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Trail Gradient Influences Load Size in Wild Leaf Cutter Ants

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Abstract Leaf cutter ants are model organisms in studies of central place foraging, but these ants carry smaller than optimal leaf fragments for their body size. Laboratory experiments show that load ratios (leaf fragment mass/ant mass) are higher on extreme downhill trails than on extreme uphill trails, which could explain deviations from optimality in the wild. Here we examine how trail gradient affects load size selection in the field for the first time. We measured load ratios on extreme natural gradients in the mountains of Monteverde, Costa Rica. In general, load ratios were found to be higher on downhill trails than on uphill trails as found in laboratory studies. Not all colonies showed this pattern, indicating that other factors may interact with trail gradient to determine load size selection. The results confirm that extreme trail gradients can contribute to determine optimal load sizes in central place foraging in natural conditions.

Keywords Leaf cutter ants · central place foraging · leaf size selection · load ratio

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Introduction

Central place foraging theory, an extension of the more general optimal foraging theory (Orians and Pearson 1979), focuses on how animals maximize energy intake per unit of time when foraging on patches located at some distance from a fixed location where they must return, such as a nest or colony (Schoener 1979; Houston and McNamara 1985; Rozen-Rechels et al. 2015). The theory has been supported by studies in mammals (Jenkins 1980; Huntly et al. 1986; McAleer and Giraldeau 2006), birds (Bryant and Turner 1982; Kacelnik 1984; Tamm 1989), and social insects (Traniello 1989; Holway and Case 2000). There have also been, however, multiple studies in which empirical data did not fit the predictions of the original theory, such as optimal food size carried back to a central place (Holder and Polis 1987; Kacelnik 1993; Alma et al. 2017).

The disagreement between empirical data and the predictions of the central place foraging theory with regard to food size carried may be caused by biotic and abiotic constraints not considered in the original theory (Alma et al. 2017). Examples of biotic constraints include information transfer rate (Roces and Núñez 1993), food processing time (Burd and Howard 2005), intraspecific competition (Adler and Gordon 2003), density-dependent habitat selection (Rozen-Rechels et al. 2015), predation risk (Olsson et al. 2008), metabolic or missed opportunity costs (Shrader et al. 2012), and differences in walking speed between foragers of different size that cause bottlenecks (Farji-Brener et al.

2011). Comparatively fewer studies have been conducted on the effect of abiotic constraints in the context of central place foraging (Alma et al. 2017). Examples of abiotic constraints include wind (Alma et al. 2017) and gravity (Lewis et al. 2008; Norton et al. 2014). To our knowledge, the effect of gravity has been tested only in laboratory settings.

Leaf-cutter ants (genus *Atta* and *Acromyrmex*) are ideal central-place foragers for studies on the effects of abiotic constraints on central place foraging due to their abundance, because they are amenable to manipulation, and because they carry smaller food fragments than predicted by the central place foraging theory (Burd 1996, 2001; Burd and Howard 2005; Lewis et al. 2008; Alma et al. 2017). They routinely clear long-lasting trails in the forest understory or grasslands that can stretch for hundreds of meters from a central permanent nest (Hölldobler and Wilson 1990). Forager ants cut and carry vegetable material, mostly leaf fragments, from canopy trees (Rockwood and Hubbell 1987; Burd 1996; Wirth et al. 1997) in their mandibles back to their nest, where they cultivate a specific fungus that constitutes the only food for their larvae (Hölldobler and Wilson 1990). Ants vary greatly in size within a nest, and the selection of fragment size by individual workers varies in relation to the size of the ant; i.e. larger ants carry larger fragments (Burd 1995).

Evidence on the effect of gravity on leaf cutter ant load size selection comes from two relatively recent laboratory studies. Lewis et al. (2008) demonstrated that laden *Atta cephalotes* ants caught on horizontal natural trails in the field decrease or increase their speed when put on artificial uphill or downhill gradients, respectively. In the lab, Lewis et al. (2008) manipulated trail gradients using short trails and a single young colony of *A. cephalotes* to study variation in load size selection in relation to trail gradient. They found that ants clearly carry the highest load to body size ratio (hereafter load ratio) when the trail that connects the food source with the nest was 90° downhill (−90°) in comparison to −45°, 0°, 45° and 90°. Load ratios were lower on uphill trails (45°, 90°) compared to horizontal (0°) and extreme (−90°) downhill trails. In addition, Lewis et al. (2008) did not find differences in ant mass between gradients, ruling out the possibility that the colony recruit larger foragers to uphill trails to increase leaf transport rates. Norton et al. (2014) found similar results with a laboratory colony of *Acromyrmex octospinosus*,

finding that load ratios where higher for ants returning to the nest vertically downwards (−90°) than for ants returning horizontally (0°) or vertically upwards (90°). These findings from two colonies that span both genera of leaf cutter ants suggest that these ants in general may adjust the size of the load they cut in response to the most extreme part of the trail (Lewis et al. 2008; Norton et al. 2014). Field studies are necessary to evaluate the generality of these results on large mature natural colonies with a full range of worker sizes that travel on long, real trails (Lewis et al. 2008; Norton et al. 2014).

In this field study, we tested the result from Lewis et al. (2008) and Norton et al. (2014) that *A. cephalotes* colonies would cut leaf sizes based on the most extreme part of the gradient that connects the food source to the nest. We specifically tested whether loading ratio was highest on the most extreme downhill trail found in the field in relation to the most extreme uphill trail. We also evaluated whether ant mass does not differ between downhill and uphill trails, as found by Lewis et al. (2008) and Norton et al. (2014).

Materials and Methods

Data Collection

Data were collected from three mature colonies of *A. cephalotes* found on hilly terrain in the premontane wet forests of Monteverde Costa Rica. Colonies sampled was several kilometers away from each other. Colonies were located on forest edges. Data were collected during the month of November 2018 between 9 a.m. and 1 p.m. on sunny days at an elevation of 1200–1300 m in the Premontane wet forest life zone (Haber 2000). This life zone constitutes the upper elevational limit for *A. cephalotes* in Monteverde (Longino 2000). Data were collected on mornings with no rain. Wind can affect load size selection in *Atta* (Alma et al. 2017), so we collected data only on days with very low wind velocities.

Two trails, one uphill and one downhill in relation to ants returning to the nest were chosen for each colony. The two trails include the most extreme gradient sections available for each colony. Uphill trails were mostly steep but included flat sections; downhill trails were mostly steep but also included flat sections. Trail lengths were not measured but uphill

and downhill trails were visually similar in length within each colony. Visually, trails ranged between 20 and 50 m between colonies. If anything, the uphill trails were slightly longer. The uphill and downhill trail for each colony were located in the same kind of habitat, either closed canopy or open areas near forest edge for both trails. Specific gradients of each trail were measured using a clinometer on the steepest part of the trail and are reported in Table 1. The slope did not vary greatly along the length of the trail, so the ants experienced the reported slope for at least 20 m.

Ant weight and load size (leaf weight) were recorded from ants carrying leaves back to the nest. Ant and load weights were recorded separately to the nearest 0.001 g in the field using a portable scale. Ants were sampled systematically by selecting the first laden ant to pass a fixed point on the trail following the weighing and release of the previously sampled ant. If more than one ant was present at the mark at one time, the ant with the larger leaf was chosen.

Trails were sampled daily in a pairwise fashion. Each day, approximately thirty ants were sampled from the uphill trail and thirty from the corresponding downhill trail of one colony. Two colonies were sampled for four days, and one colony was sampled for three days. In total, 656 ants were sampled across colonies.

Data Analysis

Load ratios were calculated by adding ant mass and load mass and dividing this quantity by ant mass (Lewis et al. 2008). Linear mixed models (LMMs) were used to compare load ratio and ant mass, individually, between trail type while controlling for dependency of data points collected from the same colony on the same day (i.e. colony identity and collection date were included as random effects in the models). Models were also fit using colony identify as a fixed effect to test for variation between

Table 1 The most extreme gradients found on uphill and downhill trails connecting the trees where leaf cutter ants (*Atta cephalotes*) foraged with their nest. Three wild colonies were sampled in Monteverde, Costa Rica

Gradient	Colony 1	Colony 2	Colony 3
uphill	55°	50°	60°
downhill	35°	30°	15°

colonies in the measured variables. Hence, two models were fit for each variable (load ratio, mass): a model that included colony identify as random effect (general model), and a colony that included colony as fixed effect (model to test for colony differences). Models were implemented using the R package "nlme" (Pinheiro et al. 2018). Variables were log transformed to meet the assumption of homogeneity and normal distribution of residuals. *P*-values correspond to analyses of deviance conducted with the function "Anova" of the R package "car" (Fox et al. 2011). Pairwise post-hoc comparisons of means were conducted using Tukey tests with the R package "emmeans" (Lenth et al. 2018). All analyses were conducted in R 3.5.1 (R Core Team 2018).

Results

Load Ratio

In general, *A. cephalotes* adjusted their load ratio differently between uphill and downhill trails (LMM: Chisq = 68.966, df = 1, $p < .0001$). Ratio for uphill trails was 13% lower than downhill trails (Fig. 1a). The LMM that included colony as a fixed effect revealed that the difference in load ratio between up and down trail was colony specific (colony identity and trail-gradient interaction term: Chisq = 9.3232, df, = 1, $p = 0.002$). Specifically, load ratios were higher in downhill trails for only 2 of the 3 colonies (Fig. 1b). Colony 1 did not show differences between the uphill and downhill trails even though the difference in steepness between trails was very similar to colony 2 (Table 1), which was the colony that showed the largest difference in load ratio between trails (Fig. 2). The only particularity of colony 1 in relation to the other two was that the uphill trails was visually more active, i.e. it consistently had more laden ants moving along the trail, compared to the other five trails studied.
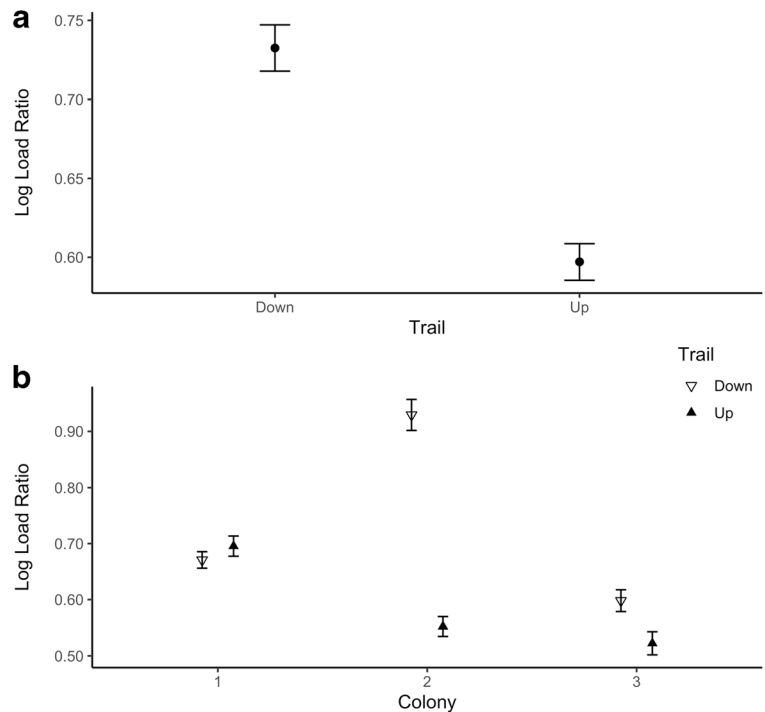
Ant Mass

In general, *A. cephalotes* mass was not found to be significant between trail gradients (LMM: Chisq = 2.1999, df = 1, $p = 0.138$; Fig. 2a). The LMM that included colony as a fixed effect revealed that the difference in ant mass between up and down trail was colony specific (colony identity and trail gradient

Fig. 1 Wild leaf cutter ants (*Atta cephalotes*) usually adjust their load ratio ([ant mass + load mass]/ ant mass) in response to trail gradient in a tropical premontane wet forest in Costa Rica. Load ratios are presented for uphill and downhill trails across the three colonies sampled (**a**) and separated by colony (**b**). Load ratios in (**b**) were different between uphill and downhill colonies for all comparisons except for colony 1 (Tukey post hoc test comparison, $p < 0.05$). Error bars represent one standard error

interaction term: Chisq = 15.8122, df, = 1, $p < 0.0001$). Specifically, ant mass was not different between trail gradients in one colony, higher in the

downhill trail of another colony, and higher in the uphill trail of the third colony (Fig. 2b). It is worth noticing that the only colony that showed higher

Fig. 2 Wild leaf cutter ant (*Atta cephalotes*) colonies usually adjust the mass of foraging in response to trail gradient in a tropical premontane wet forest in Costa Rica. Ant masses are presented for uphill and downhill trails across the three colonies sampled (**a**) and separated by colony (**b**). Masses in (**b**) were different between uphill and downhill colonies for all colonies except for colony 1 (Tukey post hoc test comparison, p < 0.05). Error bars represent one standard error. Masses were originally measured in grams
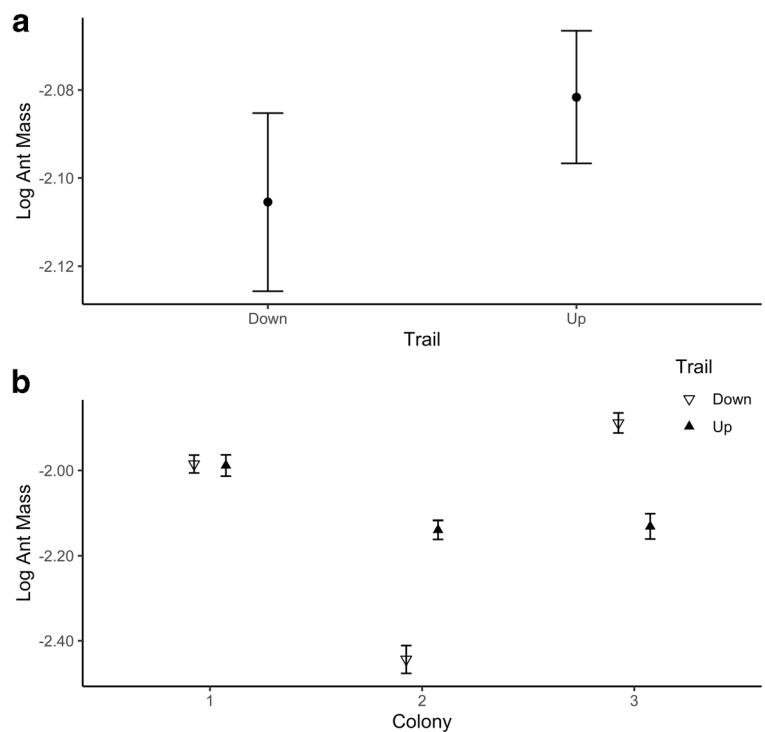
average ant mass on the uphill trail (colony 2) also showed a lower load ratio on the uphill trail (Fig. 1b).

Discussion

We evaluated whether wild *A. cephalotes* vary the size of the leaf fragments they carry in response to the gradient of the trail that connects the trees where they forage with the nest. We found that laden ants traveling downhill 15—35° tended to carry heavier loads than laden ants carrying leaf fragments uphill 50—60°. The absolute values and the magnitude of the difference in load ratio between extreme uphill and downhill trails in our field study match the values reported by Lewis et al. (2008) for *A. cephalotes* on more extreme gradients under laboratory conditions (−90° vs 45° and 90°). Lewis et al. (2008) did not find a difference in load ratio between −45° and 45° gradients, which are more similar to the extreme gradients we found in the field. This discrepancy between laboratory and field could be due to trail distance and environmental conditions. Trail distance in the laboratory setting of Lewis et al. (2008) was one meter long, whereas natural trails in this study where at least 20 times longer. The cost of transporting food to the nest on a steep uphill trail should be lower for shorter distances compared to longer distances (Orians and Pearson 1979; Roces 1990) so it is not surprising that the ants show a difference in load ratio only between very extreme gradient conditions, such as −90° vs 45° in the study by Lewis et al. (2008). In the field, the combined effect of long distance and steep gradient may force the ants to reduce load ratio at less extreme conditions (e.g. -50° vs 30°). Environmental conditions may also contribute to this reduction in load ratio. Our study site is located at the upper limit of elevational distribution for *A. cephalotes* in the region (Longino 2000). At this elevation either the ants or the fungus they grow may be challenged by low temperatures and humidity (Mueller et al. 2011), which may also force them to reduce load size on moderately steep uphill gradients.

In general, the results of our field study support the results from previous studies conducted in the lab. Both *Atta* and *Acromyrmex* respond to gravitational cues (Vilela et al. 1987) and the results from Lewis et al. (2008) and Norton et al. (2014) lab studies confirmed by our study in the field indicate that leaf cutter ants alter their load ratios according to the most extreme gradient

of the trail. In particular, ants cut and transport smaller loads back to the nest when travelling uphill in response to the difficulty imposed by the gradient, which make them walk slower (Lewis et al. 2008; Norton et al. 2014). They also seem to take advantage of downhill trails to carry heavier loads (Lewis et al. 2008; Norton et al. 2014). In the case of extreme downhill gradients, such as a vertical tree trunk, the slope may add torque created by the weight of the load size carried overhead, which may destabilize the ant, forcing it to limit the weight of the load carried. In any case, the load carried on downhill trials would be heavier than on uphill trails of similar length on the same type of substrate and under the same environmental conditions. The natural trails cleared by leaf cutter ants consist of multiple up and down sections, so adjustment of load size in response to gravity are likely very common in nature (Lewis et al. 2008), especially in hilly areas. Leaf cutter ants usually forage in the canopy (Rockwood and Hubbell 1987; Burd 1996; Wirth et al. 1997) so extreme downhill trail sections (i.e. tree trunks) are common in their foraging patterns even in flat areas. The response to trail gradient seems to be an individual foraging decision. *Atta* ants are capable of both individual and collective flexible foraging decisions (Dussutour et al. 2008). A collective decision could involve sending larger ants to uphill trails because larger ants generally walk faster and can carry heavier loads (Rudolph and Loudon 1986; Shutler and Mullie 1991; Burd 1995), which would compensate for reduced leaf transport rate on uphill trails (Lewis et al. 2008). The general lack of difference in ant mass between trail gradients across this and the laboratory studies, however, suggests that the decision is individual (Lewis et al. 2008; Norton et al. 2014). The only colony that recruited larger ants to the uphill trail in our study also showed lower load ratio on that trail in relation to the downhill trail, not supporting the idea of collective responses at the colony level.

We found that not all colonies behaved in the way expected following the laboratory results. One of the three colonies studied did not show a difference in load ratio between the uphill and downhill trails. Ant mass was very similar between trails in this colony, indicating a lack of a collective response to the gradient at the colony level as well. The difference in gradient between the uphill and downhill trail in this colony was very similar to the difference observed in one of the colonies that showed the expected difference in load ratio between trails. This suggests that among-colony

differences in steepness between up and downhill trails cannot explain variation in the response to trail gradient among colonies. The uphill trails of the colony that did not show differences appeared more active, i.e. it showed higher traffic, in relation to the other trails studied. *Atta* ants are expected to reduce the size of their loads in conditions of high traffic to prevent bottlenecks and increase the rate of food delivery to the colony (Farji-Brener et al. 2011). If the ants had responded to higher traffic on the uphill trail, they should have reduced load size on that trail, increasing the difference in load ratio with the downhill trail. High traffic on the uphill trail cannot therefore explain the observed lack of difference between trails in this colony. Traffic levels on trails of *A. cephalotes* in Monteverde are generally low, likely due to below optimal temperatures and humidity levels at the highest elevational limits of their distribution (Freeman and Chaves-Campos 2016). Hence, traffic congestions are unlikely to explain any of the results observed in this study. Among-colony variation in load size detected in this study indicates that the response to trail gradients in the field is complex, and that it may interact with biotic and/or abiotic factors not identified in this study.

In conclusion, we have shown that trail gradient contributes to variation in load size in wild leaf cutter ants. Biotic factors, such as food processing time at the nest (Burd and Howard 2005) may also contribute to load size selection in central place foragers but our results indicate that the physical characteristics of the environment significantly influences load size selection in the wild. More generally, our results support recent studies (Lewis et al. 2008; Norton et al. 2014) that suggest that the physical properties of the environments traveled by central place foragers, should be considered in models that predict optimal foraging.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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