Ant-diaspore interactions during secondary succession in the Atlantic forest of Brazil

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Abstract: Animal-plant interactions are important for the recovery of diversity and processes in secondary forests, which increasingly dominate the tropical landscape. We used a combination of observational and experimental approaches to study the interactions of ants with diaspores across a successional gradient of forests in Southern Brazil, from August 2007 to April 2008. In addition to diaspor removal rates, we assessed the species richness, diversity and behaviour of ants interacting with diaspores, in three replicated sites of four successional stages of forests. We recorded 22 ant species interacting with diaspores (an estimated 15% of the total species pool in the region). Species richness and diversity did not differ among successional stages but the behaviour of ants towards diaspores changed with the age of secondary forests. In old successional stages the removal of entire diaspores was more common than in young successional stages of forests. Concordantly, diaspor removal rates were lowest in the youngest successional stage of secondary forests and increased with the age of forests. These results indicate that ant-diaspore interactions in secondary forests are disturbed and lower removal rates in secondary forests are likely to constrain the recruitment of plant populations during secondary succession. Rev. Biol. Trop. 60 (2): 933-942. Epub 2012 June 01.

Key words: ant-plant interactions, forest recovery, seed dispersal, seed predation, succession, tropical secondary forests.

Tropical old-growth forests are vanishing at alarming rates and are converted into pastures and agricultural lands (Wright 2005, Chazdon et al. 2009). The total area covered by forests, however, is increasing in tropical countries across the globe (Wright & Muller-Landau 2006). This situation arises because of secondary forests, regenerating on former agricultural land increasingly dominate the tropical landscape (FAO 2007). Animal-plant interactions are important for the recovery of diversity and ecological processes in secondary forests (Guariguata & Ostertag 2001, Chazdon 2003). Secondary forests emerging in human-impacted landscapes do not match the original old-growth forests in species composition (Hobbs et al. 2006). Therefore, it is still uncertain to what extent secondary forests will maintain similar processes with respect to new combinations of species.

Most plants in tropical forests produce diaspores (fruits, seeds) adapted for animal dispersal (Frankie et al. 1974, Howe & Smallwood 1982). Both seed dispersal and seed predation are important ecological processes during forest regeneration as they influence the spatial distribution and survival of seedlings, and ultimately the structure of plant communities (Hulme 1998, Guariguata & Ostertag 2001, Wang & Smith 2002). The disruption of
these processes may have serious consequences for tropical forest regeneration. For instance, the extinction of animal dispersers in secondary forests can lead to arrested succession (Sarmiento 1997, Holl 2000), or even to cascading co-extinctions (Koh et al. 2004). Thus, a better understanding of how these ecological processes recover during the regeneration of tropical forests is an important step toward conservation and management of these forests (Guariguata & Ostertag 2001).

Ants (Hymenoptera: Formicidae) are important dispersers and predators of diaspores in tropical forests (Levey & Byrne 1993, Pizo & Oliveira 2000, Passos & Oliveira 2003). Ants contribute to the plant reproductive cycle by transporting fallen diaspores from the forest floor or acting as secondary dispersers transporting diaspores from vertebrate feces into their nests, where they either store them or remove the remaining fleshy pulp for consumption and afterwards discard the seeds in refuse piles (Beattie 1985, Kaspari 1993, Vander Wall & Logland 2004, Rico-Gray & Oliveira 2007). Ants may also remove the fleshy part of diaspores in situ promoting better conditions for germination and establishment of seedlings (Ohkawara & Akino 2005). The diversity of ground foraging ants in secondary forests is often lower than in old-growth forests (Dunn 2004, Silva et al. 2007) and we found the same diversity pattern of ants for the Atlantic forest in Southern Brazil (Bihn et al. 2008). The interactions between ants and non-myrmecochorous diaspores in tropical forests are mostly not species specific, i.e. ants interact with diaspores of many plant species and vice versa (Pizo & Oliveira 2000, Passos & Oliveira 2003). Thus, the reduced species diversity of ants in secondary forests might have no consequences for the interactions between ants and diaspores as long as a minimum subset of species that interact with diaspores is retained in secondary forests. Instead, the rate of seed removal and dispersal by ants might be a function of ant activity and relative abundance (Gove et al. 2007, Manzaneda & Rey 2008, Zelikova & Breed 2008). In the Atlantic forest of Southern Brazil (Bihn et al. 2008) and in other tropical forests (Armbrecht et al. 2004, Silva et al. 2007), the abundance of ant species increases with successional age of forests. Thus, each diaspore on the ground of mature tropical forests might have a higher chance of being encountered, removed and possibly dispersed by ants, than diaspores on the ground of early successional stages of secondary forests. Therefore, we expected that diaspore removal rates are highest in old-growth forest and will increase with increasing age of secondary forests.

We used a combination of experimental and observational approaches to study shifts in ant-diaspore interactions across a successional gradient in the Atlantic Forest of Southern Brazil. Exclusion experiments were used to estimate rates of diaspore removal in secondary forests differing in age since abandonment, and in old-growth forests. Additionally, we assessed the diversity and behaviour of ants interacting with diaspores on the forest floor in different successional stages of forests.

MATERIALS AND METHODS

Study region and sites: Field studies were conducted at the Rio Cachoeira Nature Reserve (25o18’51” S - 48o41’45” W) in the state of Paraná, Southern Brazil, from August 2007-April 2008. The regional climate is classified as humid sub-tropical (Köppen’s Cfa). The area receives an annual rainfall of 2 600mm without a marked dry season, less rain occurs between May and August. The mean temperature varies between 16.2°C in July and 24.5°C in February (IPARDES 1991). Tropical lowland and submontane forests originally covered the area, but these suffered intense exploitation and large portions had been converted to pastures. The resulting landscape mosaic consisted of recently abandoned pastures, secondary forests in various stages of succession, and old-growth forests (Ferretti & Britz 2006).

The experiments were carried out in four successional stages: secondary forests of 9–11 years, 15–20 years, and 40–55 years since abandonment of pastures, and old-growth
forests (sensu Clark 1996). Old-growth forests had not been logged for at least 100 years. Three study sites were selected for each successional stage. Land-use history for study sites was established through interviews with residents and reserve staff corroborated by inspection of high resolution, geocoded orthophotos from the years 1952, 1980 and 2002 in a GIS environment. Replicated sites of a particular successional stage were separated by an average distance of 4km (range: 1-6km). Study plots were never situated in one continuous patch of the same vegetation type, but separated by areas of different successional stages, pastures, among others (Bihn et al. 2008 for a map of the reserve and the location of the study sites within it).

Ant-diaspore interactions: To determine which ants interact with diaspores on the forest floor, as well as how they interact with diaspores, we made observations at experimental diaspore depots, mimicking clumps of dispersed diaspores. In March and April 2008 we established five experimental diaspore depots, separated by a minimum of 10m, in each study site. Diaspore depots consisted of 30 diaspores of three tree species (10 diaspores of each species) placed on a plastic plate (8.5cm diameter) on the forest floor. Diaspores were collected in the study region from at least three different fruiting individuals during their natural dispersal period and were from the following species: Pera glabrata (Schott) Baill., Hyeronima alchorneoides M. Allemão and Trema micrantha (L.) Blume. We used only diaspores collected on the same day in our experiments because stored diaspores or diaspores that had been refrigerated could suffer from desiccation and chilling injuries thus changing some of their characteristics and introducing bias (Theilade & Petri 2003). The plant species were selected because they were fruiting during the study period, were common in the study area and are known to be attractive to ants (Pizo & Oliveira 2000, Passos & Oliveira 2003), which we confirmed in preliminary experiments. The selected species are canopy trees, fruiting from January to April, their diaspores are primarily dispersed by birds and they have a wide distribution within the reserve and the Atlantic Forest. P. glabrata fruits consist of a dry unpalatable outer covering that splits when mature exposing three to four diaspores. The diaspores consist of brilliant black seeds (3mm diameter, 0.02g fresh weight; Passos & Oliveira 2003), which are partially surrounded by a cup-like, red, fleshy, aril-like endocarp. H. alchorneoides has roundish fruits (8mm diameter, 0.04g fresh weight; Flores 1993) consisting of a reddish fleshy pulp and one single seed. T. micrantha fruits are the smallest fruits used in the experiment (3mm diameter, 0.01g fresh weight; Silveira et al. 2003). The fruit consists of a green fleshy pulp that turns orange when mature and encase a single seed.

Observations at diaspore depots were made between 10:00 and 14:00. Each diaspore depot was checked after 15, 30, 45, 60, 75 and 90min. The species and the abundance of ants interacting with diaspores were recorded as well as their behaviour towards the diaspores. To determine whether the species richness and the diversity of ants interacting with diaspores increases during secondary forest succession, we applied a Poisson generalized linear model (GLM) with a logarithmic link function in the analysis of ant species richness among successional stages. In the analysis of the diversity of ants interacting with diaspores we calculated the Shannon-Weiner diversity index (H’) and evenness (E_H) (Krebs 1989), the diversity and evenness of ants were tested with a Gaussian generalized linear model (GLM).

The behaviour of ants interacting with diaspores was classified as “seed cleaning” (one or more ants removing the fleshy part of diaspores, but the diaspore is not removed) or “removal of diaspores” (one or more ants removing diaspores from the depot). For each ant species recorded we counted the number of stations where we observed these ant-diaspore interactions. Then we summed up the number of behaviour observations for each successional stage. Because the frequency of observations was low for some combinations of behaviour
and successional stage, we pooled the frequencies of the two youngest and the two oldest successional stages. To determine whether the behaviour of ants towards diaspores differed among successional stages we compared the rates of the two behaviours with Fisher’s Exact test. Voucher specimens of ants were collected, identified to species/morphospecies and deposited at the Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Brazil (DZUP).

**Diaspore removal experiment:** Rates of diaspore removal by ants in different successional stages were measured in an exclusion experiment. At each site we set up 10 sampling stations separated by a minimum of 10m. At each station we placed one plastic plate (8.5cm in diameter) with 1g of milled Tartary buckwheat seeds (*Fagopyrum tataricum* (L.) Gaertn.). Seed fragments ranged from 1-4mm in diameter and had a mean dry mass of 12mg±1 SE (n=50). We used relatively small seed sizes because the removal of small diaspores is not constrained by the size of the ant but large diaspores tend to be removed only by larger ants (Pfeiffer *et al.* 2006). As in many other seed removal experiments worldwide (Thompson *et al.* 1991, Christianini & Galetti 2007) we used diaspores that are not native to the study region. Diaspores of native species were not used due to the large amount of diaspores needed in order to replicate the experiment across all sites and successional stages and because native diaspores were not available during two seasons of the year (summer and winter). In preliminary diaspore removal trials we confirmed that seed fragments of Tartary buckwheat were attractive to ants. Thus, we could assess diaspore removal rates in an unbiased way across different stages of forest succession and across two seasons.

Plates were covered with a wire cage (15cm x 15cm x 15cm, 1cm mesh size) to exclude vertebrates but allowing ants to access the diaspores. Cages were secured to the ground with wire anchors. We placed a transparent plastic cover over each plate to prevent the diaspores from being washed away by rain. Sampling stations were placed at least 50m from trails, or any other different habitat (e.g. forest gaps) to reduce edge effects. We placed 1g of diaspores on each plate, arranged in a pile of ~1cm in diameter. After 48hr diaspores were carefully collected into plastic bags, oven-dried (at 60°C for three days) and reweighted. We used the difference in weight as a measure of diaspores removed. Diaspore removal trials were performed in August 2007 (winter) and March 2008 (summer). Sampling stations were placed at different locations within sites during winter and summer sampling. To reduce possible variation in diaspore removal due to weather conditions, we performed experiments in one site of each successional stage on the same day. Trials were performed in the absence of heavy rain.

Diaspore removal rates were analyzed with a two-factor nested ANOVA. The main factor in this analysis was “successional stage” (fixed factor; levels: 9-11yr, 15-20yr, 40-55yr, >100yr) with three “sites” (random factor) nested within each level of the main factor and 20 replicated observations for each combination of “successional stage” and “site”. We tested for a linear trend in changes of diaspore removal along the successional gradient employing polynomial contrast coefficients that were coded according to the age of the successional forest stages. Data on diaspore removal were log10-transformed prior to analysis to reduce skewness of the distribution.

**RESULTS**

**Ants interacting with diaspores:** We found ants interacting with diaspores at 56 of 60 diaspore depots. Ants discovered diaspores rapidly and in the majority of cases we observed interactions within 15min. after exposure of diaspores. We recorded 22 ant species from seven genera and four subfamilies interacting with diaspores at depots (Table 1). Species richness of ants did not differ among successional stages (GLM: p=0.32). We recorded 2.7
(±1.2 SE) species in study sites of 9-11 years old secondary forests, 4.3 (±0.9 SE) species in 15-20 years secondary forests, 5.7 (±1.5 SE) species in 40-55 years old secondary forests, and 3.7 (±0.9 SE) species in old growth forests (n=3 for each mean).

The diversity and evenness of ants interacting with diaspores did not differ across the successional gradient (GLM: p=0.30 and p=0.19, respectively). In secondary forests of 9-11 yr, $H^'=0.74(±0.5$ SE) and $E_H^'=0.97(±0.02$ SE), in 15-20 yr secondary forests $H^'=1.36(±0.2$ SE) and $E_H^'=0.95(±0.01$ SE), in 40-55 yr secondary forests $H^'=1.53(±0.2$ SE) and $E_H^'=0.93(±0.01$ SE) and $H^'=1.13(±0.3$ SE) and $E_H^'=0.91(±0.03$ SE) in old growth forests (n=3 for each mean).

In all successional stages “seed cleaning” was the most frequent behaviour of ants interacting with diaspores. Nevertheless, the proportion of seed depots where we observed ants removing diaspores increased from 19% in the youngest successional stages to 38% in the oldest successional stages (Fig. 1). This difference in the frequency of ant behaviour towards diaspores was significant (Fisher’s Exact test, p=0.049).

**Diaspore removal rates:** Diaspore removal rates in the 12 study sites varied from 22%-61% depending on successional stage. In general, removal rates increased with an increase in age of successional stage (Fig. 2;
Average diaspore removal rates in old growth forests (45%) were higher than in 40-55yr, 15-20yr and 9-11yr forests (33%; 26% and 25%, respectively). Diaspore removal in old growth forest was 1.8 times higher than in the 9-11yr forest.

**DISCUSSION**

Animal-diaspore interactions play an important role in the dynamics of tropical forests and their disruption may have negative consequences for forest regeneration. We found no changes in the richness of ants interacting with diaspores along a gradient of forest succession in the Atlantic forest of Southern Brazil. However, the behaviour of ants interacting with diaspores changed along the successional gradient, “diaspore removal” was more frequent in older successional stages. Concordantly, diaspore removal rates, assessed in the removal experiment, were lower in secondary forests than in old-growth forests. These changes in the interactions between ants and diaspores are likely to have implications for the population dynamics of plants in secondary forests and thus the conservation and recovery of tropical forests.

Species richness and diversity of ants interacting with diaspores was similar in all successional stages of forests. This contrasts to the reduced richness and diversity of the entire ground foraging ant fauna in secondary forests of the Atlantic forest in Southern Brazil (Bihn et al. 2008, 2010). Ant species interacting with diaspores accounted for an estimated 15% of the total species pool in the region (Bihn et al. 2010). Many ant species we found interacting

**Table 2**

Results of a two-factor nested ANOVA and test for linear trend on the effects of successional stage on diaspore removal rates with three replicated sites

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successional stage</td>
<td>3</td>
<td>0.97</td>
<td>2.49</td>
<td>0.13</td>
</tr>
<tr>
<td>Linear trend (successional stage)</td>
<td>1</td>
<td>2.81</td>
<td>25.37</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site (successional stage)</td>
<td>8</td>
<td>0.39</td>
<td>3.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Error</td>
<td>227</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
with diaspores were relatively common in the study region (JH Bihn, unpublished results), especially species of *Pheidole* which accounted for the majority of ant-diaspore interactions in our and other studies in neotropical forests (Pizo et al. 1998, Pizo & Oliveira 2000, Passos & Oliveira 2003, Fornara & Dalling 2005). All of the five most common species at diaspore depots, which accounted together for the majority of the observed ant-diaspore interactions, were present in at least two successional stages. In addition, interactions between ants and diaspores are mostly facultative and often involve ants with omnivorous diets (Pizo & Oliveira 2000, Passos & Oliveira 2003). Therefore, the habitat specialization of ants interacting with diaspores seems to be relatively low, which might indicate that the guild of ant species interacting with diaspores is rather resistant to habitat disturbance.

The behaviour of ants towards diaspores changed along the gradient of forest succession. In all stages of forest succession removal of the fleshy part of the diaspore in situ was the most common behaviour but the proportion of diaspores entirely removed increased from early to late successional stages. Both behaviours, seed cleaning and removal of diaspores, have implications for seed fate and plant establishment. Seed cleaning results in higher germination rates due to reduced fungal attack (Ohkawara & Akino 2005, Christianini et al. 2007) but seeds remain available for other guilds of seed predator, e.g. rodents, marsupials and birds. Diaspore removal often leads to seed predation but a small number of seeds are left intact and are transported to protected microsites with favourable conditions for germination (Levey & Byrne 1993, Pizo 2008). Because we did not track the fate of diaspores we cannot infer about the proportion of diaspores that reached favourable microsites for germination. Levey & Byrne (1993) estimated that up to 39% of removed seeds are deposited in microsites with favourable conditions for germination, and Pizo (2008) found 93% of diaspores deposited in favourable conditions, e.g. inside ant nests. If the proportion of seeds that reach favourable microsites for germination after seed removal is similar among successional stages, the increased seed removal in late successional stages could result in more seeds in microsites important for the recruitment of plant populations. It seems worth investigating in further studies, whether changes in the behaviour of ants towards diaspores in different successional stages of forests influence the number of seeds that become established as seedlings.

Diaspore removal rates were lower in young secondary than in old-growth forests and gradually increased with the age of secondary forests. Studies on invertebrate diaspore removal in different successional stages of tropical forests are few and report contradictory results. Notman & Gorchov (2001) found higher removal rates in mature forests but other studies report higher removal rates in secondary forests than in old-growth forests (Peña-Claros & de Boo 2002, Andresen et al. 2005, Zelikova & Breed 2008). However, removal rates are not directly comparable among these investigations because each study uses a different set of diaspore species in the experiments and it has been demonstrated that removal rates of diaspores depend on diaspores identity (Notman & Gorchov 2001, Pizo & Oliveira 2001).

What are the implications of the observed changes of the behaviour of ants towards diaspores and the increased diaspore removal rates during forest regeneration? The most likely fate of removed diaspore is predation. However, various studies have documented that ants transport undamaged diaspores to safe and favourable microsites for germination (Levey & Byrne 1993, Pizo 2008). Although this outcome of ant-diaspore interactions might be rare, it might still be important for the recruitment of plant populations (Levey & Byrne 1993). This is especially true for the interactions of ants and non-myrmechochorous diaspores in tropical forests, which are still largely unexplored (Pizo et al. 1998, Passos & Oliveira 2003). Lower removal rates in young secondary forests could potentially increase sibling competition among seedlings and thus have negative effects on the recruitment of plant populations, slowing down
the regeneration of these forests. Only careful tracking of diaspore fate, dispersal distance and germination success can illuminate the benefits and costs for the recruitment of the involved plants. The small diaspores, with which ants typically interact, are difficult to track in the opaque medium of the leaf litter in tropical forests but such studies seem worthwhile especially in the context of forest regeneration.

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**RESUMEN**

Las interacciones entre animales y plantas son importantes para la recuperación de la diversidad y los procesos en los bosques secundarios, los cuales cada vez más tienden a dominar el paisaje tropical. Nosotros utilizamos una combinación de métodos experimentales y observaciones para estudiar las interacciones entre hormigas y diaspores a través de un gradiente de sucesión en los bosques del sur de Brasil, entre agosto 2007 y abril 2008. Además de las tasas de eliminación de diaspores, evaluamos la riqueza de especies, la diversidad y el comportamiento de las hormigas que interactúan con las diaspores, con tres repeticiones por sitio de sucesión del bosque. Se registraron 22 especies de hormigas que interactúan con diaspores (se estima un 15% del total de especies en la región). La riqueza y diversidad de especies no varían entre las etapas de sucesión, pero el comportamiento de las hormigas hacia las diaspores cambia con la edad de los bosques secundarios. En las etapas sucesionales avanzadas del bosque la eliminación de diaspores fue más común que en las etapas tempranas. En concordancia, las tasas de eliminación de diaspores fueron menores en la etapa más temprana de la sucesión de bosques secundarios y aumenta con la edad de los mismos. Estos resultados indican que las interacciones hormigas-diaspores en los bosques secundarios son alteradas y las menores tasas de extracción en los bosques secundarios es probable que limiten el reclutamiento de las poblaciones de plantas durante la sucesión secundaria.

**Palabras clave:** interacciones planta-hormiga, recuperación del bosque, dispersión de semillas, depredación de semillas, sucesión, bosques tropicales secundarios.
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