

Collective Response of Leaf-Cutting Ants to the Effects of Wind on Foraging Activity

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ABSTRACT: One advantage of sociality is to mitigate environmental restrictions through collective behavior. Here we document a colony-level response of leaf-cutting ants to wind, an environmental factor that impedes foraging. Given that larger ants adhere more strongly to the substrate, increasing forager size in windy conditions should reduce the negative effect of wind. We tested this idea for *Acromyrmex lobicornis* in windy regions of Patagonia. We examined (1) whether the fraction of larger ants versus smaller ants increased in windy conditions and (2) whether the effect of wind on the ants' movement was lower for larger ants. The size–frequency distribution of foragers was skewed more toward larger ants in nature under more windy conditions. Under windy conditions in the field, the mobility of smaller ants was more reduced than that of larger ants. The change toward larger foragers in windy conditions reduced the negative effect of wind by 32%, illustrating how a social organism can collectively mitigate the adverse effects of the environment.

Keywords: *Acromyrmex lobicornis*, collective solutions, environmental restrictions, Patagonia.

Introduction

Animals deal with situations that alter the normal progress of their activities and require special responses (hereafter, problems). The absence of appropriate solutions for these problems often implies a cost. For example, if ant workers do not remove the litter that falls on a trunk trail, the rate of food delivery to the nest is reduced (Howard 2001). One advantage of social organisms is that they can solve problems collectively (Anderson and Franks 2001). For instance, ants can cooperatively transport prey that exceed their individual carrying capacity (Czaczkes and Ratnieks

2013), and leaf-cutting ants increase the number of ants with small loads when an object above their trails limits the passage of ants with larger loads (Dussutour et al. 2009). Collective solutions in ants have been studied mainly in circumstances of foraging and colony defense (Hölldobler and Wilson 1978; Elizalde and Folgarait 2012; Czaczkes and Ratnieks 2013). Here, we determined how the leaf-cutting ant *Acromyrmex lobicornis* collectively responds to the negative effect of wind, an environmental constraint poorly studied in terrestrial arthropods.

In regions with frequent strong winds, foraging of *A. lobicornis* decreases, because wind reduces the ants' speed and increases the number of ants displaced from the trail (Alma et al. 2016a). Recently, it has been discovered that, in leaf-cutting ants, contact area per tarsus and the total force of adhesion increase with body size not only because of the positive allometry of the ants' adhesive pads but also due to an increase in pad efficiency (Labonte and Federle 2015). Accordingly, one possible colony response to mitigate the negative effect of wind could be to increase the frequency of larger foragers under windy conditions. We tested this prediction by analyzing (1) whether the size distribution of both outbound and inbound laden ants was more skewed toward larger ants under windy conditions and (2) whether larger ants were less affected by wind than smaller ants. In addition, to test whether the allocation of larger ant foragers mitigates the effect of wind, we estimated the rate of food input (RFI) on the basis of ant size distributions found under windy and windless conditions and the effect of wind on movement of ants and load area.

Methods

Study Area and Ants

Acromyrmex lobicornis inhabits windy regions of Patagonia (Farji-Brener and Ruggiero 1994). Fieldwork was performed during Austral spring and summer during 2013–2014 in El

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Chocón, Neuquén (39°16' S, 68°47' W), where the mean wind speed (\pm SE) is 20 ± 20 km/h with a maximum of 56 km/h (measurements at 10 m above the ground; Autoridad Interjurisdiccional de Cuencas). At ground level, the mean speed of wind (\pm SE) is 2.5 ± 1.6 km/h with gusts of 20 km/h, and during the activity period of *A. lobicornis* (October–May), the windiest month is December, which has a mean wind speed (\pm SE) of 4.4 ± 1.7 km/h.

Collective Response

To evaluate whether larger ants are more likely to forage in windy conditions than in windless conditions, we filmed 10 nests (one trail per nest) in naturally windless and windy conditions (wind speed: 0 and 4–9 km/h, respectively) for 150 s with a ruler placed alongside the trail; we used a Cannon Rebel T3i video camera at 60 frames per second and $1,280 \times 720$ pixels (high definition). Windless conditions occurred during mornings or afternoons when no wind was blowing. We recorded the average wind speed at ground level with a digital anemometer (Lutron LM-81AM, range: 0.4 to 30 m/s, resolution: 0.1 m/s) while recording. To determine whether the higher frequency of larger inbound foragers was a consequence of smaller ants being more affected by wind (e.g., blown off the trail by the wind while foraging), we filmed trails 2 m from the nest entrance and measured the size of inbound loaded ants and outbound ants. In total, we randomly selected and measured 206 loaded ants and 354 outbound ants in each wind condition, and we measured the body length (from the head to the tip of the gaster) with the aid of the ruler using Kinovea 0.8.15 software (<http://www.kinovea.org/en>). To avoid off-axis distortion, we placed the ruler parallel in relation to ant walking direction and filmed from above and perpendicular to the trail (video A1, available online).

Wind Effect Varies with Ant Size

To assess whether larger ants were less affected by wind than smaller ants, we generated wind with small fans (from computers) that allowed wind speed to be adjusted in 12 trails from 12 adult colonies (one trail per colony) on windless days (wind speed: 0 km/h). The fans were placed 5 cm from the trail to generate wind perpendicular to the ant movement (fig. A1, available online). For each trail, a 10-cm section was protected from the wind generated by the fans, and an adjacent section of 10 cm was exposed. The maximum wind speed at which ants continued foraging was 4 km/h (speed comparable to natural wind at ground level); at higher wind speeds, ants either walked away from the trail or went behind the fans. Anemometers on trail sections that were not exposed to artificial wind always registered

0 km/h. We measured ant speed, number of times that each ant fell or was displaced at least 1 cm from its trajectory, and time spent by ants to cross from a windless to a windy condition (hereafter, crossing time). Delays crossing from a windless to a windy section were evidently generated by wind (see video A1). We obtained these measurements in the trail sectors with and without wind for large (5–7-mm body length, $n = 51$) and small (2–4-mm body length, $n = 36$) loaded ants. We sampled only larger and smaller ants, because we found a shift in the distribution of ant sizes, where very small ants (<3.5 mm) were almost entirely absent in windy conditions, and very large ants (>6.5 mm) were present only in windy conditions (see “Results” below). Using the extremes of ant size distribution allowed us to focus on sizes that were less abundant but were the ones that were more important to testing our hypothesis. We estimated ant speed (in centimeters per second) using the time spent by each ant to walk 10 cm of trail in both sections (with and without artificial wind). Ant speed was measured when the ant was not displaced by wind to discriminate the effect of wind on speed and displacements. The crossing time was compared with the time that each individual spent crossing an imaginary line between two windless sections. In addition, we estimated from the videos the area of the load carried by each ant, because the effect of wind increases with the exposed area (Alma et al. 2016a). We measured the length and width of loads with the aid of a ruler, using Kinovea 0.8.15 software (<http://www.kinovea.org/en>). We calculated the load area as length \times width or $\pi \times$ radius² depending on its shape.

To calculate the measurement error of the variables estimated from the videos, we filmed and collected 49 ants foraging with their loads from 12 nests. From the videos, we measured the body length of ants as explained above. In the laboratory, we obtained the same measurements with a stereoscopic microscope. Then we calculated the measurement error as the difference between the value measured from the videos and its value measured with the microscope. Mean (\pm SE) measurement error for ant body length was 0.4 ± 0.05 , representing $\sim 8\%$.

To determine how assignment of larger ants in windy conditions mitigates the negative effect of wind, we estimated the RFI per minute considering the proportional flux of small and large ants in windy and windless conditions, both with the effect of wind on ant’s movement. This approach allowed us to estimate the RFI if the colonies were affected by wind but did not respond collectively. We used the following equation:

$$\text{RFI} = \frac{\text{load size}_{\text{small ants}} \times \text{proportional flux}_{\text{small ants}}}{\text{trail length/speed}_{\text{small ants}}} + \frac{\text{load size}_{\text{large ants}} \times \text{proportional flux}_{\text{large ants}}}{\text{trail length/speed}_{\text{large ants}}}.$$

We estimated the mean flux of ants per minute, trail length, load sizes, and mean speed of large and small laden ants in windy conditions. In addition, to estimate the proportional flux of large and small ants found in windy and windless conditions, we estimated the percentage of large and small ants on the basis of the actual distributions of ant sizes in windy and windless conditions (table A1; tables A1, A2 available online).

Statistical Analysis

To evaluate whether larger ants are more likely to forage in windy conditions than in windless conditions, we used a Kolmogorov-Smirnov test to compare the distributions of body lengths in windless and windy conditions for both laden and outbound ants. To assess whether larger ants were less affected by wind than smaller ants, we calculated the differences in speed, number of displacements, and crossing time between windless and windy sections (i.e., larger values representing stronger wind effect) for every ant. These response variables were log transformed and analyzed using

linear mixed-effect models. Ant size was considered as a categorical fixed factor (small and large ants, 2–4 and 5–7 mm, respectively), load area as a covariate, and nest identity as a random factor. We tested whether the random effect was significant using log-likelihood ratio tests, with P values corrected for testing on the boundary (Zuur et al. 2009). Finally, we used a t -test to evaluate differences in load size between large and small ants using data from 71 ants and load collected (load length and width were measured in a stereoscopic microscope). Statistical analyses were performed in the R environment (R Development Core Team 2010).

Results

The distribution of ant size was skewed toward larger ants in windy conditions, compared with windless conditions, for both outbound and loaded inbound ants (Kolmogorov-Smirnov test, loaded ants: $D = 0.4$, $P < .0001$, 4.2 ± 0.07 and 5.2 ± 0.06 mm; outbound ants: $D = 0.27$, $P < .0001$, 4.2 ± 0.05 and 5.0 ± 0.06 mm; fig. 1). Very small ants (<3.5 mm) were almost absent in windy conditions, and

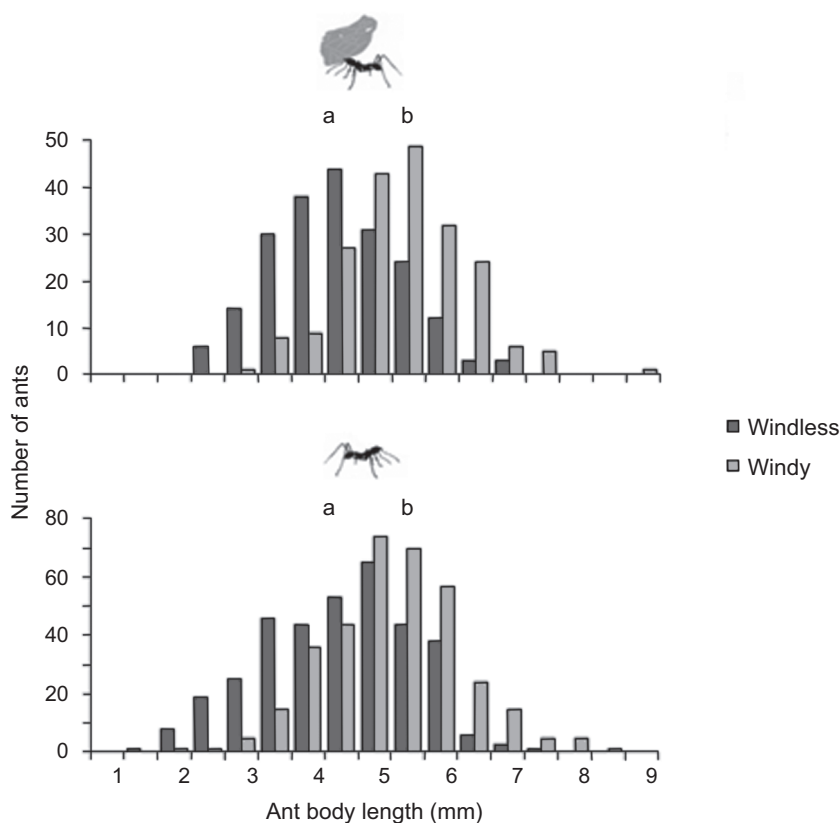


Figure 1: Body-length distribution of loaded ants (*top*) and outbound ants (*bottom*) in windless (*a*) and windy (*b*) conditions (wind speed: 0 and 4–9 km/h, respectively). Different letters denote statistically different groups and distributions (Kolmogorov-Smirnov test, loaded ants: $D = 0.4$, $P < .0001$; outbound ants: $D = 0.27$, $P < .0001$). Data underlying this figure are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d22f7> (Alma et al. 2016b).

very large ants (>6.5 mm) were present only in windy conditions. The effects of wind on all behavioral response variables were lower for larger ants than for smaller ants (fig. 2; table A2). Thus, while wind reduced the speed of smaller ants by 43%, this reduction was only 21% for larger ants (mean \pm SE, 0.53 ± 0.07 and 0.32 ± 0.12 cm/s, respectively; $F = 3.35$, $df = 73$, $P = .07$). Wind increased by 1.2 ± 0.3 times the number of displacements per ant for smaller ants but only by 0.23 ± 0.01 times for larger ants ($F = 9.97$, $df = 73$, $P = .002$). Finally, smaller ants took 9.4 ± 3.7 s to cross from a windless to a windy section, whereas larger ants took 1.7 ± 0.06 s ($F = 9.87$, $df = 73$, $P = .002$). In all models, the ant load was not significant (all $P > .12$; table A2), and these differences occurred despite the fact that large ants, compared with small ants, transported loads with larger areas (mean area \pm SE: 19 ± 2.1 vs. 11 ± 2.3 mm²; $t = 2.24$, $df = 34.9$, $P = .03$). Data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d22f7> (Alma et al. 2016b). The higher frequency of larger foragers under windy conditions increased the RFI by an estimated 32% compared with that if the same ant size frequency had occurred in windless conditions (table A1).

Discussion

One advantage of group living is the option to collectively solve adverse conditions. Here, we described how colonies of the leaf-cutting ant *Acromyrmex lobicornis* reduced the problems imposed by wind by increasing the frequency of the type of workers that are better able to deal with this environmental factor. The size distribution of loaded and outbound ants was skewed toward larger sizes in windy conditions, and larger ants were less affected by wind than smaller ants with respect to speed, frequency of displacement from the trail, and crossing time. These differences are not attributable to ant allometry (i.e., larger legs in bigger ants; Zollikofer 1994b; Kaspari and Weiser 1999; Farji-Brener et al. 2004), because in our experimental design, each ant was used as its own control. The high frequency of larger foragers in windy conditions appears to be a colony response that reduces by 32% the negative effect of this environmental factor on their foraging.

The size distributions of both laden and outbound ants in windy conditions were similar, which suggests that the higher representation of larger ingoing foragers in windy periods was not a consequence of smaller ants having been blown away by wind when foraging. The fact that fewer smaller ants left the colony to forage on windy days suggests that some kind of communication occurred inside the nest. There are many examples where interactions and communication occur inside the nest. Harvesting ants regulate the activation of waiting foragers and the number of

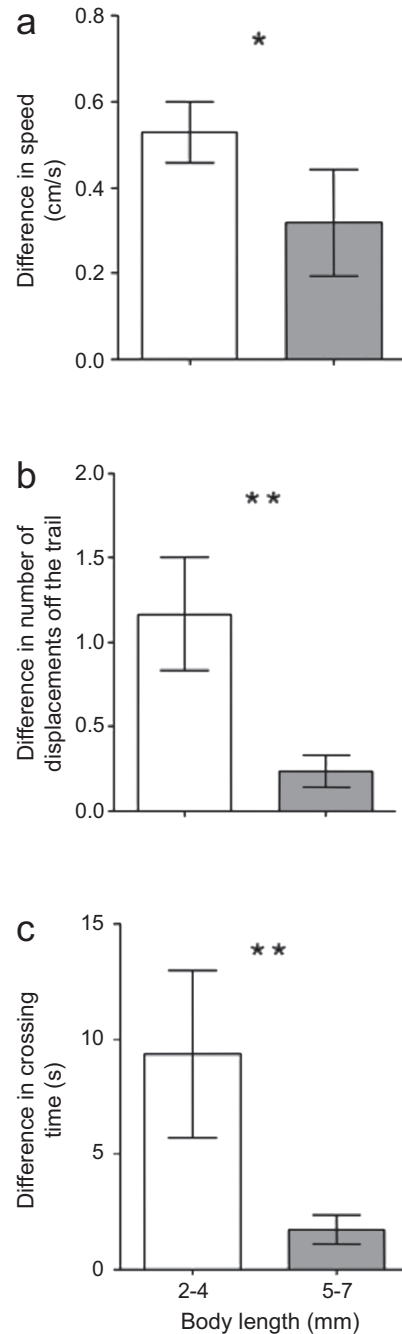


Figure 2: Differences in speed (speed without wind minus speed with wind; *a*), number of displacements (number with wind minus number without wind; *b*), and crossing time (i.e., crossing time from windless to windy condition minus crossing time between two windless conditions expressed as mean \pm SE; *c*), for smaller (2–4 mm; $n = 36$; gray bars) and larger (5–7 mm; $n = 51$; white bars) ants. One asterisk indicates $P = .07$, two asterisks indicate $P < .01$; see table A2. Data underlying this figure are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.d22f7> (Alma et al. 2016b).

foragers available to be activated through interaction near the nest entrance (Pless et al. 2015), and in leaf-cutting ants, gardening ants may transmit information on the unsuitability of a substrate to foragers inside the nest (North et al. 1999; Herz et al. 2008). Laboratory studies will be necessary to evaluate possible mechanisms of communication inside the nest regarding changes in the ant size frequency correlated with wind conditions. The activity of parasitic phorid flies, individual experience, information flow, waste management, and type of resource are known to affect division of labor in ants (Orr 1992; Clark 2006; Ballari et al. 2007; Evison and Ratnieks 2007; Ravary et al. 2007; Burd and Howard 2008; Röschar and Rocés 2011). As far as we know, this is the first study showing that environmental factors may also affect division of labor.

The delaying effect of wind on foragers was lower for larger ants, despite the fact that they carried bigger loads. This was so even though it is known that large loads impose higher resistance to wind (Alma et al. 2016a) and reduce an ant's stability (Röschar and Rocés 2002). The mechanism behind this pattern is probably that large ants, compared with small ants, can resist stronger detachment forces, such as wind gusts, because their adhesion to the ground is stronger (Zollikofer 1994a; Labonte and Federle 2015). When wind blows, the lower adhesion force of smaller ants may make it necessary to keep larger areas of their legs in contact with the ground (Moll et al. 2013), maybe adopting a "freezing reflex" (i.e., standing motionless with their legs spread in contact with the ground; Federle et al. 2000; see also video A1). Responses of these sorts could help to explain why, under windy conditions, smaller ants transport food more slowly than larger ants.

A previous study determined that leaf-cutting ants can individually decrease the effect of wind by selecting loads that reduce the wind interception (e.g., smaller loads; Alma et al. 2016a). Here, we documented that ant colonies can also reduce the negative effect of wind on foraging by increasing the number of workers that deal better with windy conditions (i.e., larger foragers). Furthermore, this collective solution might compensate for the reduction in load size (individual response to wind), because large ants are able to better resist the effect of wind and carry larger loads than small ants. This illustrates how social organisms can collectively mitigate the negative effect of environmental factors, highlighting one of the most important advantages of group living.

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Foraging activity of the leaf-cutting ant *Acromyrmex lobicornis* in Patagonia. Photo credit: Alejandro G. Farji-Brener.