

animal associations. The technology is suitable for long-term deployment on relatively small animals, and overcomes key constraints of other data-collection methods. Importantly, with its high sampling rates and excellent tag-to-tag detection range, Encounternet can generate time-resolved association data needed to link network topologies to biological processes — a key challenge in social network analyses [2,3].

#### Supplemental Information

Supplemental information including experimental procedures and two figures can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2012.06.037>.

#### Competing Interests

Encounternet was conceived and developed at the University of Washington, Seattle, USA, and BO, JBU and JBo are currently working on the technology's commercial exploitation.

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## Wingless virgin queens assume helper roles in *Acromyrmex* leaf-cutting ants

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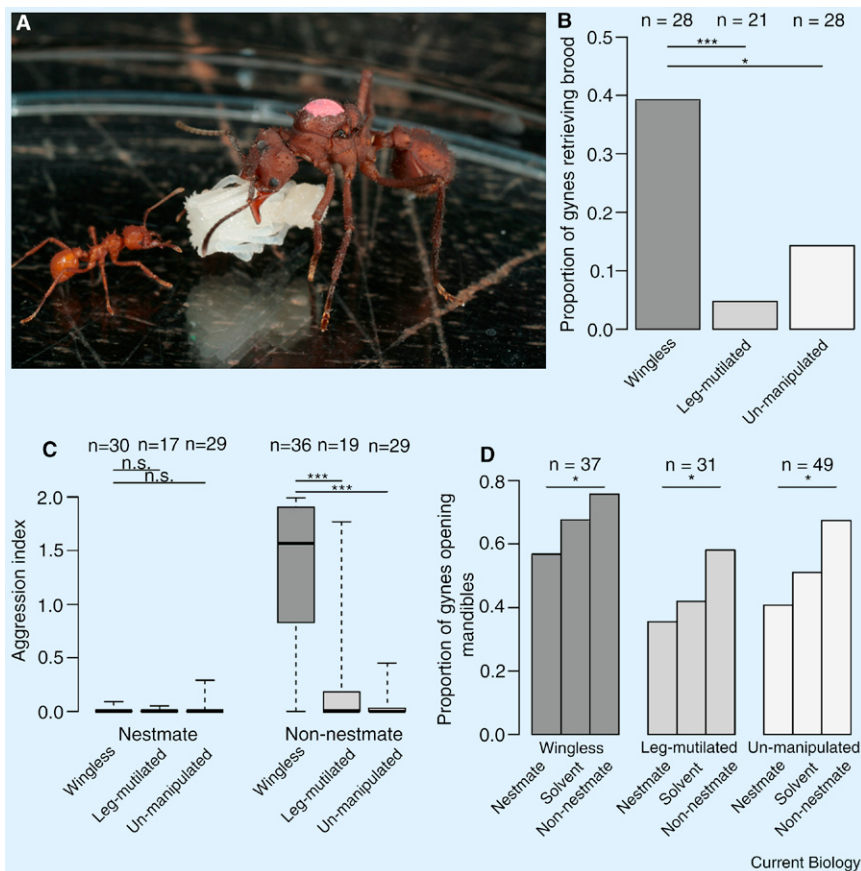
Division of labour is the hallmark of advanced societies, because specialization carries major efficiency benefits in spite of costs owing to reduced individual flexibility [1]. The trade-off between efficiency and flexibility is expressed throughout the social insects, where facultative social species have small colonies and reversible caste roles and advanced eusocial species have permanently fixed queen and worker castes. This usually implies that queens irreversibly specialize on reproductive tasks [2]. Here, we report an exception to this rule by showing that virgin queens (gynes) of the advanced eusocial leaf-cutting ant *Acromyrmex echinator* switch to carrying out worker tasks such as brood care and colony defence when they fail to mate and disperse. These behaviours allow them to obtain indirect fitness benefits (through assisting the reproduction of their mother) after their direct fitness options (their own reproduction) have become moot. We hypothesize that this flexibility could (re-)evolve secondarily because these ants only feed on fungal mycelium and thus could not benefit from cannibalising redundant gynes, and because queens have retained behavioural repertoires for foraging, nursing, and defense, which they naturally express during colony founding.

Permanence of caste is the most fundamental difference between eusocial and cooperative breeding [2]. Helpers of the latter category may become breeders later in life and thus retain full flexibility towards the eventual pursuit of direct fitness. Illustrative examples are cooperatively breeding birds, paper wasps and some Ponerinae ants [3–5]. Workers of eusocial ants and honeybees, by contrast, at best retain some phenotypic

plasticity for adopting specific behaviours depending on their age and the needs of the colony [6], but their reproductive potential is always permanently reduced. The behavioural repertoires of queens tend to be limited to mating, colony founding and egg-laying, and this never changes in response to queen condition. This should imply that gynes — unmated virgin queens — should be cannibalized or otherwise disposed of when they somehow fail to disperse on a mating flight, similar to infertile diploid males [7,8]. Uselessness for both colony-founding and altruistic behaviour means that failed queens have zero fitness and might thus be even expected to die voluntarily, as recycling of their tissues and prevention of further consumption of scarce resources will offer them at least some indirect fitness when their resources will benefit properly endowed siblings.

We observed field colonies of the leaf-cutting ants *Acromyrmex echinator* and *A. octospinosus* that contained several non-inseminated wingless gynes besides the mother queen (Supplemental Information published with this article online). As these queens had been allowed to live, we hypothesized that they might still be worth their keep if they would assume helper roles that were of value for their colony. To test this hypothesis, we experimentally removed the wings of normal gynes and quantified worker-like behaviours. As controls, we used both completely un-manipulated gynes, and gynes with one middle leg removed. This mutilation was not expected to affect normal behaviour as queens missing a leg are occasionally observed as functional colony mothers.

*Acromyrmex* gynes with their wings experimentally removed were more likely to conduct worker tasks: unlike the control gynes, they took care of brood that we introduced into experimental nests (Figure 1A,B) and they attacked non-nestmates (Figure 1C). A second nestmate recognition experiment, in which tethered gynes were presented with nestmate or non-nestmate odour, showed that both the control and wingless gynes were equally able to discriminate between nestmate and non-nestmate cues (Figure 1D).



**Figure 1. Virgin queens (gynes) as helpers in *Acromyrmex* leaf-cutting ants.** Wingless *Acromyrmex* gynes carry out worker behaviour in contrast to winged control gynes (leg-mutilated and un-manipulated). (A and B) They transported brood items back to their fungus garden (three colonies tested) and (C) attacked non-nestmate intruders, while control gynes rarely engaged in these behaviours (boxplots show interquartile ranges and medians; whiskers cover all data points; four colonies tested in the field and laboratory, see also Figure S1). Gynes hardly ever attacked nestmates ( $p < 0.001$ , interaction gyne type  $\times$  intruder  $p < 0.001$ ). (D) Tethered wingless gynes and control gynes were all able to discriminate non-nestmates from nestmates: all three types of gynes were more prone to opening their mandibles (showing threat) when confronted with non-nestmate than with nestmate odour or solvent ( $p < 0.05$ , three colonies). Wingless gynes were generally more likely to open their mandibles than other gynes ( $p < 0.05$ ), consistent with expression of more aggressive behaviour; the ability to discriminate between nestmate and non-nestmate odours was, however, similar for all gynes (interaction odour  $\times$  gyne type  $p > 0.05$ ). Sample sizes are indicated above the bars and boxes (\*\*\*)  $p < 0.001$ , \*  $p < 0.05$ , n.s.  $p > 0.05$ ).

This indicates that the lack of aggression in control gynes is not due to their inability to recognise intruders (differential perception of recognition cues), but to gynes avoiding intruders (differential decision rule) when dispersal and reproductive potential is intact. Only after the option to disperse and mate was lost did gynes switch to the more risky behaviour of attacking intruders, a task that their larger body size allows them to do more effectively than large workers (*Acromyrmex* never evolved a soldier caste as their sister genus *Atta* did).

As comparative context for our experimental results we provide a summary of cases where ant queens have been reported to perform worker behaviour (Supplemental Information). They all concerned ants with either many queens per colony or special gyne-morphs that can later become replacement queens when they mate in the nest, but no case reminiscent of *Acromyrmex* where colonies are predominantly (*A. echinaior*) or almost exclusively (*A. octospinosus*) headed by a single queen and where queens always have a dispersal flight and never mate in the nest. We believe that this

is not an oversight by generations of natural historians, but that there are likely to be two selection forces that make working/defending gynes particularly probable in fungus-growing ants such as *Acromyrmex*. First, *Acromyrmex* queens are semiclaustral, which means that they forage during colony founding, unlike the claustral queens of most ants that seal their founding burrow to live on body reserves until their first workers hatch [9]. *Acromyrmex* gynes have thus retained a working and defending repertoire that becomes temporarily expressed in response to dispersal and insemination, so could easily be co-opted. Second, attine ants consume almost exclusively fungal tissue, and are thus likely to have lost the ability to digest animal tissue. *Acromyrmex* fungus-gardens have also never been observed to contain parts of dead insects as substrate in any appreciable amount [10]. This means that workers cannot recoup investments that went into raising reproductives, so they may as well let wingless gynes serve the colony while living on the fat and carbohydrate reserves that were deposited to prepare them for founding a new colony. This would imply that wingless gynes expressing worker behaviour are not expected to be fed, because their existence only makes sense when their siblings profit at no cost. However, our current set-up did not allow us to investigate this possibility.

We hypothesize that the same logic may apply in other attine ants and that soldier/nurse gynes might occur also in related higher attine genera such as *Trachymyrmex* and *Sericomyrmex* that rear very similar fungal symbionts. However, we expect that such gynes will never be found in *Atta*, as this genus secondarily evolved claustral colony founding, so that gynes in any condition may no longer have a worker-like behavioural repertoire to activate either after or before mating. Consistent with this idea, we have never observed wingless queens when digging out mature *Atta* colonies but we have regularly observed *Atta colombica* workers dumping dead reproductives on their above ground compost heaps shortly after a mating flight,

confirming that their resources cannot be recycled.

#### Supplemental Information

Supplemental Information including experimental procedures, a figure and a table can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2012.064.038>.

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## The first World Cell Race

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Motility is a common property of animal cells. Cell motility is required for embryogenesis [1], tissue morphogenesis [2] and the immune response [3] but is also involved in disease processes, such as metastasis of cancer cells [4]. Analysis of cell migration in native tissue *in vivo* has yet to be fully explored, but motility can be relatively easily studied *in vitro* in isolated cells. Recent evidence suggests that cells plated *in vitro* on thin lines of adhesive proteins printed onto culture dishes can recapitulate many features of *in vivo* migration on collagen fibers [5,6]. However, even with controlled *in vitro* measurements, the characteristics of motility are diverse and are dependent on the cell type, origin and external cues. One objective of the first World Cell Race was to perform a large-scale comparison of motility across many different adherent cell types under standardized conditions. To achieve a diverse selection, we enlisted the help of many international laboratories, who submitted cells for analysis. The large-scale analysis, made feasible by this competition-oriented collaboration, demonstrated that higher cell speed correlates with the persistence of movement in the same direction irrespective of cell origin.

The race track consisted of 4  $\mu\text{m}$ - and 12  $\mu\text{m}$ -wide fibronectin lines

printed in multi-well glass-bottomed cell-culture wells (see Supplemental Experimental Procedures and Figure S1A in the Supplemental Information available online); 54 different cell types from various animals and tissues were provided by 47 laboratories. Genotypically, cells were wild type, transformed or genetically engineered (Table S1). The cells were distributed to six organizing laboratories (two in the USA, and one each in the UK, France, Germany and Singapore), who prepared cell-culture stocks using the frozen samples received from participating laboratories and plated these onto the race tracks under identical culture conditions. Cells were allowed to adhere overnight and cell motility was recorded for 24 hours using an inverted video microscope (Figure 1B, Movie S1). Cell morphology (length, shape, symmetry, and nucleus position) varied greatly from one cell type to another (Figure 1A). Cell nuclei were stained by incubating live cells with 5 ng/ml Hoechst dye diluted in normal growth medium. Cell displacements were monitored every 10 minutes. Nuclei images were segmented and geometric centers were tracked with a global minimization algorithm in order to track automatically individual cell displacements (see Supplemental Experimental Procedures and Figure S1B). The motility of over 7,000 cells was compared, with an average of 130 cells analyzed per cell type. Detailed statistical analyses were used to characterize cell motility parameters for each cell type (see <http://www.worldcellrace.com/ResultFiles>).

The mean instantaneous speed of individual cells is computed by averaging the cell displacements between consecutive frames over time. The distribution of mean instantaneous speeds for each cell type was asymmetric (Figure 1C) and non-Gaussian (Figure S1C). Interestingly, we observed that a higher mean speed for a given cell type did not reflect a global shift of the speed distribution, but rather the spreading of the distribution due to the presence of faster moving cells (Figure 1C and Figure S1C). In order to identify the 2011 World Cell Race winner, only cells with an effective overall displacement of at least