-balanced numbers of individuals among nests, with more ants moving to higher-quality cavities (reviewed

in *Debout et al.* 2007). In contrast, *C. rohweri* colonies consistently defended a large number of new cavities using fewer soldiers than they had for the original cavities (Fig. 4). Ultimately, the colony must grow to fill new cavities more completely. Nevertheless, our results do show a clear and stable cavity acquisition stage of colony growth, wherein a large overall commitment of soldiers (Fig. 5) was spread thinly across many new cavities. Before discussing this pattern further, it is important to reiterate that the number of soldiers in a cavity reflects the level of defence that cavity receives. During our laboratory experiments and censuses of field-collected colonies, soldiers that did not fit in the entrance hole formed additional lines of defence from behind. The front-most soldiers are not invincible, so subsequent lines of defence can be critical for cavity survival. Indeed, a field experiment revealed that even when the head of one soldier perfectly fits a cavity entrance, nine additional soldiers still cannot prevent some cavities from being usurped by enemies (Powell 2009). Given the functional relationship between soldier number and level of defence, why would *C. rohweri* favour limited defence of many cavities when acquiring new cavities for colony growth?

Considered in a broader context, soldier deployment in *C. rohweri* appears to be consistent with the general life history strategy of ‘conservative bet hedging’ (Philippi & Seger 1989; Olofsson et al. 2009). Bet-hedging theory addresses how organisms should maximize their fitness in variable and unpredictable environments. Although most often considered for reproductive strategies, bet hedging is applicable to the growth strategies we focus on here and any other life history trait (Philippi & Seger 1989; Olofsson et al. 2009). Generally speaking, limiting the variance of fitness between life history events will maximize lifetime fitness in variable environments. Conservative bet hedging is one strategy for achieving this, and it involves always limiting short-term risk, or ‘always playing it safe’ (Philippi & Seger 1989; Olofsson et al. 2009). But how does cavity acquisition in *C. rohweri* fit this scenario?

As noted, cavity acquisition is critical for colony growth and reproduction in turtle ants (Powell 2009), but the canopy is an unpredictable and dangerous nesting environment (Carroll 1979; Hasegawa 1993; Philpott & Foster 2005; Powell 2009; Powell et al. 2011). *Cephalotes rohweri* always ‘plays it safe’ during cavity acquisition by only establishing low-level defence of new cavities. If usurpation pressure on a new cavity is moderate, low-level defence will most likely hold it for subsequent growth. If, however, usurpation pressure on a new cavity is high, the cavity is likely to be lost regardless of how many soldiers are present (e.g. survival experiment in Powell 2009). More soldiers in new cavities would also reduce the level of defence in original cavities. The observed strategy may therefore strike a balance between a moderate level of defence and a minimal level of potential loss. Put more generally, the variance in fitness gains between cavity acquisition attempts should be greatly minimized by this strategy, assuming unpredictable levels of cavity usurpation pressure, and long-term fitness should be maximized. Future studies addressing cavity survival under different deployment patterns will be important in testing this hypothesis directly. Nevertheless, our current findings provide further suggestions that the soldier deployment strategy in *C. rohweri* is an example of conservative bet hedging.

Larger cavities can yield greater fitness gains by providing more space for growth and reproductive output, much like additional cavities (Powell 2009). Consequently, within the context of a conservative bet-hedging strategy that generally limits soldier deployment to new cavities, we might still expect selection to favour greater soldier deployment to larger cavities. Concordantly, colonies showed a clear preference for larger cavities in our cavity quality experiments (Fig. 3b) and defended them with more soldiers (Fig. 4b). The capacity to collectively select larger spaces

has been demonstrated before in ants, but in the entirely different context of catastrophic cavity loss (e.g. Mallon & Franks 2000; Franks et al. 2006). We show this ability in normal colony growth, and establish how it is intimately connected with a context-sensitive, soldier-based defence strategy. This demonstrates that while the risk of new cavity acquisition is generally limited, greater risk is taken for higher-quality cavities. Future experimental studies will be valuable for testing whether this greater risk ultimately results in greater fitness, as we would expect for an adaptive bet-hedging strategy.

While soldier deployment is remarkably dynamic in *C. rohweri*, our findings also suggest that soldier availability ultimately constrains the total number of soldiers deployed to new cavities (Fig. 5). Total deployment, in turn, appears to limit the number of new cavities defended and their average level of defence (Fig. 6), with potentially real fitness consequences for the colony. In general terms, the soldier availability, dictated by inflexible production rates, thus limits the scope of flexible deployment strategies. Interestingly, the relatively static nature of soldier availability in ants may also limit the types of deployment strategies that can evolve. A theoretical alternative to ‘conservative bet hedging’ is ‘diversified bet hedging’, where individuals invest simultaneously in different, contrasting strategies (Philippi & Seger 1989). In turtle ants, the alternative to the observed uniform, low-level deployment would be to combine it with immediate high-level deployment to some new cavities. However, this strategy would require a much larger surplus of soldiers, or the ability to increase soldier production rapidly. As already discussed, these properties are unlikely in ants and almost certainly absent in turtle ants.

Broadly, our findings provide novel insights into how the deployment of soldiers, a key defence trait in the evolution of insect societies, adjusts to environmental contingencies. More specifically, we show that soldier-based defences track changes in resource availability and quality, via a process of dynamically adjusting the deployment of their existing soldiers among cavities. Most notably, adjustments to soldier deployment appear to conform to a risk-limiting ‘conservative bet-hedging’ strategy that is ultimately constrained by overall soldier availability. This underscores the importance of considering both soldier availability and deployment when addressing the adaptive defence strategies of insect societies. While availability, and especially the integral process of soldier production, has been a focus of past research (e.g. Wilson 1985; Walker & Stamps 1986; Kaspari & Byrne 1995; Passera et al. 1996; Harvey et al. 2000; McGlynn & Owen 2002), deployment has been largely overlooked. This more complete view of defence strategies in social insects may greatly facilitate future work on the evolution of defence across levels of biological organization. Comparisons with defence in plants may be particularly valuable (see Kaspari & Byrne 1995), as they share similar modular design and specialized defensive traits that respond to environmental contingencies. We anticipate that a combined understanding of soldier availability and deployment may reveal many more similarities in the evolution of defence in insect societies and plants.

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