

The Influence of Fruit Morphology and Habitat Structure on Ant-Seed Interactions: A Study with Artificial Fruits

by

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ABSTRACT

Artificial fruits chemically designed to simulate lipid-rich diaspores were used to test for the effect of fruit morphology and habitat structure on ant-seed interactions in the Atlantic Forest of SE Brazil. The outcome of the interaction (i.e., if the “fruit” was removed, cleaned by ants on the spot or had no interaction with ants) and the time of ant response were the investigated variables. Two fruit models simulating “drupes” and “arilate” diaspores were used to test for morphological effects and four habitat attributes (litter depth, number of logs, number of trees, and percentage of bromeliad coverage on the forest floor), which are likely to be correlated with the diversity and abundance of ants in the study site, were measured to test for the effect of habitat structure. The proportion of fruits removed or cleaned did not differ between the two morphological models. Sites in which fruits were cleaned had a higher number of trees than those in which no interaction occurred, which may be a result of the foraging behavior of arboreal ants that frequently descend to the forest floor to exploit fleshy diaspores. Sites in which model removal occurred had lower litter depth than both those in which models were cleaned and those in which no interaction occurred. A negative correlation was observed between litter depth and ant response time. Accumulation of leaf litter on a given spot may have constrained the movements of large ants in general, and ponerine ants (that are important seed removers) in particular. We concluded that small-scale patchiness in habitat structure influences

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the probability of occurrence and the ecological outcome of interactions between ants and non-myrmecochorous, fleshy diaspores.

Key Words: seed removal, seed cleaning, *Odontomachus chelifer*, *Pachycondyla striata*, Ilha do Cardoso

INTRODUCTION

Ants are ecologically dominant organisms playing a key role in the fate of plant seeds. The evolutionary and ecological consequences of ant-seed interactions have been widely studied worldwide, especially for myrmecochorous species – i. e., those plants whose diaspores present morphological and chemical traits thought to be adaptations that have evolved in a context of mutualism with seed-dispersing ants (Beatie 1985, Hölldobler & Wilson 1990, Hughes & Westoby 1990, Mark & Olesen 1996). In the last ten years several studies have focused on the interactions of Atlantic Forest ants and non-myrmecochorous, primarily vertebrate-dispersed plant species (Oliveira *et al.* 1995, Pizo & Oliveira 1998, 2000, 2001, Passos & Oliveira 2002, 2003, 2004, Guimarães & Cogni 2002). Ants exploit the fleshy portion (pulp or aril) that surrounds non-myrmecochorous seeds, and may carry diaspores to their nests or exploit it on spot. Ant-seed interactions are known to have diverse impacts on the survival of seeds and seedlings of various Neotropical tree species. For instance, seed cleaning by ants facilitates the germination of several non-myrmecochorous plants (Oliveira *et al.* 1995, Pizo & Oliveira 1998, Guimarães & Cogni 2002, Passos & Oliveira 2003, 2004). Moreover, diaspores transported to ant nests may also escape vertebrate predation and be deposited in nutrient enriched sites in which seedling survival may be higher (Passos & Oliveira 2002, 2004).

It has been demonstrated that small diaspores are usually carried by ants to their nests, while large diaspores tend to be exploited by workers on the spot (Pizo & Oliveira 2001). However, diaspores may also differ in the chemical content, amount, and distribution of the fleshy portion covering the seed. In non-myrmecochorous diaspores, the seeds may be partially covered by fleshy tissues (as in *C. vernalis*, Guimarães & Cogni 2002), or may be totally covered by the pulp, as in drupes and berries. In the case of true myrmecochorous species, diaspore morphology has been shown to influence diaspore selection and removal by ants (Hughes & Westoby 1992, Gorb & Gorb 1995, Mark & Olesen 1996). However, it is still unknown if, other things being equal, ants are able to distinguish between these patterns of resource distribution in fleshy diaspores, and eventually behave differently in response to them. Another poorly-explored aspect of the interaction between ants and

non-myrmechochorous diaspores in Neotropical forests is the influence of habitat structure on the outcome of the interaction (Pizo & Oliveira 1999). The composition of local ant assemblages, as well as ant abundance, are directly constrained by the availability of key resources, such as prey and nesting sites, which, in turn, are influenced by habitat structure (Kaspari 1996). Given that different ant species treat diaspores in different manners (Pizo & Oliveira 2000), and habitat structure influences the distribution and abundance of ant species, the fate of a seed is likely to depend on the characteristics of the site in which it lands on the forest floor.

In this paper we investigated the effects of (1) resource distribution (diaspore morphology) and (2) habitat structure on the output of ant-seed interactions (categorized as seed removal, seed cleaning on the spot, or no interaction) using artificial fruit models, a new approach to the study of ant-seed interactions in the field that permitted the control of highly variable features of true diaspores (e.g., size and chemical composition). The following questions were addressed (a) is the fate of a diaspore affected by its morphology? (b) can small-scale variation in habitat structure affect the fate of the diaspore by locally constraining the kinds of ant-diaspore interactions?, and (c) is the time spent by ants to find and interact with the models related to variations in habitat structure?

METHODS

Study area

Field work was undertaken in May 2002 in the Ilha do Cardoso State Park (25° 03'S, 47° 53'W), São Paulo State, SE Brazil. The park is located in an oceanic island of about 22.500 ha covered by relatively undisturbed Atlantic Rainforest. The experiments were performed in an area of sandy forest (*restinga*). This forest grows on sandy soils (2-3 m a. s. l.) and is characterized by a diverse tree flora that forms an open canopy (5 to 15 m high). Terrestrial bromeliads are abundant and cover large areas of the *restinga* floor [for additional details on the study area see Barros *et al.* (1991).

Experiment

Influence of diaspore morphology on ant-seed interactions

Artificial fruits were made by the combination of two components. The "resource" was represented by small portions (76.4 ± 11.2 mg, $n = 30$) of a white mass made with cotton fat SC (75%), casein (7%), calcium carbonate (3%), fructose (4,8%), sucrose (0,5%), glucose (4,7%), and maltodextrin (5%). The mass was developed by ITAL (Institute of Food

Technology, Campinas, Brazil), and simulates the chemical composition of lipid-rich diaspores, which usually attract a great number and variety of ants (Hughes *et al.* 1994, Pizo & Oliveira 2001). Small plastic spheres (65.3 ± 8.1 mg, diameter of 0.5 cm) were used as “seeds” in the models. Therefore, the total weight of artificial fruits was about 150 mg, which is within the size range of many fleshy diaspores with which ants interact at the study site (Passos & Oliveira 2003).

In the experiment we evaluated the effect of diaspore morphology on the kind and time of ant response to diaspores. Two categories of fruit models were used. In “drupe” models the resource (white mass) covered the entire seed (plastic spheres), while in the “arilate” model the resource covered half the seed. The amount of resource did not differ between the two categories of models. Thirty replicates of each fruit model were alternately placed in 60 experimental stations set 10 m apart along a 600-m transect, each station receiving only one fruit model. Stations were checked one, three, and six hours after the experiment started (from 0900 to 1500) in order to record ant response (i.e., seed removal, cleaning, or no interaction). To evaluate if the frequencies of each kind of response differed between arilate and drupe models we used a permutation test for contingency tables (Blank *et al.* 2001).

Influence of habitat structure on ant-seed interactions

The experiment also explored the influence of habitat structure on (1) the kind of ant response, and (2) the time spent by ants to interact with the models. In order to select habitat attributes to be measured we considered information available on the natural history of the most common ant species in the Cardoso's restinga. Local myrmecofauna includes the giant ponerine predators *Pachycondyla striata* Fr. Smith and *Odontomachus chelifer* (Latreille), which feed on small soft-bodied litter insects, specially termites (Fowler 1980, Medeiros 1997) and are known to nest and forage in the leaf litter (Medeiros 1997, Fowler 1980). The small, group-recruiting, ponerinae *Gnamptogenys moelleri* (Forel) is an example of a bromeliad-nesting species found in the site (Cogni & Oliveira, in press), while several *Crematogaster* species make their nests on trees. All these ant species are known to exploit non-myrmecochorous diaspores on the floor of the study site (Passos & Oliveira 2003).

The selected habitat attributes were (a) leaf-litter depth (measured with a ruler in the central point of each station), (b) number of logs, (c) number of trees (dbh > 1 cm), and (d) percentage of bromeliads coverage on forest floor (estimated in categories of 0-25, 26-50, 51-75, 76-100%). The last three habitat descriptors were measured in a 2 m radius area centered at each station.

To test if the values of habitat descriptors as well as ant responses are distance independent, we applied Paired Quadrat Variance (PQV) tests with the software Passage 1.0 (Rosenberg 2003), and also the Run Test suggested by Knight (1974). PQV was used for spatial autocorrelation tests with quantitative data while the Run Test was applied for binary data. The Run Test corresponds to a Join Counts test for transects (Dale *et al.* 2002). Although PQV is not a spatial autocorrelation analysis, it should be interpreted as such since a variogram and a PQV for transects are equivalent (Ver Hoef *et al.* 1993, Dale *et al.* 2002). Significance tests (95%) for the PQV were obtained through 999 randomizations. The values of each habitat descriptor were compared among stations in which models were removed, cleaned, or that had no interaction, through a Kuskal-Wallis test. Pairwise comparisons of the three possible combinations of interaction categories (removal, cleaning, no interaction) were used as *a posteriori* tests to access the significance of observed differences in the value of each habitat descriptors among interaction categories (Zar 1998).

To evaluate if the time spent to record ant- fruit models interactions (1h, 3h, 6h, > 6h) was correlated to the values of habitat descriptors, we

Table 1. Ant species observed removing/cleaning artificial fruits in an area of sandy forest (*restinga*) in the Ilha do Cardoso State Park, SP, Brazil. Observations were performed one day after the experiment but using the same stations and models. (*) Species observed cleaning fruit models. (**) Species observed removing fruit models.

Ant species	Frequency
FORMICINAE	
<i>Paratrechina</i> sp. 1	2*
<i>Paratrechina</i> sp. 2	1*
<i>Paratrechina</i> sp. 3	2*
<i>Brachymyrmex</i> sp.	1*
MYRMICINAE	
<i>Crematogaster</i> sp.	2*
<i>Pheidole</i> sp. 1	4*
<i>Pheidole</i> sp. 2	1*
<i>Solenopsis</i> sp.	3*
<i>Acromyrmex</i> sp.	1?
PONERINAE	
<i>Pachycondyla striata</i>	2**
<i>Odontomachus chelifer</i>	1**
<i>Gnamptogenys moelleri</i>	1*

performed Spearman rank correlations. All permutations tests were made using the software Resampling Stats (Blank *et al.* 2001).

RESULTS

Twelve species of ants were recorded interacting with fruit models (Table 1). After 6 hours, cleaning or removal of fruits models were recorded in the majority (92 %) of the stations, with the relative proportion of removed models increasing through time (Table 2). Models were removed only by large ants (> 2 cm body length), such as the ponerines *Odontomachus chelifer* and *Pachycondyla striata*. Smaller species, however, such as *Crematogaster* spp., *Pheidole* spp., and *Paratrechina* spp., recruited numerous nestmates that monop-

able 2. Temporal variation in observed ant interactions with artificial fruits in a sandy forest (Ilha do Cardoso State Park, São Paulo, Brazil). Values are percentages based on observations of 60 fruit models distributed along a transect in the forest floor.

Kind of interaction	After 1h	After 3 h	After 6 h
Model removed	33.3	50	60
Model being cleaned	55	33.3	32
No interaction	21.7	17.7	8

lized the models and remained cleaning them during a period of up to 6 h.

The proportion of models removed, cleaned, and with no interaction did not differ between drupe and arilate models (Contingency Table, 1000 permutations, $P = 0.836$). The time spent to record ant-models interactions did not differ between both morphological models ($P = 0.921$ for seed removal and $P = 0.584$ for seed cleaning, considering only results observed in the first-hour of experiment, since after that the high number of fruit models removed precluded the analysis). PQV analysis did not detect spatial dependence between the values of habitat descriptors, nor did the Run Test between ant responses in neighbor stations. Since spatial autocorrelation was not detected, and no correlation was found between any pair of habitat descriptors (Spearman Rank Correlation), we proceeded to explore correlations between each habitat descriptor and the time of ant responses assuming that any station is a true replicate.

A significant difference in the number of trees was observed among stations in which models were removed, cleaned, and with no interaction ($H = 6.78$; $df = 2$; $P = 0.034$). Stations in which models were cleaned presented more trees than those in which no interaction was observed ($P = 0.013$; *a posteriori test*), but no difference was found between stations with models removed and those with models cleaned ($P > 0.05$; *a posteriori test*). The Kruskal-Wallis test also pointed a difference in leaf litter depth among stations in which models were removed, cleaned, and with no interaction ($H = 14.21$; $df = 2$; $P < 0.001$). Leaf-litter depth was lower in stations in which models were removed than in stations in which they were cleaned ($P < 0.001$; *a posteriori test*) or that had no interaction ($P < 0.01$; *posteriori test*). A negative correlation was observed between litter depth and ant response time ($r_s = 0.58$; $P < 0.01$ $N = 26$).

DISCUSSION

Contrary to overall diaspore size, the amount of resource, and the resource/seed ratio (Hughes & Westoby 1992, Gorb & Gorb 1995, Mark & Olesen 1996, Pizo & Oliveira 2001), our results revealed that the distribution of resource in the artificial fruit is not a morphological feature that matters for the interaction with ants. These results suggest that, other things being equal, drupes that have the seed totally enveloped by the pulp, and arilate diaspores that have the seed only partially covered by the aril have the same opportunity to interact with ants.

The positive relationship between tree density and ant-model interaction may be a result of the foraging behavior of small, arboreal ants, such as *Crematogaster*, which frequently descend to the forest floor to exploit fleshy diaspores (Passos & Oliveira 2003). A high density of trees in the immediate vicinity of a given diaspore probably increases the probability of interaction with arboreal ants. Accumulation of leaf litter on a given spot, on the other hand, may constrain the movements of larger ants in general, and ponerines (mainly *Odontomachus chelifer* and *Pachycondyla striata*, two important seed removers) in particular, producing the negative relationship among litter depth and both ant response time and model removal.

The influence of litter density on ant foraging patterns was recently illustrated by an experiment with tropical litter ants (Farji-Brener *et al.* 2004) showing that small-bodied ants were more likely to first reach food in ground plots with high litter rugosity (similar to 1 kg of litter fall), while large-bodied ant species were dominants in plots in which litter was removed. Since foraging movements of ants are constrained by ground patch structure, the forest floor can be interpreted as a mosaic of “hot” and “cold” spots for both seed removal (Crist & Wiens 1994) and cleaning by ants. Even if these “hot” and “cold” spots, rather than being fixed, shift with time, as noted for seed-dispersing Australian ants (Hughes & Westoby 1990), some diaspores are more likely to be rapidly removed/cleaned than others simply because of their spatial location. The availability of seed removal hot spots are likely to be important for several plant species, since rapid removal by ants may be crucial to avoid seed predation by vertebrates (Hughes & Westoby 1990).

In conclusion, we have found that patchiness in habitat structure at a small spatial scale influences the probability of occurrence and the outcome of interactions between ants and artificial fruits that simulate flesh, non-myrmecochorous diaspores. How such influence varies over broad spatial scales is the next step to be pursued in the search for

patterns in the interaction between ants and non-myrmecochorous diaspores in Neotropical forests (Pizo *et al.* 2004). For this, experiments with artificial fruits are especially useful because it circumvents the problem of finding a fruit whose morphological and chemical features do not vary geographically.

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