Convergent evolution of an ant–plant mutualism across plant families, continents, and time

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ABSTRACT

Questions: How often has dispersal of seeds by ants evolved in monocots and is the timing of origins associated with changes in the ant community or instead with the rise of forests? Are patterns in the origin of elaiosomes (the trait associated with the dispersal of seeds by ants) through time similar to those for the origins of fleshy fruits?

Data studied: We estimate the timing of the origin of elaiosomes and fleshy fruits respectively by mapping seed morphology onto a recent phylogeny based on *ndhF* sequence data for the monocots (Givnish *et al.*, 2005). For comparison, we use fossil data on ant relative abundance through time and phylogenetic data for the timing of the origin of seed-dispersing ant lineages.

Search method: We mapped origins of both elaiosomes and fleshy fruits onto the phylogeny using parsimony in the program Mesquite (Maddison and Maddison, 2005). We analysed the relationship between ant relative abundances, the number of origins of seed-dispersing ants, and the rate of origination of elaiosomes using randomization-based Monte Carlo regression in the program R (Cliff and Ord, 1981). Using the program Discrete (Pagel, 2006), we test whether fleshy fruits or elaiosomes and shaded forest understoreys show correlated evolution.

Conclusions: Morphological features for the dispersal of seeds by ants (myrmecochory) have evolved at least twenty times within the monocots. Origins of myrmecochory are not associated with the rise of forests during the Cretaceous or with subsequent transitions of plant lineages into closed canopy habitats, nor are they contemporaneous with the origins of fleshy fruits. Instead, the origins of myrmecochory are closely associated with the rise in relative abundance of ants (proportion of all individual insects in fossils) towards the end of the Eocene and more recently.

Keywords: ant, convergent evolution, dispersal, diversity, Formicidae, mutualism, myrmecochory, seed.

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INTRODUCTION

A major challenge for ecology and evolutionary biology is to understand why some traits and interactions have evolved multiple times while others have not and the contexts of such origins. Convergent traits in both animals and plants often involve many tens and even hundreds of origins (Patterson and Givnish, 2004; Givnish *et al.*, 2005; Losos *et al.*, 2006; Richmond, 2006; Wiens *et al.*, 2006). Combined with phylogenetic approaches, such instances of repeated convergence may now offer excellent opportunities for a quantitative understanding of convergence and its drivers. To better understand convergence we need to know which traits are truly convergent and then for those traits the factors that drive such convergence (Ackerman, 1995; Givnish *et al.*, 2005).

Seed dispersal mutualisms are an ideal context in which to understand the factors driving trait convergence because of their multiple origins, simple morphological bases, diffuse suites of animal partners, and ecological importance (Tiffney, 1984; Tiffney and Mazer, 1995). Ant-mediated dispersal or myrmecochory is one of the more enigmatic animal–plant mutualisms. To date, estimates of the number of origins of elaiosomes have been based purely on the number of families in which the trait occurs. Nonetheless, the number of plant families in which myrmecochory occurs (>70) indicates that it may have evolved frequently (Handel and Beattie, 1990) and that the selection pressures acting on the mutualism are or were strong. When myrmecochory is successful for the plant, ants take the entire seed (or, more generally, diaspore) back to the nest, where they remove the elaiosome and discard the seed in a midden or outside the nest. In any given site, multiple species may disperse seeds, but one or a few ant species tend to be numerically dominant in terms of the number of seeds removed (Beattie, 1985; Gove *et al.*, 2007).

The benefits of dispersal of seeds by ants to modern plants appear to vary among ecosystems (Giladi, 2006). Attempts to understand the evolution of myrmecochory have focused on two research strategies. Studies have either compared the features of ecosystems in which myrmecochory is rare with another in which it is common (Milewski and Bond, 1982; Beattie, 1983; Bond *et al.*, 1991; Westoby *et al.*, 1991b), or have followed the fate of seeds dispersed and not dispersed by ants to infer the current selective pressures on ant-dispersed seeds (Davidson and Morton, 1981; Slingsby and Bond, 1985; Christian, 2004; Manzaneda *et al.*, 2005). To date, most research has tended to highlight the role of site effects. Ant nests are thought to be preferred sites for seed germination or storage because of reduced risk of mortality due to fire [Mediterranean ecosystems (Auld, 1986)], predation (Heithaus, 1981) or nutrient limitation (Westoby *et al.*, 1991a) relative to areas outside the nest. Alternatively, dispersal by ants may actually offer benefits in terms of dispersal distance. Several authors (Gomez and Espadaler, 1998; Whitney, 2002) have suggested that for the relatively small plants that tend to be dispersed by ants, a distance of even a metre may represent several plant widths and so still yield substantial fitness benefits. Although average dispersal distances are typically on the order of just a few metres, longer-distance dispersal events can be achieved, either by ants with large foraging areas (Whitney, 2002) or through removal of the seed from the ant nest after initial dispersal (personal observation).

When and in response to what factors ant dispersal evolved have never been explicitly considered. Comparisons of when, where, and how often dispersal has evolved in a phylogenetic context (Givnish *et al.*, 2005) offer hope of a better understanding of the evolution of this globally common mutualism. Givnish *et al.* (2005) lumped elaiosome-bearing plants with other plants and tested the hypothesis that actively dispersed fruits or seeds evolved in response to the rise of closed canopy forests and the consequent reduction in wind forces

and hence dispersal by wind. Givnish and colleagues' (2005) overall results were highly significant, but by lumping ant-dispersed species with vertebrate-dispersed species, the authors may have obscured differences between the two groups. If the rise of forests and the consequent reduction in wind triggered the evolution of both vertebrate-dispersed fruits and elaiosomes, then both groups should show evidence of correlated evolution between transitions into forest and the evolution of active dispersal. If, on the other hand, it is not simply distance that is important but site effects, then the origin of elaiosomes might be expected to more closely track changes in the condition of undispersed seeds (which we don't test here) or changes in the ant community itself.

To study the origin of elaiosomes in the monocots, we use the well-resolved monocot phylogeny of Givnish *et al.* (2005) based on *ndhF* sequence variation. Importantly, the monocots contain roughly 60,000 species, are morphologically diverse, are evolutionarily old [roughly 160 million years (Bremer, 2000)], and contain many examples of ant dispersal of seeds. We have identified known examples of plant genera that include species with morphological adaptations for dispersal by ants to examine the independent origins and losses of ant-mediated dispersal. By combining the phylogeny of Givnish *et al.* (2005), our data on myrmecochory, a recent ant phylogeny (Moreau *et al.*, 2006), and a review of patterns of ant abundance through time (Grimaldi and Agosti, 2000), we examine the number and timing of origins of elaiosomes. We compare the number and timing of those origins to origins of a better studied dispersal syndrome, fleshy fruits. We then consider the hypothesis that myrmecochory evolved in concert with transitions in the composition of ant communities. We examine two aspects of changes in ant communities through time: ant abundance (relative to other taxa), and hence dependability as mutualists, and the number of origins of seed-dispersing ant lineages in series of ten million year time intervals. We also examine an alternative but not mutually exclusive hypothesis that the origin of elaiosomes was linked to the rise of closed forests, and hence a reduction in wind dispersal. We found that elaiosomes have evolved more than twenty times within monocots. These origins of myrmecochory are closely associated with the rise in relative abundance (proportion of all individual insects in fossils) and diversity of ants towards the end of the Eocene and more recently.

METHODS

Ant-dispersed plants almost universally have morphological adaptations to encourage dispersal by ants. The one exception to this general pattern is in ant-garden epiphytes, where seeds dispersed by ants have chemical attractants but no elaiosome *per se* (Davidson, 1988). Thus with the exception of the ant-garden epiphytes (Davidson, 1988), it is possible to determine the dispersal mode of a seed by examining its morphology alone. The most conspicuous morphological adaptation of seeds dispersed by ants is an elaiosome (Sernander, 1906b). The elaiosome is a lipid-rich, nitrogen-poor appendage of multiple developmental origins that ants use as a handle to carry the seed back to the nest and which subsequently serves as a reward for the ants (Sernander, 1906b; Berg, 1975; Beattie, 1985). Although some elaiosomes are technically 'fleshy fruits', for the sake of simplicity when we refer to fleshy fruits herein we are referring to fleshy fruits exclusive of those that are simply seeds with elaiosomes.

Taxon sampling and phylogenetic hypotheses

We test whether ant dispersal is convergent by reconstructing the trait on the monocot tree. We derived a maximum parsimony tree for the monocots based on the *ndhF* sequences used in Givnish *et al.* (2005). Sequence data were included for 290 species that represented 78 of 92 monocot families and all 12 orders. Few families are missing from the sample (Givnish *et al.*, 2005), but these do include ant-dispersed lineages. We discuss the implications of exclusion of these lineages later. Phylogenies were reconstructed using *Ceratophyllum* as the out-group. The phylogenetic hypotheses for the monocots are those used in Givnish *et al.* (2005). A consensus tree was used for all concordance analyses. For dating the tree, we optimized branch lengths onto one arbitrary maximum parsimony tree using maximum likelihood and an HKY85 substitution model using PAUP*4b10.

We use the ages of six Cretaceous plant fossils and the inferred divergence of *Acorus* from other monocots to date the tree: earliest common ancestor of Poaceae-Joinvilleaceae-Flagellariaceae-Restionaceae-Typhaceae-Sparganiaceae and Arecales (69.5 mya), Zingiberales and Tofieldiaceae (83 mya), Arecales (89.5 mya), *Acorus* split (134 mya) (Herendeen and Crane, 1995; Bremer, 2000). All fossil dates are set as minimum dates. The *Acorus* divergence date was fixed at 134 million years ago based on the analysis by Bremer (2000).

We use penalized likelihood (PL) in r8s to correct for rate variation across the tree and calibrate against the reference dates (Sanderson, 2002), using the cross-verification algorithm to find the optimal value of the smoothness parameter. We mapped origins of both elaiosomes and fleshy fruits onto the phylogeny using parsimony in the program Mesquite (Maddison and Maddison, 2005).

Origins and the ant community

One explanation for the observed patterns in the origination of elaiosomes is that elaiosomes evolved in response to changes in ant communities. To test this, we compare the origination rate of elaiosomes (as measured above) to the relative proportion of ants in fossil and amber samples through time, and the number of origins of seed-dispersing ant lineages. For the seed-dispersing ant lineages, dates are estimated from a recent phylogeny (Moreau *et al.*, 2006) using the authors' dated phylogeny based on maximum fossil ages. Qualitatively identical results are obtained using minimum ages. The identity of seed-dispersing ant lineages is based on a global survey of field studies of dispersal by ants, available from the authors upon request. These lineages include, among others, *Formica*, *Aphaenogaster*, *Myrmica*, *Rhytidoponera*, and *Anoplolepis*.

Data on ant abundance through time come from a review in Grimaldi and Agosti (2000) of ant relative abundance in compression fossil and amber samples. Data for the different time periods are from, respectively, New Jersey amber-Turonian (Grimaldi *et al.*, 2000), Tamyr amber-Santonian (Grimaldi and Agosti, 2000), Alberta amber-Campanian (Pike, 1995), Sakhalin Island amber-Paleocene (Dlussky, 1988), Oise, France amber-late Eocene (Nel *et al.*, 1999), Arkansas amber-middle Eocene (Saunders *et al.*, 1974; Wilson, 1985), Washington compression fossils-middle Eocene (Lewis, 1992), Wyoming compression fossils-middle Eocene (Dlussky and Rasnitsyn, 1999), Baltic Amber-middle Eocene (Rasnitsyn and Kulicka, 1990), Sicily amber-Oligocene (Skalski and Veggiani, 1990), Florissant, Colorado compression fossils-late Eocene (Carpenter, 1930), Dominican Republic amber-Miocene (Rasnitsyn and Kulicka, 1990), and Dominican Republic amber-Miocene (Grimaldi and Agosti, 2000).

The trend across studies is for a dramatic increase in ant relative abundance at about the end of the Eocene. This trend appears relatively general and is not simply due to a few outlying samples. There are six geographically widespread samples that span the period from the Turonian to the beginning of the middle Eocene. In none of these samples do ants represent more than 7% of individuals. The three lowest relative abundances for ants are all from the earliest samples where ants represent less than a tenth of one percent of individuals in samples. In contrast, for none of the six samples since the end of the middle Eocene do ants represent less than 17% of all individuals (Grimaldi and Agosti, 2000). In addition, the general pattern holds for both compression and amber fossils.

The relative abundance of ants in samples could be biased by large changes in overall insect abundance, which would lead to changes in relative ant abundance without changes in ants per given area. Thus we cannot conclusively disentangle the role of ant abundance and ant relative abundance in our analyses. That said, we know of no evidence that overall insect abundance has changed systematically over the study period.

Because data on the relative abundance of ants are not available from every ten million year time step, we interpolated abundance data for the only time bin without data (30–40 mya, the early Eocene). To check the validity of this interpolation, we conduct all regressions with and without the interpolated time bin. The results were qualitatively identical. Modern data are from one low-elevation tropical site (Olson, 1994) and a temperate forest (Hunter *et al.*, 2003), where ground-foraging ants and other invertebrates were sampled using pitfall traps and litter bags respectively.

Although our analyses extend only to the level of plant genera (as in Givnish *et al.*, 2005), consideration of additional published phylogenies within genera reveals that elaiosomes have also been gained and lost within genera. Such a bias would tend to lead to over-estimates on our part of the age of elaiosome origins. Our estimates of the dates for the origins of elaiosomes may be overestimates for another reason, namely that in those monogeneric clades in which elaiosomes are now present elaiosomes could have evolved at any time between the divergence of that genus from its sister taxon and the present day. Our overall bias is thus towards overestimating the rate of origin of elaiosomes early in monocot evolution and underestimating the rate of origin of elaiosomes in the last 20 million years. To account for the potential effects of this bias, we run all regression analyses using maximum and minimum dates. To estimate minimum dates, we shift all of the origins in genera where elaiosomes are clearly not basal to the most recent time bin (0–10 mya). For one ant-dispersed genus, *Uvularia*, for which recent analyses (Vinnersten and Bremer, 2001) indicate a passively dispersed sister taxon not included in our analyses and a recent divergence date (15 mya), we use that divergence date as the time of origin.

We analyse the relationship between ant relative abundance and the number of origins of seed-dispersing ants and the rate of origination of elaiosomes (origins/ten million year window/lineage) using randomization-based Monte Carlo regression in the program R (Cliff and Ord, 1981), with Pearson's product–moment correlation (r) as the test statistic. Data points for the analyses thus correspond to those 14 bins between today and 140 million years ago (or 13 bins when the interpolated bin was omitted). Because ant relative abundance and the number of origins of seed-dispersing ant lineages were uncorrelated ($\rho_s = 0.115$, $P = 0.696$), we included both variables in our analyses. To test for temporal autocorrelation, we plot the autocorrelation and partial autocorrelation functions for the residuals of a generalized linear model, and examine the 95% confidence intervals (Box and Jenksin, 1970). Because there was no significant level of temporal autocorrelation, regression
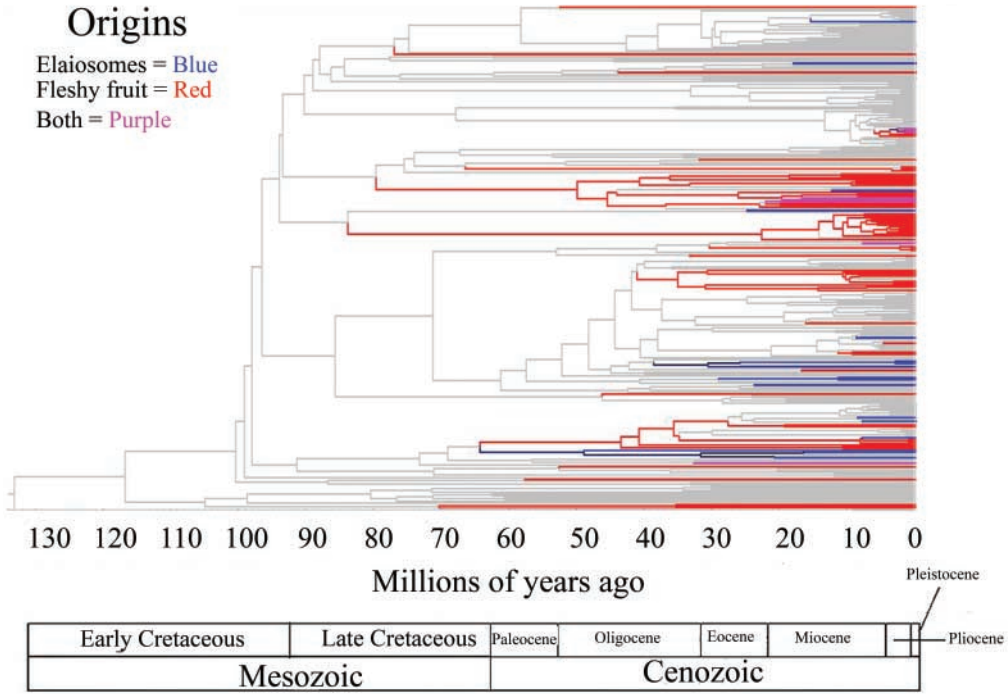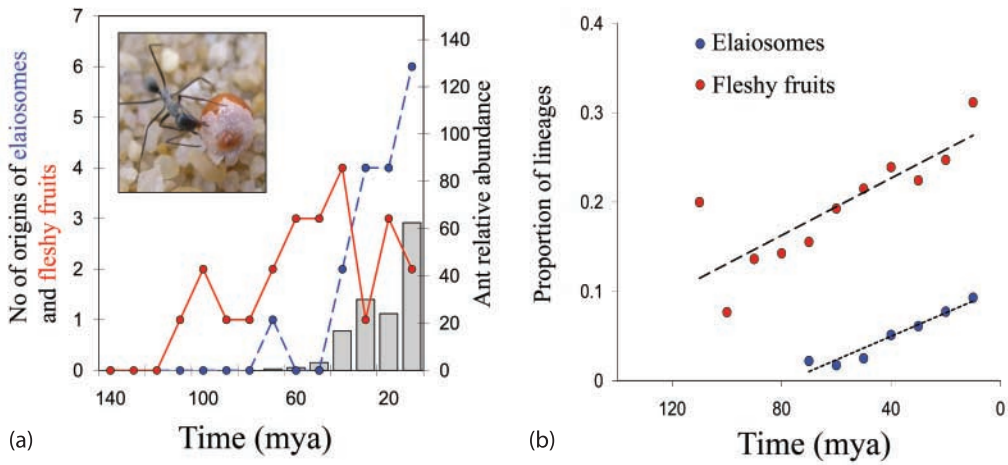
Figure 1

(a)

(b)

Figure 2

models that account for autocorrelation were not necessary. Results are qualitatively similar whether we use the Monte Carlo non-parametric analyses discussed here or generalized linear models.

Origins and forest cover

An alternative to the hypothesis that ant communities have driven patterns of origination of elaiosomes is that changes in forest structure and climate have played a more important role. More specifically, it has been hypothesized that in closed canopy shaded habitats, dispersal by animals, including dispersal of seeds by ants, is more likely to evolve than in open canopy habitats (Sernander, 1906a; Givnish *et al.*, 2005) due to the relative absence of wind. Angiosperm-dominated closed canopy forests became more abundant during the Late Cretaceous-Paleocene (Crane *et al.*, 1995). If elaiosomes evolved primarily due to this transition, the expectation is that there will be a pulse of origination of elaiosomes in the Late Cretaceous-Paleocene. We test for such a pulse by comparing the timing of the expansion of forests to the timing of the origination of elaiosomes (Fig. 1).

Similarly, if the benefit of dispersal by animals in closed forests is modest dispersal distance (a few metres) (Andersen, 1988; Whitney, 2002) in the relative absence of wind, then ant-dispersed species should also show a consistent pattern whereby elaiosomes evolve with transitions of lineages into closed forest habitats. Using the program Discrete (Pagel, 2006), we test if the evolution of fleshy fruits, fleshy fruits + elaiosomes, and elaiosomes alone correlate with occupancy of shaded forest understoreys. More specifically, we ask if transitions into shaded habitats in monocots are associated with the evolution of fleshy fruits, elaiosomes or elaiosomes + fleshy fruits more consistently than expected by chance and, conversely, if transitions out of shaded habitats are associated with the loss of elaiosomes more often than expected by chance.

RESULTS

We find that within monocots adaptations for seed dispersal by ants have evolved no fewer than 24 times, similar to the roughly 21 origins and 11 losses of vertebrate-dispersed fleshy fruits (Fig. 1, Table 1). Of the 24 origins of elaiosomes, 19 were detected based on our phylogenetic analyses and 5 additional origins were inferred based on other published phylogenies (Table 1). Parsimony mapping of elaiosomes onto the phylogeny resulted in

Fig. 1. Origins and losses of fleshy fruits (red) and elaiosomes (blue). Purple clades are those in which both elaiosomes and fleshy fruits are present. Ages are calibrated based on plant fossil data.

Fig. 2. (a) Number of origins of elaiosomes and fleshy fruits (per ten million year interval) and ant relative abundance through time, and (b) the proportion of extant lineages with fleshy fruits or elaiosomes. In (a), each dot represents the number of gains of elaiosomes (blue) or fleshy fruits (red) per ten million year time window. Each bar represents the proportion of insects in samples that were ants for the same time intervals. Origins of elaiosomes are maximum dates. In (b), dots represent the proportion of lineages surviving to the present that include elaiosomes (blue points) or fleshy fruits (red points) through time. The proportion of lineages with active dispersal (elaiosome or fleshy fruit) increases through time at the expense of lineages with other dispersal modes.

Table 1. Origins and losses of elaiosomes by habitat sun/shade and life form

	Gains	Sun	Shade
Order	**Family (Genus)**		
Asparagales	Hypoxidaceae (*Curculigo*)		Tropical understorey (Aus)
Asparagales	Tecophilaeaceae (*Cyanastrum*)		Tropical understorey (Af)
Asparagales	Hemerocallidaceae (*Johnsonia* + *Hensmannia* + *Caesia*)	Mediterranean (Aus)	
Asparagales	Amaryllidaceae (*Leucojum*)	Mediterranean (Eur.)	
Asparagales	Iridaceae (*Iris*)	**Mediterranean (Israel)**	
Liliales	Colchicaceae (*Uvularia*)		Temperate understorey (NA)
Liliales	Liliaceae (*Erythronium*)		**Temperate understorey (NA/Eurasia)**
Liliales	Liliaceae (*Gagea*)		Temperate understorey (Widespread)
Liliales	Liliaceae (*Scoliopus*)		Temperate understorey (NA)
Liliales	Melanthiaceae (*Trillium*)		Temperate understorey (NA/Asia)
Liliales	Colchicaceae (*Androcynbium* + *Alstroemeria*)	Mediterranean (Af)	
Poales	Cyperaceae (*Carex*)		**Temperate understorey (NA/Eur)**
Poales	Poaceae (*Panicum*)	**Mediterranean (Aus)**	
Poales	Bromeliaceae (*Aechmea*)	**Tropical epiphyte (Neo)**	
Poales	Bromeliaceae (*Nidularium*)	**Tropical epiphyte (Neo)**	
unplaced	Dasypogonaceae (*Dasypogon*)	Mediterranean (Aus)	
Zingiberales	Costaceae (*Costus*)		Tropical understorey (Neo)
Zingiberales	Marantaceae (*Calathea*)		Tropical understorey (Neo)
Zingiberales	Zingiberaceae (*Globba*)		Tropical understorey (Asia)

Note: Results are based on parsimony analysis of ancestral traits using the tree in Fig. 1. Under Sun/Shade, names in parentheses correspond to the ecological regions in which the genus primarily occurs. Eur = Europe, Neo = Neotropical, Aus = Australia, Af = Africa. Note that the habitats in which ant dispersal has evolved are a non-random subset of global habitats. Clades in **bold** are those in which elaiosomes are unlikely to be basal.

Table 2. Likelihood ratios from correlated evolution analyses

	Shade/elaiosomes	Shade/fleshy fruits	Shade/fruit
Likelihood ratio	0.822	35.61	25.62
Significance	0.6	<0.0001	<0.0001

Note: The table shows the results for concerted convergence analyses in Discrete (Pagel, 2006), for fruit type and habitat specificity (shade/sun). Tests were run separately for the presence/absence of elaiosomes, presence/absence of a fleshy fruit (as in Givnish *et al.*, 2005), and the presence/absence of a 'fruit', be it a fleshy fruit or an elaiosome.

three clades in which there was more than one most parsimonious result. Additional phylogenetic data suggested that a loss was more likely in two of these cases, and two independent gains were most likely in the third case.

Most origins of myrmecochory are associated with transitions from passive dispersal (including both wind and gravity) to myrmecochory. Only four cases were observed where myrmecochorous seeds evolved from vertebrate-dispersed fleshy fruits. The highest observed rate of origination of ant dispersal was substantially later (essentially the present) than that for fleshy fruits (Fig. 2a).

Origins of elaiosomes (per time interval, per lineage) were not correlated in time with origins of fleshy fruits (per time interval, per lineage), either when maximum dates for the origins of elaiosomes were used ($r = 0.36$, $P = 0.2$) or when minimum dates were used ($r = 0.299$, $P = 0.306$; Fig. 2a). Because ant-mediated dispersal evolved more recently than fleshy fruits, the number of genera with fleshy fruits is greater than the number of genera with elaiosomes. The proportion of lineages with fleshy fruits or elaiosomes has increased through time and appears to be continuing to increase at the expense of the number of wind- and passively-dispersed lineages (Fig. 2b).

The number of origins of elaiosomes (per ten million years, per lineage) is strongly correlated with the relative abundance of ants in compression fossil and amber deposits (for maximum dates: $r = 0.729$, $P = 0.0022$; for minimum dates: $r = 0.928$, $P < 0.001$), but is not correlated with the number of new origins of seed-dispersing ant lineages (for maximum dates: $r = -0.021$, $P = 0.502$; for minimum dates: $r = -0.372$, $P = 0.210$) (Fig. 1). The relationship between ant relative abundance and the number of origins of elaiosomes/interval/lineage remained significant when the one data point (30–40 mya) for which relative abundance data had to be interpolated was excluded (for maximum dates: $r = 0.73$, $P = 0.002$; for minimum dates: $r = 0.95$, $P = 0.001$).

Fleshy fruits evolved along with transitions into closed canopy habitats more often than expected by chance (Tables 1 and 2). Evolution of genera with species bearing elaiosomes showed no correlation with occupation of either shaded or open habitats: elaiosomes were equally likely to be possessed by taxa with open or shaded habitat preferences (Tables 1 and 2).

DISCUSSION

We find that both elaiosomes and vertebrate-dispersed fleshy fruits have arisen more than twenty times in monocots. We undoubtedly underestimate the number of origins of elaiosomes and fleshy fruits because transitions in dispersal mode occur within some genera (Givnish *et al.*, 2005). The proportion of lineages dispersed by ants (elaiosome-bearing seeds) or vertebrates (fleshy fruits) has increased and continues to increase through time, such that the

general story of dispersal in the monocots appears to be one of increasing reliance on animal vectors. The two dispersal modes, however, show very different patterns of origination through time. Whereas the peak in origination rates (per lineage) of fleshy fruits was early (85 mya) and has slowed, elaiosomes evolved no earlier than the end of the Eocene, and their rate of origination continues to be high on a per lineage basis (Fig. 2). Because this pattern is a consequence of the temporal order of branching on the tree (rather than the dates themselves), it will not change with modifications of the dating of the branching events.

The rate of origin of elaiosome-bearing lineages is strongly correlated with the relative abundance of ants through time. We must interpret this relationship with caution given potential biases in when and which insect species are preserved. But, as Grimaldi and Agosti (2000) put it, while 'each deposit is biased by the local paleoenvironment and mode of preservation, overall the pattern is striking'. Ants go from representing less than 1% of individual insects in samples from the Cretaceous ambers (Zherikhin and Eskov, 1999) and just over 1% of a Paleocene sample (Zherikhin and Eskov, 1999) to more than 40% of individual insects in amber and compression fossils at the end of the Eocene (Grimaldi and Agosti, 2000). Ants are numerically dominant in samples of nearly any kind today, whether from arboreal, litter or soil habitats and in biomes ranging from taiga to desert (Allred, 1973; Kharboutli and Mack, 1991; Punttila *et al.*, 1996; Kaspari and Valone, 2002). Therefore, it is unlikely that multiple geographically widespread samples from before the Eocene would have all yielded extremely low estimates of ant abundance due simply to taphonomic biases. Furthermore, ants were rare before the Eocene not only within samples in which they occur, but also in terms of the proportion of amber inclusions that contain ants (Nel *et al.*, 2004). Like Grimaldi and Agosti (2000), we think the data on relative ant abundance are sufficient to have confidence that there was a dramatic shift in the abundance of ants during the Eocene.

A strong temporal correlation between the rise in the ants and the rise in the origination rate of elaiosomes could exist for any of several different reasons. It has been speculated that the rise in the incidence of elaiosomes could have helped to precipitate the rise of ants (Wilson and Holldobler, 2005). We believe this is unlikely for a variety of reasons. Even those relatively few species of ants that consume large numbers of elaiosomes depend on them for only a small part of their total diet (e.g. *Rhytidoponera* spp., *Aphaenogaster* spp., *Myrmica* spp., *Anoplolepis* spp., and *Formica* spp.) (Majer, 1982; Morales and Heithaus, 1998). Only one study has shown a fitness consequence to ants from shifts in the number of seeds available (Morales and Heithaus, 1998) and even then only for the main seed-dispersing ant species at the study site, not the tens of other ant species present in the region. Elaiosome-bearing plants are geographically restricted (Dunn *et al.*, in press) and so could not account for a geographically widespread increase in the relative abundance of ants. In addition, in our analyses, most seed-dispersing ant genera evolved before the majority of origins of elaiosomes. Finally, no morphological or behavioural adaptations in ants have yet been documented for specialization on elaiosome collection.

A second explanation, and the one that we favour, is that the rise in abundance of ants in the Late Eocene made ants dependably available as dispersers for the first time. Most ant genera that now disperse seeds were already present well before the first origin of elaiosomes (Moreau *et al.*, 2006), but overall ants represented less than 1 in a 100 individual insects found in amber deposits (Grimaldi and Agosti, 2000). Consequently, the probability that an ant would be the first animal to encounter a seed would have been low. During the Eocene, ants made the transition from abundant to numerically dominant (Grimaldi and Agosti, 2000). Nearly all of the origins of myrmecochory occur in the late Eocene or more recently, once ants were

numerically dominant. In addition, the first records of phasmid eggs with capitula also come from the late Eocene (Sellick, 1997). Capitula are elaiosome-like structures on phasmid eggs that function in the same way as elaiosomes, to attract ants to remove the eggs to the protected environment of the nest (Hughes and Westoby, 1992). We suggest that it is only once ants were numerically dominant that investment in an appendage for dispersal by ants (be it an elaiosome, or for that matter a capitula) would more consistently lead to whatever population or evolutionary benefits accrue to plants with seeds dispersed by ants. While recent work has shown that the radiation of ants may have been precipitated by the radiation of angiosperms (Moreau *et al.*, 2006), our results suggest that the origin of plants with elaiosomes was precipitated by the rise in abundance and hence dependability of ants.

In contrast to our results for elaiosomes, the origin of fleshy fruits can plausibly be linked to global transitions from predominately open habitats in the mid-Cretaceous to closed forests in the late Cretaceous. Reduced wind speeds in closed habitats have long been suggested to favour active, rather than passive, dispersal (Givnish *et al.*, 2005). Like previous authors (Givnish *et al.*, 2005), we find strong correlated evolution between habitat preference and the presence/absence of fleshy fruits. Fleshy fruits evolved along with transitions into closed canopy habitats more often than expected by chance (Table 2). Thus the expansion of forest habitat may have triggered the evolution of fleshy fruits (Tiffney, 2004) both because seeds needed to be larger to germinate in shaded understoreys (Crane, 1985; Givnish, 1998; Tiffney, 2004) and because the lack of wind in those understoreys necessitated active dispersal (e.g. Givnish *et al.*, 2005). When elaiosomes are considered on their own, we find no relationship between habitat openness and the presence/absence of elaiosomes.

Overall our analyses indicate that a high abundance of ants was necessary for the evolution of myrmecochory. Once ants were dependably present, we suggest that elaiosomes were favoured wherever conditions in ant nests were better for seed and seedling fitness than conditions outside ant nests. We cannot rule out the idea that one of the advantages of dispersal by ants is dispersal distance in the absence of wind, but we can at least show that such an advantage, if present, is likely contingent on the composition of the ant community itself. Origins of elaiosomes, while not explicitly tied to either open or closed habitat types, occur more frequently in some habitats than others. All of the origins of elaiosomes in open habitats were in fire-prone, Mediterranean ecosystems ($n = 11$) or tropical forest canopies ($n = 2$). Within Mediterranean ecosystems, origins were primarily in two regions, the Fynbos of South Africa and the heathlands of Australia. While elaiosomes arose often in temperate forests ($n = 7$), no origins of elaiosomes were associated with temperate open habitats such as the grasslands and prairies that neighbour the temperate forest habitats in which elaiosomes frequently evolved. These results are very different than the pattern seen in fleshy fruits, where the origins of fleshy fruits in monocots are almost exclusively restricted to closed habitats such as forest understoreys.

Overall, we show the repeated and relatively recent origin of elaiosomes in concert with changes in the ant community. While we have documented many independent origins of myrmecochory in the monocots, these origins are just a small part of the total number of origins in the angiosperms since ant dispersal is arguably more widespread in dicots than in monocots (Beattie, 1983). If we take an even broader perspective, we suspect that many of the diverse plant adaptations for relationships with ants first evolved during the late Eocene and more recently. Once ants became abundant, they would have been dependable agents not only of seed dispersal but also of other processes including plant protection and homoptera defence.

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