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The critical role of ants in the extensive dispersal of *Acacia* seeds revealed by genetic parentage assignment

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Abstract

Ants are prominent seed dispersal agents in many ecosystems, and dispersal distances are small in comparison with vertebrate dispersal agents. However, the distance and distribution of ant-mediated dispersal in arid/semi-arid environments remains poorly explored. We used microsatellite markers and parentage assignment to quantify the distance and distribution of dispersed seeds of *Acacia karina*, retrieved from the middens of *Iridomyrmex agilis* and *Melophorus turneri perthensis*. From parentage assignment, we could not distinguish the maternal from each parent pair assigned to each seed, so we applied two approaches to estimate dispersal distances, one conservative (CONS), where the parent closest to the ant midden was considered to be maternal, and the second where both parents were deemed equally likely (EL) to be maternal, and used both distances. Parentage was assigned to 124 seeds from eight middens. Maximum seed dispersal distances detected were 417 m (CONS) and

423 m (EL), more than double the estimated global maximum. Mean seed dispersal distances of 40 m (±5.8 SE) (CONS) and 79 m (±6.4 SE) (EL) exceeded the published global average of 2.24 m (±7.19 SD) by at least one order of magnitude. For both approaches and both ant species, seed dispersal was predominantly (44–84 % of all seeds) within 50 m from the maternal source, with fewer dispersal events at longer distances. Ants in this semi-arid environment have demonstrated a greater capacity to disperse seeds than estimated elsewhere, which highlights their important role in this system, and suggests significant novel ecological and evolutionary consequences for myrmecochorous species in arid/semi-arid Australia.

Keywords: Seed dispersal; Myrmecochory; Semi-arid zone; Plant density; Parentage assignment;

Introduction

Dispersal is the movement of individual organisms away from their origin (Clobert et al. 2001). Although established plants are immobile, their seeds can be dispersed by external vectors that transport them varying distances and in idiosyncratic patterns (Nathan and Muller-Landau 2000; van der Pijl 1982). Characterising the distance and distribution of seed movements provides insight into fundamental questions in ecology and evolutionary biology, including the drivers of genetic diversity and its spatial structuring within and among populations (Nathan and Muller-Landau 2000). Characterising seed movements is also of critical importance for conservation and natural resource management (Lengyel et al. 2010; Trakhtenbrot et al. 2005), particularly in the modelling of species extinction risks under fragmentation and climate change scenarios (Keith et al. 2008), and in predicting the impact of invasives (Gibson et al. 2011; Montesinos et al. 2012; Wandrag et al. 2013).

Modes of seed dispersal include gravity, wind, water, vertebrates (externally or internally) and ants (myrmecochory) (van der Pijl 1982). Myrmecochory is a mutualism whereby ants obtain nutrition from lipid rich elaiosomes on the seed body (Bronstein et al. 2006; Giladi 2006) while plants obtain dispersal benefits while include reduced competition from parents or siblings (Andersen 1988; Bond and Stock 1989; Boyd 2001; Kalisz et al. 1999), deposition in nutrient-rich micro-sites for

germination and establishment (Beattie and Culver 1983; Culver and Beattie 1978; Hanzawa et al. 1988), burial at depths that avoid incineration during fires (Boyd 2001; Majer 1982; Majer et al. 2011), and escape from predators because seeds are located underground in nests (Culver and Beattie 1978; Heithaus 1981). Myrmecochory is an important part of the demographic cycle of many plant species and, worldwide, ants are estimated to disperse the seeds of at least 11,000 species, of which 90 % are native to the southern hemisphere (see Lengyel et al. 2009, 2010).

In general, ants disperse seeds over short distances. A global meta-analysis of observational studies of ant behaviour found a mean seed dispersal distance by ants of 2.24 m (±7.19 SD) and a maximum of 180 m (Gómez and Espadaler 2013). However, there is some evidence to suggest that mean and maximum seed dispersal distances are greater in Australian arid systems than elsewhere (Davidson and Morton 1981; Whitney 2002). For example, the mean dispersal distance for seeds of *Acacia ligulata* by workers of *Iridomyrmex viridiaeneus* in Australia was 93.9 m (±11.1 SE), with a maximum of 180 m (Whitney 2002). Outside Australia, the highest dispersal distance recorded was 70 m (Gómez and Espadaler 2013). Accordingly, the distances, nature, and consequences of seed dispersal by Australian arid zone ants may be quite different to elsewhere, and global generalizations may be in error (Whitney 2002). This runs counter to the suggestion that reduced dispersal capacity is a feature of old landscapes such as arid Australia, leading to high endemism and plant species diversity (Hopper 2009). Clearly, quantitative data that characterise myrmecochorous seed dispersal in Australian arid zone species is necessary to resolve these apparent contradictions.

The application of highly polymorphic molecular markers and associated statistical analyses for parentage assignment overcomes many of the difficulties of visual tracking of individual seed movements (Hamrick and Trapnell 2011; Jones et al. 2010; Selkoe and Toonen 2006). Genetic assignment approaches enable an accurate estimation of not only dispersal distance but also o other important characteristics, such as the spatial distribution of seed collection, the influence of plant density on seed movement and the genetic composition of seeds within middens. For example, a key ecological outcome of ant-mediated seed dispersal is the effect it has on the degree of relatedness of conspecific seeds aggregated in space (Andersen 1988; Bond and Stock 1989; Kalisz et al. 1999).

Changing the genetic environment (level of relatedness) of seeds is important, as experimental studies have shown that decreased genetic relatedness is correlated with increased productivity (Young 1981), survival in spatially or temporarily heterogeneous environments (Hanski and Saccheri 2006) and individual fitness responses to herbivory or pathogen attack (Koelewijn 2004). Thus, molecular markers can provide substantial information about dispersal and its influence on the genetic and evolutionary characteristics of plant populations.

Here, we examine and quantify realised patterns of seed dispersal by ants using microsatellite markers and parentage assignment in the semi-arid zone shrub, *Acacia karina* (Maslin and Buscumb 2007). The study is located in the South West Australian Floristic Region, 1 of 34 internationally recognised biodiversity "hotspots" (Mittermeier 2004; Myers et al. 2000) and also an international "hotspot" for ants (Heterick 2009) and ant dispersal (Gove et al. 2007). Of the >7500 plant species in the South West Australian Floristic Region, more than one-third have arillate diaspores, which are thought to be myrmecochorous (Lamont et al. 1977). Previous observational studies on the behaviour of myrmecochorous ant species in the South West Australian Floristic Region found that ants remove most of the *Acacia* seeds (~70 % in the first 30 h) and store them in galleries underground (Majer 1982; Majer et al. 2011). Middens are only a manifestation of what is in the nest underneath and consist of seeds which have been disposed of following consumption of the edible parts. Middens are dynamic, eroding constantly, then reforming in the same place or nearby (i.e. within 20 cm) if the ant relocates its nest entrance. Viable seeds that have not been predated by insects germinate in nests and on middens after infrequent fire events (Majer 1982, Majer et al. 2011; Gove et al. 2007; Schatral et al. 1994).

The broad objective of this study is to harness the power of a genetic assignment approach to characterise realized dispersal of seeds by ants and address hypotheses on the global significance of myrmecochory in the Australian arid/semi-arid zone. Specifically, we directly quantify realised seed dispersal distances, compare the seed-collecting outcome of two ant species for *A. karina*, examine the multiple maternal origins of seeds in ant middens, assess how plant density influences the pattern of myrmecochory, and ultimately consider patterns of fine-scale spatial genetic structure of adult *A.*

karina in light of these findings. Our findings draw on the existing knowledge concerning ant–seed interactions in this region.

Materials and methods

Study system

Acacia karina is a recently described conservation priority species endemic to the Midwest region of Western Australia, where it is predominantly associated with banded ironstone formations (Maslin and Buscumb 2007). *Acacia karina* is a spreading shrub (1–3 m tall) that occurs in naturally disjunct patches, with plants distributed in multi-age groups of different densities within a patch. Seed set in acacias is highly variable and rainfall-dependent, and in *A. karina*largely takes place in the driest and hottest period of the year, between November and March, following flowering through the winter (Maslin and Buscumb 2007). *Acacia* seeds can be dispersed short distances by gravity or forcible ejection from dehisced pods and has no specialised appendages for wind dispersal. The presence of an elaiosome, and our observations of seed movement, indicate that ants are the primary dispersal vectors for *A. karina*.

Our study was conducted at the base of John Forrest Lookout, an undisturbed banded ironstone formation outcrop approximately 400 km northeast of Perth, Western Australia (centred on -29°18′40.88″ S, 116°43′53.25″ E) (Fig. 1). The region has a semi-arid climate with hot dry summers and cooler winters, when most rainfall occurs (average annual rainfall 250–300 mm) (Maslin and Buscumb 2007). The vegetation at the study site consists of an open thicket or shrub land, dominated by *Acacia* species. The study site is flat with a slight north-to-south slope. The study site contains a small disjunct cluster of <200 *A. karina* plants spread over ~11.25 ha (Fig. 1), and the nearest occurrence of further *A. karina* is at least 2 km to the east. These features of the cluster facilitated the complete sampling of adult plants and suitability of parentage assignment, as exhaustive sampling of the local cluster enables for parentage conclusions to be drawn with confidence.

Sample collection

Phyllodes (a flattened leafstalk that functions as a leaf) were collected in January 2013 from all known *A. karina* shrubs (*n* = 196) within the study area (Fig. 1). It is difficult to determine whether an *A. karina* individual is reproductively mature at early stages of development. Therefore, we collected phyllodes from all individuals in the cluster and both adults and juveniles are referred to as "shrubs".

A total of 28 active ant middens in January 2013 and 9 active middens in January 2014 were assessed by walking transects through the site in a grid pattern. Ant nests were identified by searching for ants moving in and out of their nests during periods of peak activity (morning and early evening). Once the nests were identified, the accompanying middens were inspected to establish whether the accompanying midden contained acacia seeds and, if present, seeds were collected from the midden. In 2013, two ant middens were found to contain *A. karina* seeds (middens A and B) and in 2014, six ant middens were found to contain *A. karina* seeds (middens C, D, E, F, G and H). From these eight middens, a total of 124 seeds were genotyped. Ants sampled from each of the middens indicated that *Iridomyrmex agilis* was responsible for the seeds collected in middens A, F, G and H and *Melophorus turneri perthensis* was responsible for seeds collected from middens B, C, D and E (Fig. 1). Five ants from each of these target nests were collected to serve as a reference species collection, with ants stored in vials containing 100 % ethanol and later identified by Dr. Brian Heterick of Curtin University. Seeds were identified as those of *A. karina* using morphology, and, where identifications were ambiguous, by a DNA barcoding approach for mid-west acacias developed by Nevill et al. (2013) (data not shown). Global positioning system (GPS) coordinates of each individual shrub and ant midden were recorded.

Microsatellite genotyping

Total genomic DNA was extracted from plant phyllodes and seeds collected from the ant middens using a modified hexadecyltrimethylammonium bromide (CTAB) extraction protocol (He et al. 2004). Seeds were germinated on filter paper and DNA extracted from 3-week-old seedlings and 0.2 g of

phyllodes material was ground in liquid nitrogen prior to DNA extraction. Adults and seeds were genotyped at seven nuclear microsatellite loci previously developed for *A. karina* (Nevill et al. 2010). Five microsatellite loci (A*k*C104, A*k*A112, A*k*C103, A*k*B10 and A*k*C108) were amplified by PCR in a multiplex reaction using the QIAGEN (USA) Multiplex kit (Type-it) in 12-µl volumes containing: 10 µl PCR master mix; 1.5 µl primer mix (each primer final concentration 2.5 µM) and 2 µl 5–20 ng DNA. PCR cycling was performed in a Veriti Thermal Cycler (Applied Biosystems, USA) using the following cycling conditions: Taq activation at 95 °C for 15 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s, and extension at 72 °C for 90 s; followed by a final extension at 72 °C for 10 min. Two microsatellite loci (A*k*B114 and A*k*B108) were amplified by PCR in single reactions. Locus A*k*114 and A*k*108 were amplified in 10-µl reaction volumes containing 3.72 µl of 5× PCR buffer (Fisher Biotec, Australia), a primer concentration of 0.2 µM for each forward (labelled) and reverse primer, 1 unit of Taq DNA polymerase (Fisher Biotec) and 20 ng of template DNA. PCR was carried out using the following cycling conditions: initial denaturation at 94 °C for 3 min; followed by 30 cycles of denaturation at 94 °C for 40 s, annealing at 58 °C (A*k*B114) or 56 °C (A*k*B108) for 40 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 15 min. Each genotyped plate included a negative control to ensure there was no DNA contamination and a positive control to ensure consistency when scoring genotypes. PCR products were separated by electrophoresis using a Beckman 8800 sequencer and alleles were sized using the CEQ 8800 genetic analysis system v.9.0 (Beckman & Coulter, USA). We manually checked markers for the presence of null alleles by genotyping a sample of progeny from known mothers to confirm Mendelian inheritance.

Parentage assignment

To describe genetic variation, we used GenAlEx v.6.5 (Peakall and Smouse 2012) to calculate the number of alleles, heterozygosity and inbreeding in the adult plants and seeds collected from middens. A more explicit comparison of the genetic diversity of adult plants that occur within patches to the seeds contained in middens was conducted by calculating: (1) the number of alleles, heterozygosity, and inbreeding in three patches of plants [patch coordinates centred on (1) -29°18′43.82″ S,

116°43′59.84″ E; (2) -29°18′41.89″ S, 116°43′55.29″ E; and (3) -29°18′41.91″ S, 116°43′49.02″ E] from which 20 individuals were randomly selected; and (2) three middens with matching sample sizes (middens A; D; H).

Parentage assignment was then performed for seeds collected from ant middens against the genotypes of all genotyped adults in the 1.6-ha study site. We used the maximum likelihood-based method of Marshall et al. (1998) implemented in the program CERVUS v.3.0.3, which uses categorical allocation to assign offspring to the plant (or plant pair) that has the highest likelihood of being the parent(s) by calculating likelihood ratios (LOD). Statistical confidence in assigned parentage is based on the difference in LOD scores between the most likely and second most likely candidate (Delta) (Marshall et al. 1998). The simulation parameters for CERVUS to assign parentage to the most-likely parent pair with a known level of statistical confidence were as follows: 10,000 cycles of simulation, 196 candidate parents, 0.95 as the proportion of the cluster sampled (exhaustive sampling of all potential seed donors) and 0.01 as the proportion of mistyped loci. Confidence levels using the LOD scores were set at 95 % (strict) and 80 % (relaxed) for assignment of parentage to offspring.

Seed dispersal distances were calculated for individual seeds based on the GPS position of the ant midden and the assigned parent pair. As we could not determine which parent within each assigned (parentage analysis) parent pair was the maternal, two approaches were used to estimate seed dispersal distances. First, we followed Hardesty et al. (2006) and applied a conservative approach (CONS) that assumed the closest plant of each parent pair to the ant midden was the maternal seed source. Secondly, we applied an equal likelihood approach (EL), as we had no a priori reason for assuming one parent over the other as the maternal, and all parent-pair-to-midden distances were included in calculations of seed dispersal distance properties (average, maximum, distribution). A seed dispersal distribution of individual seed dispersal distances was plotted for all ant midden samples, and then for the middens of each ant species separately, for both approaches. The seed dispersal distribution was compared to the distance distribution of all plants from all middens, and summed across all middens. We also calculated the Spearman rank correlation coefficient (r_s) between plant density (i.e. the number of plants within 25 m of ant midden) and the genetic diversity

of seeds in ant middens (i.e. the number of parents assigned to each ant midden). We examined the relationship between maternal plants that contributed multiple seeds to a midden (CONS approach only) against the distance of maternal plants to each midden. Finally, the effect of plant density on dispersal was assessed by regression analysis of average distance over which seeds were dispersed and number of plants within 25 m, for each ant midden.

Adult genetic structure

We assessed spatial genetic structure within the *A. karina* cluster using a spatial autocorrelation analysis within GenAlEx v.6.5 (Peakall and Smouse 2012), with distance classes set at 25 m. Altering the distance classes and changing their size did not qualitatively change the outcome of the analyses. Results are presented graphically in a correlogram, which shows the genetic correlation as a function of distance class across the site. A correlogram was produced with calculated correlation coefficient *r* and upper and lower confidence limits, as generated by 999 random permutations of the data, which was bound by the 95 % confidence interval about the null hypothesis of no spatial structure. Correlation values outside the 95 % confidence interval were considered to be statistically significant (Peakall and Smouse 2012). Additionally, the strength of spatial genetic structure was quantified by calculating the S_p statistic using SPAGeDi 1.3 (Hardy and Vekemans 2002).

The S_p statistic is primarily dependent on the rate of decrease of pairwise kinship coefficients between individuals with the logarithm of the distance in two dimensions. The S_p statistic was determined as $b/(1 - F_1)$, where b is the mean regression slope with natural log of distance (ln) and F_1 is the mean pairwise kinship coefficient for the smallest distance class (Vekemans and Hardy 2004).

The S_p statistic allows a comparison to other species that is often not possible with spatial autocorrelation analysis (Vekemans and Hardy 2004).

Results

Parentage analysis and realised seed dispersal by ants

Progeny arrays showed Mendelian inheritance of all alleles (data not shown). We successfully genotyped 196 adults and 124 seeds (midden A, $n = 65$; B, $n = 6$; C, $n = 2$; D, $n = 18$; E, $n = 1$; F, $n = 8$; G, $n = 3$; and H, $n = 21$). The seven microsatellite primer pairs amplified 111 alleles (average 16, range 6–25, per locus) in the adult *A. karina*, and high levels of genetic variation were revealed in adults ($H_o = 0.690$) and seeds ($H_o = 0.632$) (Table 1). A more explicit comparison of the genetic diversity of adult plants that occur within patches to the seeds contained in middens shows that middens have marginally less genetic diversity than the patches of adults, but display a similar level of variation in inbreeding (F_{IS}) amongst each midden (Table 2).

From CERVUS, parentage was assigned for five seeds at 95 % confidence and 124 seeds at 80 % confidence. No seeds were assigned to more than two compatible parents. All subsequent analysis of dispersal patterns and distances was done using the 124 seeds. Under the CONS approach, the overall mean seed dispersal distance was 40 m (±5.8 SE) (Fig. 2), and was 37 m (±5.6 SE) and 51 m (±17.2 SE) for *I. agilis* and *M. turneri perthensis,* respectively (Fig. 3). Under the EL approach, overall mean seed dispersal distance was 79 m (±6.4 SE) (Fig. 2), and was 77 m (±6.7 SE) and 84 m (±17.8 SE) for *I. agilis* and *M. turneri perthensis,* respectively (Fig. 3). Maximum seed dispersal distances detected for *I. agilis* (CONS 265 m and EL 300 m) were shorter than for *M. turneri perthensis* (CONS 417 m and EL 423 m) (Fig. 3). However, seed dispersal for both approaches and both ant species was predominantly within 50 m of the maternal source (44–84 % of all seeds), with a lower number of longer distance dispersal events identified, although relative proportions for each distance class varied depending on approach (Fig. 3). The number of parents assigned to each midden ranged from each seed having a different parent to multiple seeds having the same parent (Table 3). Our tests for correlation between plant density (i.e. the number of plants within 25 m of ant middens) and the genetic diversity of seeds in ant middens (i.e. the number of parents assigned to each ant midden) were not significant [for EL, Spearman Rank Correlation Coefficient (r_s) = −0.216 ($n = 8$)

and for CONS, $(r_s) = 0.018$ ($n = 8$); significant r_s at $P = 0.05$ is 0.643 ($n = 8$)]. However, there was a higher probability of a plant being a maternal source of multiple seeds within a midden when the maternal plant was closer to the midden (Fig. 4). Finally, although we observed a negative trend between the density of plants within 25 m of midden and seed dispersal distance for both approaches, this association was not statistically significant (CONS: $F_{1,7} = 2.83$; $P = 0.143$; EL: $F_{1,7} = 1.89$; $P = 0.218$; Fig. 5).

Adult genetic structure

The geographic distance between all possible pairs of adult plants ranged from 0.5 to 459 m, (mean 179 ± 8 SE) and the distance between nearest neighbours ranged from 0.5 to 68 m (mean 5.8 ± 0.6 SE). Spatial autocorrelation analysis revealed significant spatial genetic correlation among individuals at distances up to 100 m ($P < 0.05$), although the absolute correlation coefficient values were low (ranging from 0.009 to 0.077) (Fig. 6). Beyond 100 m, the genetic correlation coefficient was not significantly different from zero, indicating a stabilising profile and reflecting no association between pairwise genetic association and distance beyond 100 m. The S_p statistic ($S_p = 0.013$) supported conclusions about significant spatial genetic structure among adults. Neighbourhood size, estimated as the inverse of S_p, was 76.

Discussion

Our application of a molecular parentage assignment approach to seeds recovered from ant middens has facilitated novel insights into the dispersal of seeds by ants in semi-arid Australia. We found that mean and maximum seed dispersal distances by ants for the Australian semi-arid zone shrub, *A. karina*, far exceeded previously published global means and maxima (Gómez and Espadaler 2013). Previously recorded maximum distances for arillate seed dispersal by ants in Australia include 4 m for *Pultenaea daphnoides*, 4.7 m for *Daviesia triflora*, 8.1 m for *Acacia blakelyi*, 77 m for chenopod species, and 180 m for *Acacia ligulata* (Beaumont et al. 2013; Davidson and Morton 1981; He et al. 2009; Whitney 2002). In our study, maximal seed dispersal distance detected for *A. karina* was

425 m, with a (CONS) mean of 40 m (±5.8 SE). Outside Australia, the maximum distance recorded for seed dispersal by ants is 70 m, with an overall global mean of 2.24 m (±7.19 SD) from 7889 observations and 54 studies (Gómez and Espadaler 2013). Our results are well outside the confidence intervals reported by Gómez and Espadaler (2013) and strongly support suggestions that myrmecochorous seed dispersal in the Australian arid/semi-arid zone is characterized by longer dispersal distances than those in other parts of the world.

Potential role of alternative dispersal modes

The dispersal distances estimated here are effective dispersal distances that integrate the spatial pattern of shrub-to-midden positions and also post-dispersal processes (see Nathan et al. 2013). Primary seed dispersal may have ejected seeds a small distance from the fruits; however, this is generally short in acacia (0–2 m) (Auld 1997). While it is possible that some *A. karina* seeds may be initially dispersed by other modes (water, wind or other animals, including emus, passerine birds, kangaroos and other mammals) away from the maternal plant, and then dispersed secondarily by ants, this appears unlikely. Firstly, seeds of *A. karina* mature and fall to the ground during the driest part of the year and seeds of acacia species are quickly collected by ants, often within 24 h (Majer 1980, 1984), meaning that water dispersal is an unlikely mode for consideration in this system. Also, many seeds were assigned to a maternal parent that was down-slope from the midden from which the seeds were found, negating the likelihood of surface runoff and downhill effects. Secondly, the seed weight and lack of appendages indicate that wind is unlikely to play a role in seed dispersal in acacias (for example, Marchante et al. 2010). Thirdly, although diaspores that have been ingested by vertebrates are known in the tropics to be secondarily dispersed by ants (e.g. see Passos and Oliveira 2003), seeds without food bodies have been shown to be less attractive to ants (Auld 1986; O'Dowd and Hay 1980), so it seems unlikely that seeds have been ingested by other animals and excreted, then to be collected by ants. Although it is possible that one or more of these other agents may have contributed to the longest distance dispersal events, the fact remains that the main part of the dispersal curve, and the high mean values, indicate that both ant species disperse seeds much further than previously reported for other parts of the world.

Effect of plant density and ant body size on seed dispersal distances

Plant density can impact the distance to which seeds are dispersed, with increasing plant density often promoting shorter dispersal distances (Jansen et al. 2014). Interestingly, we found that plant density had no effect on seed dispersal distances. This is in contrast to the outcomes of previous studies, which showed plant density was negatively correlated with the distances seeds were carried by ants (e.g. Pudlo et al. 1980). While our results imply that ant species responsible for the dispersal of *A. karina* seeds at this site forage widely, regardless of the density of available resources, we have no data on spatial patterns of seed production for *A. karina* or other species at the site. Therefore, this conclusion must be treated with caution.

The identity of dispersing ant species is another factor that can influence the outcome of myrmecochory, as differences in body size, foraging behavior and locations where seeds are processed and deposited all have an effect on the distribution of seed dispersal (Andersen 1988; Beaumont et al. 2013; Gómez and Espadaler 1998, 2013; Ness 2004). Previous investigations have shown that mean and maximum dispersal distances increased with worker body size (Gómez and Espadaler 1998; Ness 2004); however, in our study, *M. turneri perthensis* dispersed seeds over greater distances (mean 51 m ± 17.2 SE CONS) than the slightly larger *I. agilis* (mean 37 m ± 5.6 SE CONS). Why the spatial range of seed dispersal was greater in the *M.turneri perthensis* than in *I. agilis* could be related to several factors. *M. turneri perthensis* is highly dependent on seeds, with almost half of its dietary intake being seeds and other plant parts (Majer et al. 2011), whereas *I. agilis* is more omnivorous (Heterick and Shattuck 2011; Shattuck 1992), possibly reducing the need to carry seeds over long distances due to a greater resource opportunity within a similar area. Also, unlike *I. agilis*, the seasonal activity of the thermophilic *Melophorus* is at a maximum during warmer periods (Majer et al. 2011), a period when enhanced activity may allow the carrying of seeds over longer distances. Finally, the long, linear-triangular, weakly-toothed mandibles of the *Iridomyrmex* species may well be mechanically less well-adapted to carrying, smooth, heavy seeds for long distances. The mandibles of the *Melophorus*species, by way of contrast, are sub-triangular and stout (Heterick 2009), ideally suited to the carrying of seeds.

Myrmechory, population dynamics and spatial genetic structure

Our data show that, despite evidence that a closer proximity to a midden increased the probability of a maternal plant contributing multiple seeds, the level of genetic relatedness of *A. karina* seeds in ant middens was low due to the high number of unique parents contributing to an aggregation and a completely out-crossing mating system (Pascov 2013). The comparison of the genetic diversity of adult plants that occur within patches to the seeds contained in middens showed that middens have marginally less genetic diversity than the patches of adults, but display a similar level of variation in inbreeding (FIS). Therefore, genetic patterns detected in middens are important and hold true past germination. This finding demonstrates how ants play an important role in determining the degree of relatedness of conspecific seeds aggregated in space, potentially increasing productivity (Young 1981), survival (Hanski and Saccheri 2006) and individual fitness responses to herbivory or pathogen attack (Koelewijn 2004).

Our results are largely congruent with previous studies of spatial genetic structure in *Acacia* (e.g. Millar et al. 2014) but add evidence for the importance of ant mediated dispersal in its development. Spatial autocorrelation analyses showed significant spatial genetic structure up to an interplant distance of 100 m and more generally, spatial genetic structure detected for *A. karina* ($S_p = 0.013$) was less than half the global average of $S_p = 0.030$ for 47 plant species (Vekemans and Hardy 2004). Spatial genetic structure can arise from a variety of factors, however, gene flow through pollen and seed dispersal are key determinants in its establishment (Wright 1943; Vekemans and Hardy 2004). Pollen of most *Acacia* is thought to be dispersed by a range of generalist insect pollinators, including ants, moths, wasps, beetles and bees (Stone et al. 2003), but studies of pollen dispersal distances in acacias are surprisingly limited (Millar et al. 2008, 2012, 2014) given their ubiquity in the Australian landscape. In *A. saligna*, for example, the majority of pollen is dispersed within a 50 m neighbourhood of the maternal plant and the pattern of mating is essentially random with no evidence of a majority of nearest neighbour mating (Millar et al. 2008, 2012). Whilst pollen dispersal predominantly within 50 m would produce a SGS structure similar to the one observed here, the

surprisingly high seed dispersal distances we identified highlight that ant-mediated seed dispersal is also a significant factor in the relatively large spatial scale genetic structure found in *A. karina*.

Implications

Dispersal distance per se has historically been rejected as a potential benefit of myrmecochory because ant seed dispersal distances were thought to be small in comparison with those recorded for birds and mammals. However, the large dispersal distances identified in this study, along with other studies in Australian arid/semi-arid zones (Davidson and Morton 1981; Whitney 2002), lends credence to the hypothesis that distance dispersal (in contrast to directed dispersal) could be of benefit to myrmecochorous plants. These studies combined provide ecological modellers with critical empirical data on the dispersal ability of myrmecochorous species, which will enable better predictions of the ability of species to respond to climate change by dispersing and tracking suitable climates, the impact of invasive species and impacts of habitat fragmentation/anthropogenic disturbance.

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Fig. 1 Geographic location of all *Acacia karina* plants (*n* = 196) (*open circle*) and sampled ant middens (*n* = 8). *Melophorus turneri perthensis* (*filled triangle*) and *Iridomyrmex agilis*(*open triangle*) at John Forest Lookout, southwest Australia. *Ant midden codes* match those used in Table 2
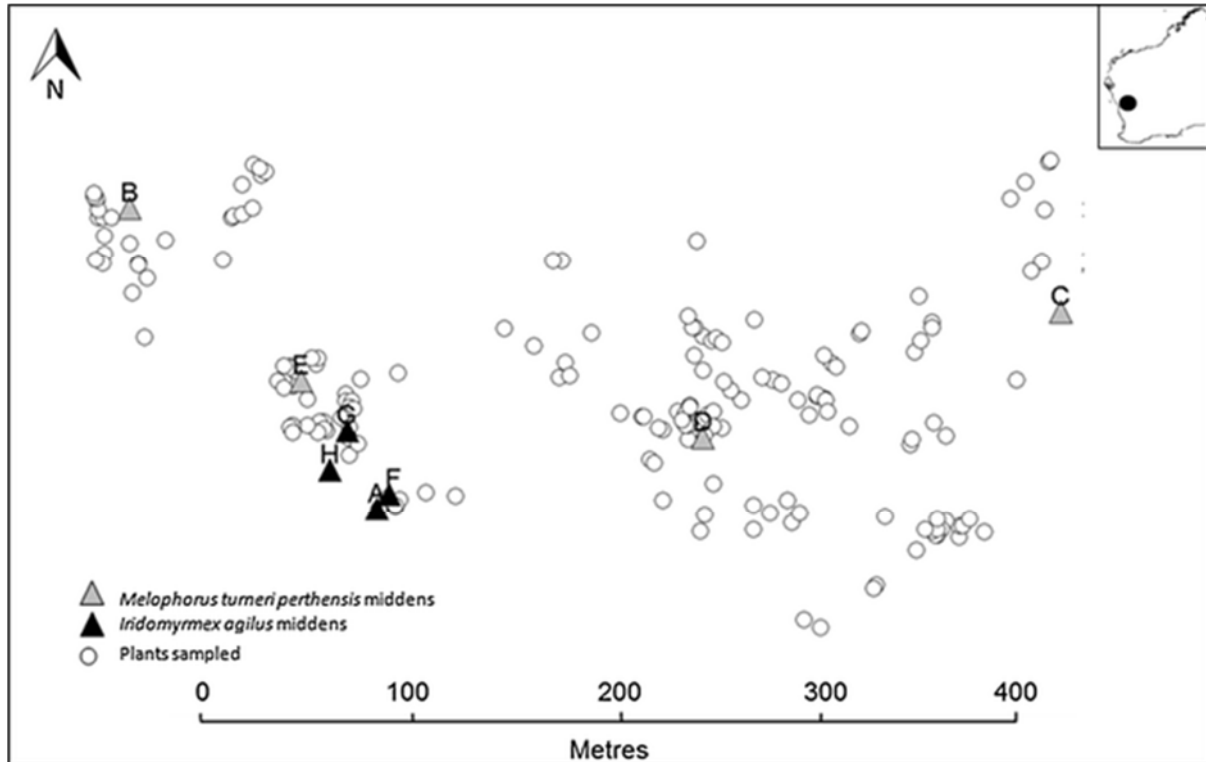
Fig. 2 Frequency distribution of seed dispersal inferred from parentage assignment of ant midden seeds (*n* = 124) within a natural cluster of *Acacia karina*, using the conservative approach (CONS) (*dark grey bars*) and equal likelihood (EL) approach (*light grey bars*). Also shown is the frequency distribution of the distance of all shrubs relative to all sampled ant middens (*white bars*). Note: CONS approach assumes as maternal the closest plant to a midden from the maximum likelihood parent pair for each seed, and EL approach includes the distances of both parents of each maximum likelihood parent pair
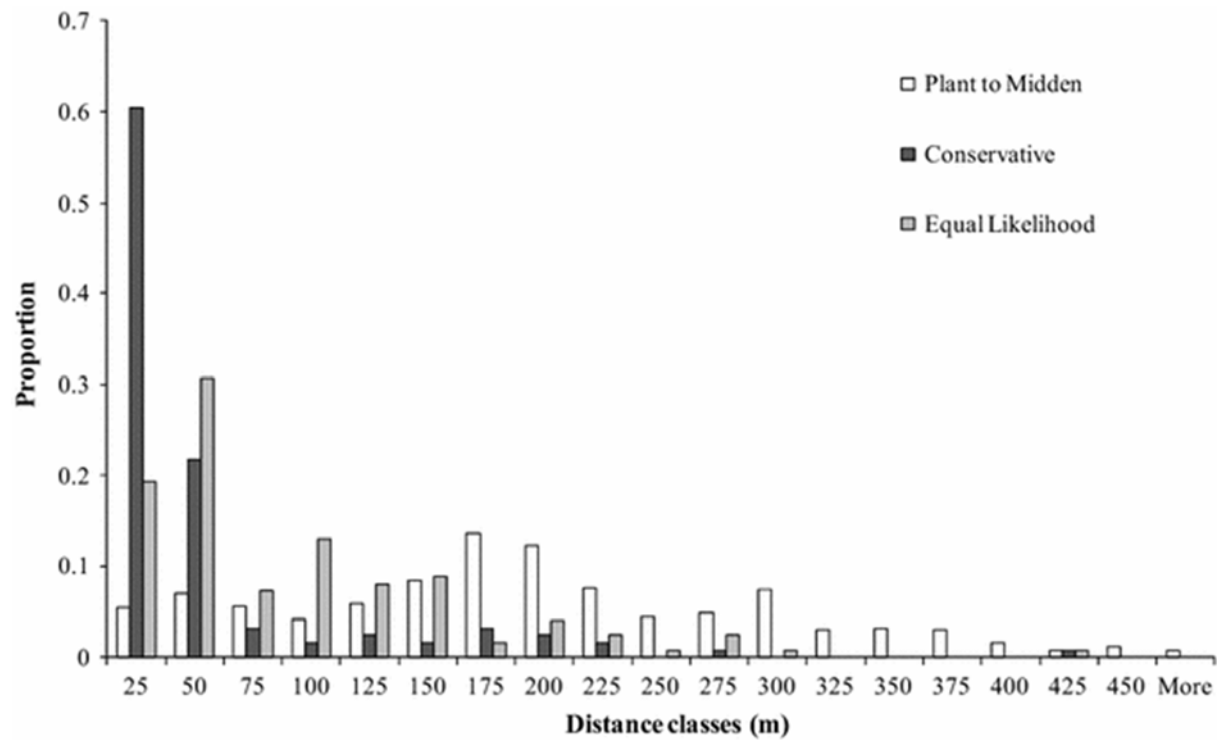
Fig. 3 Frequency distribution of seed dispersed by two ant species within a natural cluster of *Acacia karina* (*n* = 196), using the conservative (CONS) (*dark grey bars*) and equal likelihood (EL) approach (*light grey bars*). **A** *Melophorus turneri perthensis* (four middens and *n* = 27 seeds) and **b** *Iridomyrmex agilis* (four middens and *n* = 97 seeds). Note: CONS approach refers to dispersal distances calculated using the individual from the parent pair that was closest to the midden and EL refers to dispersal distances calculated based on the distance from the midden for both individuals within the parent pair
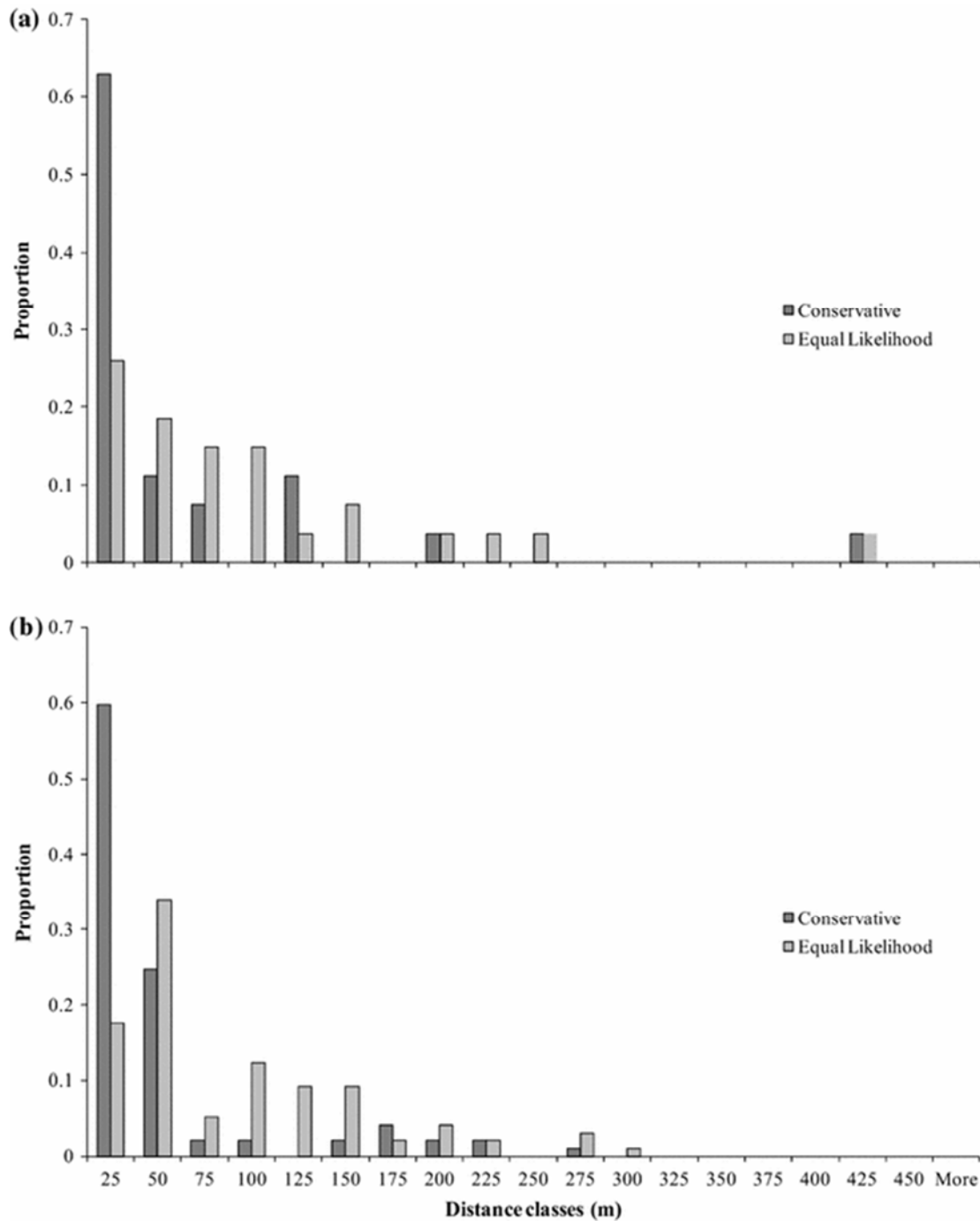
Fig. 4 The frequency of maternal assignments for *Acacia karina* seeds collected within each of eight ant middens as a function of the distance of the maternal plant from the midden
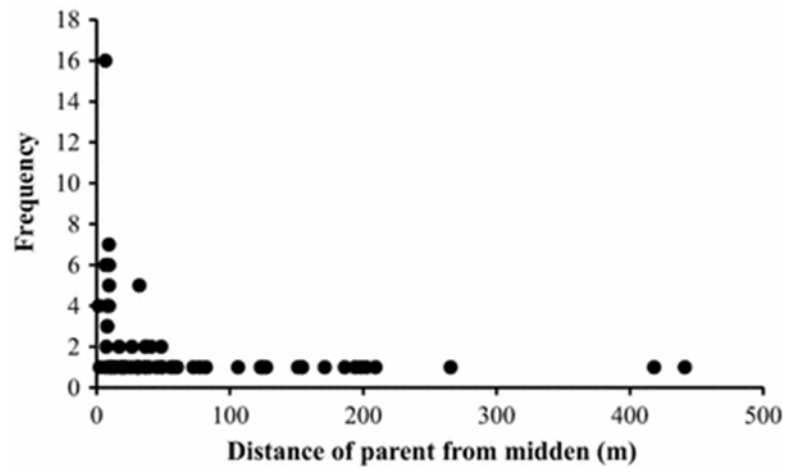
Fig. 5 Average (±SE) distance over which *Acacia karina* seeds was dispersed, and number of plants within 25 m, for each of eight ant middens, assessed by the **a** conservative parentage (CONS) approach and **b** the equal likelihood (EL) parentage approach for *Melophorus turneri perthensis* (*closed diamonds*) and *Iridomyrmex agilis* (*open diamonds*) middens. Note: CONS approach refers to dispersal distances calculated using the individual from the parent pair that was closest to the midden and EL refers to dispersal distances calculated based on the distance from the midden for both individuals within the parent pair
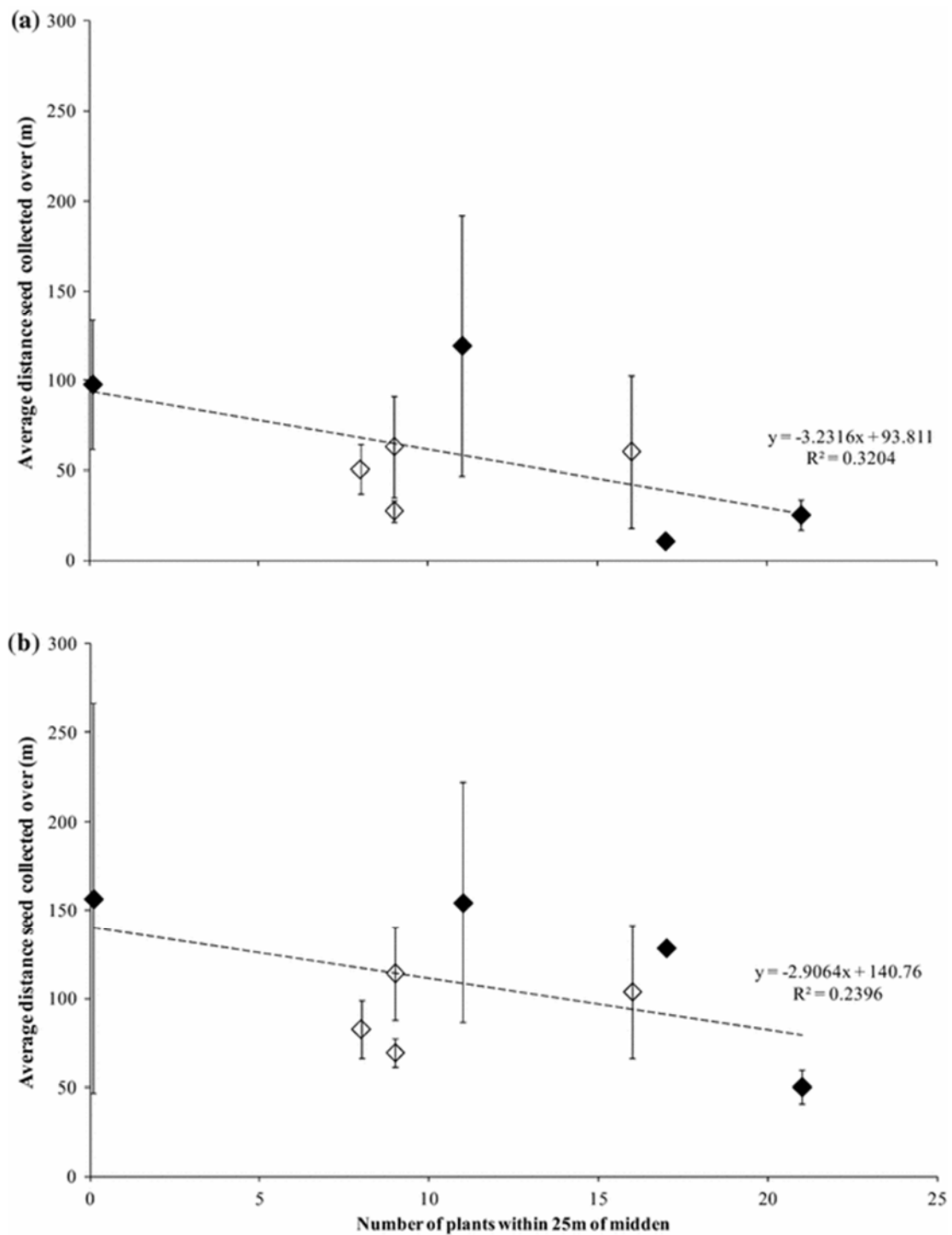
Fig. 6 Spatial autocorrelation analysis of microsatellite variation for *Acacia karina* at John Forest Lookout, southwest Australia. The genetic correlation coefficient (*r*) ±SE is plotted against distance classes. *Dashed lines* indicate 95 % confidence intervals around the null hypothesis of no spatial genetic structure
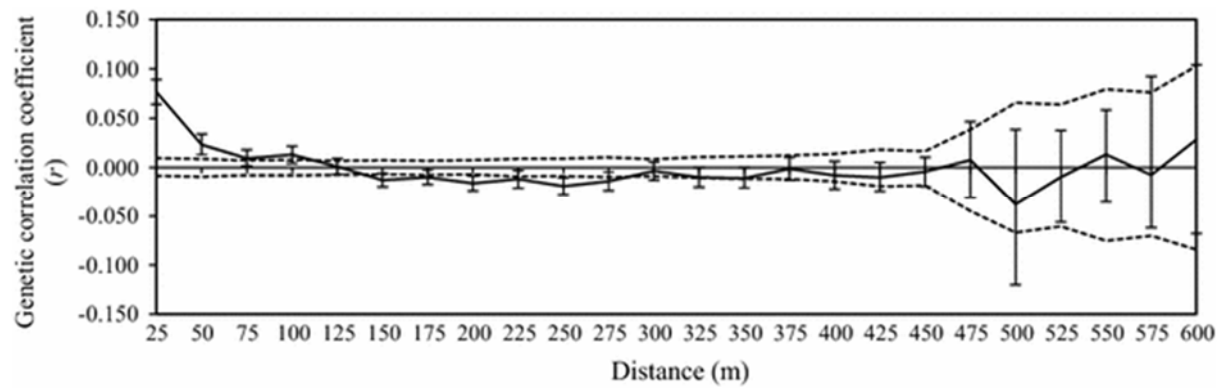
Table 1 Descriptive genetic statistics calculated for adult shrubs and seeds collected from active ant middens (two middens in 2013 and six middens in 2014) from a natural cluster of *Acacia karina* at John Forest Lookout, southwest Australia

	N_a	H_o	H_e	F_{IS}
Adults (*n* = 196)				
Mean	15.9	0.69	0.799	0.126
±SE	2.7	0.041	0.036	0.062
Seeds (*n* = 124)	0	0	0	0
Mean	16.7	0.632	0.821	0.224
±SE	1.8	0.025	0.031	0.045
Overall	0	0	0	0
Mean	16.3	0.661	0.81	0.175
±SE	1.5	0.024	0.023	0.039

N_a number of alleles per locus, H_o and H_e observed and expected heterozygosity, respectively, F_{IS} fixation index

Table 2 Descriptive population genetic statistics calculated for three patches of adult trees (1–3) and three ant middens (*A*, *D*, *H*) from a natural population of *Acacia karina* at John Forest Lookout, southwest Australia

	N_a	H_o	H_e	F_{IS}
Patch of adults				
1. Mean (*n* = 20)	8.7	0.757	0.761	−0.003
±SE	1.1	0.046	0.033	0.065
2. Mean (*n* = 20)	8.3	0.615	0.724	0.133
±SE	1.1	0.058	0.044	0.086
3. Mean (*n* = 21)	7.1	0.699	0.705	0.002
±SE	1.3	0.058	0.06	0.051
Middens				
A. Mean (*n* = 20)	6.1	0.567	0.687	0.138
±SE	0.7	0.049	0.047	0.108
D. Mean (*n* = 19)	6.4	0.605	0.701	0.125
±SE	1	0.046	0.042	0.074
H. Mean (*n* = 21)	6	0.705	0.694	−0.013
±SE	0.6	0.055	0.03	0.054

N_a number of alleles per locus, H_o and H_e observed and expected heterozygosity, respectively, F_{IS} fixation index

Table 3 Total number of different parents assigned to *Acacia karina* seeds (*n*) in each midden, dispersed by two ant species a) *Melophorus turneri perthensis* (*MT*; four middens and *n* = 27 seeds) and b) *Iridomyrmex agilis* (*IA*; four middens and *n* = 97 seeds)

Midden	Ant species	*n* assigned	Number of parents: conservative	Number of parents: equal likelihood
B	MT	6	5	10
C	MT	2	2	4
D	MT	18	12	25
E	MT	1	1	2
A	IA	65	16	51
F	IA	8	7	15
G	IA	3	3	6
H	IA	21	12	23
Total		124		