

# Ants cannot account for interpopulation dispersal of the arillate pea *Daviesia triflora*

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## Summary

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**Key words:** assignment test, ants, long-distance dispersal, metapopulation, microsatellite DNA marker, myrmecochory, nonstandard dispersal, Reid's paradox.

- Estimating distances and rates of seed dispersal, especially long-distance dispersal (LDD), is critical for understanding the dynamics of patchily distributed populations and species' range shifts in response to environmental change.
- *Daviesia triflora* (Papilionaceae) is an ant-dispersed shrub. The ant *Rhytidoponera violacea* was recorded dispersing its seeds to a maximum distance of 4.7 m, and in more intensive trials seeds of a related species from the study area, to a maximum of 8.1 m.
- Microsatellite DNA markers and population assignment tests identified interpopulation immigrants among 764 plants on 23 adjacent dunes bearing *D. triflora*, and 13 interpopulation seed dispersal (LDD) events (1.7%) were inferred. The distance between source and sink populations ranged from 410 m to 2350 m (mean 1260 m). These distances exceed ant dispersal distances by two to three orders of magnitude but are comparable with previous measurements of LDD for two co-occurring wing-seeded (wind-dispersed) species from the same system.
- The observed distances of seed dispersal in this arillate species demonstrate the significance of nonstandard dispersal mechanisms in LDD and the independence of these from primary dispersal syndromes. The likely role of emus in dispersal of the many 'ant-dispersed' species in Australia is discussed.

## Introduction

Long-distance dispersal (LDD) of seeds has important implications for invasion dynamics, population genetic structure, and species response to climate change (Cain *et al.*, 2000). Long-distance dispersal is defined as a dispersal distance of at least 100 times greater than plant height (Nathan *et al.*, 2003). The quantification of LDD is critical for understanding the dynamics of patchily distributed populations in fragmented landscapes (Sork & Smouse, 2006). Seed dispersal between widely separated habitats allows for declining populations to be rescued and unoccupied habitats to be colonized or recolonized (e.g. following local extinction). Although typically rare in comparison with local dispersal, LDD is essential for processes such as establishment of new populations, range expansion and species invasion (Clark *et al.*, 2003; Shea, 2006). Data on LDD are also being used increasingly in models of species range contraction and extinction risk under climate change scenarios (Fitzpatrick *et al.*, 2008; Keith *et al.*, 2008).

The Southwest Australian Floristic Region (SWAFR) is a 300 000 km<sup>2</sup> area of sclerophyll forests, woodlands, and shrublands (kwongan) with a Mediterranean-type climate and is an internationally recognized biodiversity 'hotspot' (Myers *et al.*, 2000). Over one-third of the 7380 native plant taxa recorded so far (Hopper & Gioia, 2004) possess arillate (elaiosomic) diaspores that are thought to be myrmecochorous (dispersed by ants; Lamont *et al.*, 1977; Milewski & Bond, 1982). Dispersal of seeds by ants is generally regarded as reaching only a few metres, which is much less than that attained by birds, mammals and other vertebrates (Andersen, 1988). A global meta-analysis of seed dispersal distances by ants reported an average distance of 0.96 m and a maximum of 77 m (Gómez & Espadaler, 1998), though more recently a maximum of 180 m has been recorded (Whitney, 2002). In general, however, the tail of the dispersal curve for myrmecochorous species remains poorly understood.

The distribution of the arillate, sub-shrub, *Daviesia triflora* Crisp (Papilionaceae) in the shrubland landscape of the

Eneabba Plain, SWAFR, corresponds to the distribution of 'habitat islands' formed by discrete dunes separated by an average of 280 m of shallow sand habitat lacking this species (Calviño-Cancela *et al.*, 2008). Interpopulation (inter-dune) seed dispersal of *D. triflora* is highly unlikely to be achieved by ant dispersal, and any local population extinction (including loss of soil stored seeds) should be permanent in the absence of LDD. Moreover, the current landscape has been geomorphologically stable for over 20 000 yr (Krauss *et al.*, 2006), providing ample opportunities for population extinction following periods of unfavourable climate and/or fire regime over that time. Thus, LDD by nonstandard vectors is essential for providing the habitat connectivity necessary for the long-term persistence of this species. Species that have a much wider distribution than can be explained by their primary dispersers are examples of Reid's paradox, and its resolution requires invoking agents of LDD (Clark *et al.*, 1998).

The LDD of seeds likely results from a number of processes, so the relationship of LDD to morphologically based dispersal syndromes is complex (Higgins *et al.*, 2003). We previously indirectly quantified LDD by molecular markers and assignment tests in two serotinous shrub species with winged seeds from the same metacommunity – the fire-killed *Banksia hookeriana* and resprouting *Banksia attenuata*, with LDD rates of 6.8% and 5.5%, respectively, with events observed up to 2.6 km from the parent population (He *et al.*, 2004, 2008b). While winged attachments may facilitate local dispersal, the LDD frequency and distance observed for *Banksia* species cannot be the result of the same processes responsible for short-distance transport (wind gusts). Instead, wind vortices and carriage by cockatoos have been invoked to account for these nonstandard distances (He *et al.*, 2004, 2008b).

In the absence of other knowledge, models of LDD for arillate species either extrapolate from the comparison of the mechanisms of local dispersal to assume that LDD rates and distances will be proportionately lower than for winged seeds (Keith *et al.*, 2008), or assume that they have no probability of LDD (Esther *et al.*, 2008). Recently, Calviño-Cancela *et al.* (2006, 2008) identified a likely mechanism for LDD in arillate-seeded species within Australia, recording seeds and pods of numerous arillate species, including three *Daviesia* species (and *Acacia blakelyi*), in droppings of the emu (*Dromaius novaehollandiae*), a large flightless bird. Here we examine the evidence for LDD in *Daviesia triflora* and the operation of nonstandard dispersal agents by comparing distances seeds are transported by ants with the metapopulation spatial/genetic structure of *D. triflora*.

Some of the processes that enable LDD are amenable to formal investigation, while others are inherently intractable. Until recently, it has been almost impossible to determine the distances travelled or the percentage of dispersal events that led to successful recruitment. Cain *et al.* (2000) suggested that genetic methods provide a broadly applicable way to monitor LDD in determining its frequency and spatial scale. Assignment

of individuals to source populations on the basis of variation at multilocus genotypes has been demonstrated as a powerful, and sometimes the only, tool to measure LDD (Berry *et al.*, 2004; Kraaijeveld-Smit *et al.*, 2007; Lee *et al.*, 2007), as well as providing important details on the extent of the tail of the dispersal curve (Sork & Smouse, 2006). Consequently, we first examined the local dispersal of seeds by ants using cafeteria (seed removal) experiments, and then used polymorphic microsatellite DNA markers and population assignment tests to estimate the frequency and spatial scale of seed dispersal between populations within a metapopulation of *D. triflora*. We specifically aimed to test the idea that seeds of myrmecochores are dispersed to distances that exceed those possible with ants, thus invoking a role for secondary vectors, such as highly mobile generalist herbivores, in explaining the distribution of arillate species across the landscape (Calviño-Cancela *et al.*, 2008). We also tested the hypothesis that the rate and distances of LDD in this species are lower than those of two co-occurring wing-seeded shrub species adapted for dispersal by wind.

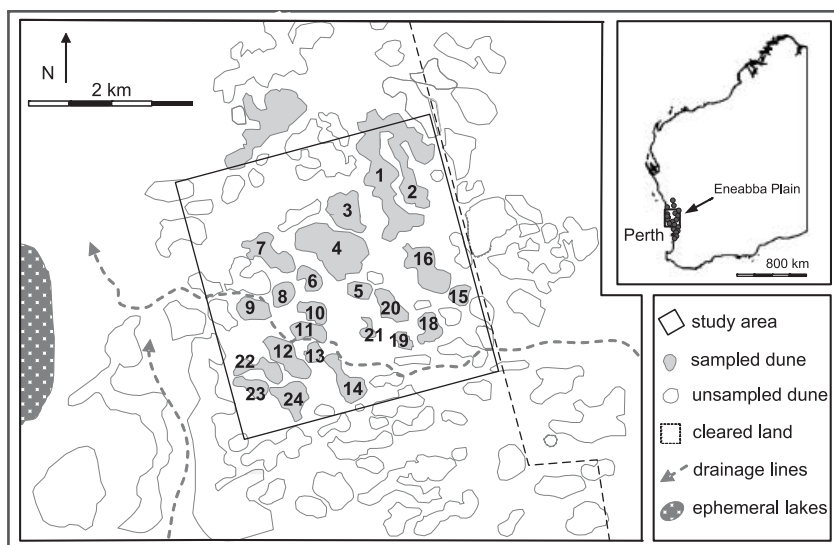
## Materials and Methods

### Study species and site

*Daviesia triflora* Crisp (Papilionaceae) is a leafless, green-stemmed sub-shrub with the ability to resprout from buried stem tissues (lignotuber) after fire. It is distributed over a 350 km latitudinal range on the near-coastal Swan and Eneabba sandplains of Western Australia (Fig. 1). It has typical pea flowers that are pollinated by small native bees (Crisp, 1995), while seed set is sporadic and seedling recruitment is rare and only occurs following fire (B. Lamont, pers. obs.). The study area covered 10 km<sup>2</sup> (3.3 × 3.3 km) centred on 115°13'54"E, 29°44'29"S, at Beekeepers Nature Reserve, 10 km north of Eneabba, Western Australia (Fig. 1). This area overlaps with that of previous studies into interactions among plant life-history, dispersal mode and LDD (He *et al.*, 2004; Calviño-Cancela *et al.*, 2008; Groeneveld *et al.*, 2008). The region has a Mediterranean climate with a hot dry summer, cool wet winter and an average annual rainfall of 504 mm yr<sup>-1</sup>. The topography is a predominantly dune (crest) and swale (low-lying) system where sands overlaying clay and laterite at depths of 60–800 cm support a dense (cover > 70%) sclerophyll shrubland rarely more than 1.5 m tall. Fire is the major form of natural disturbance on the sandplain, with a mean return interval of 13 yr observed since 1940 (Lamont *et al.*, 2003; Miller *et al.*, 2007). Most plant species show adaptations to fire, including the ability to regrow vegetatively, soil and/or canopy seed storage and fire-stimulated flowering (Gill, 1981; Enright *et al.*, 2007).

### Seed dispersal by ants

To assess the capacity of two regionally common species of the ant genus *Rhytidoponera* (*R. violacea* [Smith] and *R. metallica*



**Fig. 1** Study site near Eneabba, south-western Australia, showing the overall distribution (upper right figure indicated by black dots) and sampled populations of *Daviesia triflora*. Note: population DT17 contained a single individual in unhealthy growing condition, and was not included in the genetic analysis.

[Forel]; identified as the most important seed-dispersing genus in Australia; Gove *et al.*, 2007) to transport seeds, we conducted a cafeteria trial at 36 points within each of eight sites (summarized in Gove *et al.*, 2007) in the study area using seeds of *A. blakelyi*. This leguminous shrub co-occurs with *D. triflora* and has seeds that are similar in size, weight and aril-type (Sweedman & Merritt, 2006) but more easily collected. To confirm that *Rhytidoponera* ants were capable of carrying seeds of *D. triflora*, a cafeteria experiment was conducted at Thomas Regional Reserve (115° 50' 12" E, 32° 13' 57" S), in Banksia woodland where both *D. triflora* and *Rhytidoponera violacea*, co-occur. Five sites were established at 30-m intervals at each of which, 10 arillate seeds of *D. triflora* were placed together on bare sand at 09 : 00 h. The seeds were then monitored until to 17 : 00 h and seed-removing ants were identified and tracked to the point where their seeds were dropped or deposited in nests.

#### Microsatellite genotyping and genetic structure analysis

Stems of *D. triflora* were collected from up to 45 individuals encountered on random walks over each of 23 adjacent dunes with *D. triflora* in the study area (Table 1, Fig. 1). The population size of *D. triflora* stands was estimated from density counts for five replicate 15 × 15 m plots on each dune, and dune size (area) which was calculated from IMAGEJ (available from <http://rsb.info.nih.gov/ij>) after adapting images of dune distribution from a 1 : 25 000 colour aerial photo run (WA3970C; DoLA, 1997). Stems from each sample were kept in zipped plastic bags and stored at -80°C before genetic analysis. Genomic DNA was extracted following the protocol described in Butcher *et al.* (1998). Quantity and quality of DNA were assessed by visualization on a 1.5% agarose gel and using a Fluorometer (DQ 300; Hofer, San Francisco, CA, USA). Eleven microsatellite primer pairs were used for geno-

typing. The detailed protocol for microsatellite genotyping is described in He *et al.* (2008a).

Microsatellite data were first subjected to analysis using GENALEX 6.0 (Peakall & Smouse, 2006) for parameters of population genetic diversity: number of alleles ( $N_a$ ), expected heterozygosity ( $H_e$ ), for assessment of overall population genetic differentiation ( $F_{ST}$ ), and for the partitioning of total genetic variation into within and among population components by AMOVA. Pairwise population differentiation was estimated by standardized  $F_{ST}$  since high gene diversity can compromise the ability to accurately quantify population divergence through  $F$ -statistics (Hedrick, 2005). First, original  $F_{ST}$  and maximum  $F_{ST}$  (after recording genetic marker data using RECODEDATA, Meirmans, 2006) were calculated with FSTAT (Goudet, 1995). Standardized  $F_{ST}$  was then obtained by dividing original  $F_{ST}$  by maximum  $F_{ST}$ . The spatial structure of genetic variation within and among populations was assessed by spatial autocorrelation analysis using GENALEX 6.0, with Bonferroni corrections applied when testing for significance across distance classes.

#### Population assignment

Population likelihood assignment tests were implemented to infer the seed source population for each sampled *D. triflora* individual (i.e. first generation of migrants between populations). First, GENECLASS2 (Piry *et al.*, 2004) was used to calculate the probability of a given population being the seed source for an individual, which was established with Monte Carlo resampling methods with 100 000 simulated individuals where  $P < 0.001$  excluded a population as the source of an individual (Paetkau *et al.*, 2004). Consequently, individuals with  $P < 0.001$  for all candidate populations indicated that the individual did not originate from any of the sampled populations (i.e., originating from outside of the sampling

Code	Population size	Sample size	$N_a \pm SD$	$H_e \pm SD$	$F_{is}$	Immigrants
DT01	5200	33	9.2 ± 5.6	0.726 ± 0.148	0.054	0
DT02	2900	40	10.2 ± 5.4	0.715 ± 0.151	0.088	1
DT03	3500	38	9.5 ± 6.1	0.730 ± 0.127	0.056	0
DT04	10300	39	10.0 ± 5.6	0.738 ± 0.125	0.128	1
DT05	1000	34	9.2 ± 5.2	0.734 ± 0.140	0.040	2
DT06	2100	41	10.1 ± 6.1	0.734 ± 0.136	0.092	0
DT07	5700	40	9.8 ± 5.4	0.720 ± 0.133	0.075	1
DT08	800	25	8.9 ± 4.4	0.745 ± 0.098	0.070	1
DT09	1200	22	7.5 ± 3.5	0.731 ± 0.084	0.077	0
DT10	1000	31	9.5 ± 4.9	0.741 ± 0.122	0.105	1
DT11	260	27	9.0 ± 5.0	0.715 ± 0.147	0.072	0
DT12	5300	35	9.2 ± 4.6	0.724 ± 0.138	0.144	0
DT13	800	19	7.5 ± 3.7	0.720 ± 0.133	0.087	0
DT14	2800	40	9.3 ± 5.3	0.710 ± 0.163	0.109	0
DT15	100	37	9.1 ± 5.2	0.728 ± 0.157	0.123	0
DT16	5300	45	10.2 ± 5.8	0.720 ± 0.160	0.101	1
DT18	1200	28	8.6 ± 4.4	0.745 ± 0.126	0.120	0
DT19	1400	21	7.6 ± 3.8	0.714 ± 0.151	0.072	0
DT20	1300	34	9.7 ± 5.7	0.712 ± 0.162	0.105	0
DT21	2100	36	9.7 ± 4.9	0.750 ± 0.139	0.101	1
DT22	3300	29	8.8 ± 4.6	0.703 ± 0.175	0.095	1
DT23	1800	35	9.1 ± 4.8	0.704 ± 0.170	0.071	0
DT24	3400	35	9.9 ± 6.0	0.741 ± 0.132	0.079	1
Mean	2700	33	9.2	0.729	0.090	–

**Table 1** Summary of genetic diversity in 23 adjacent populations of *Daviesia triflora*

$N_a$ , number of alleles per locus;  $H_e$ , expected heterozygosity;  $F_{is}$ , inbreeding coefficient; SD, standard deviation.

Note: population DT17 contained a single individual with unhealthy growing condition, and was not included in the genetic analysis.

domain). Then, the log-likelihood of each individual originating from each source population was calculated following the Bayesian-based method described by Rannala & Mountain (1997), since this method performs best for any value of  $F_{ST}$  (Cornuet *et al.*, 1999).

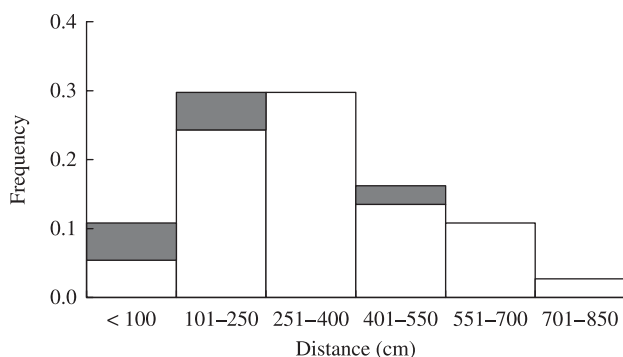
An unambiguous assignment of an immigrant was accepted when the difference between the largest and the second largest log-likelihood was above a threshold stringency level ( $\delta$ ). Adopting a low level of stringency (i.e.  $\delta$  near 0) increases the risk of falsely assigning immigrants because of small genotyping errors and/or inclusion of interpopulation hybrids resulting from interpopulation pollen flow (Roques *et al.*, 1999). Conversely, too high a level of stringency (e.g.  $\delta = 3$ , i.e.  $10^3$  times more likely to originate from the population with highest log-likelihood than from the second highest log-likelihood) unduly reduces assignment efficiency (in terms of the fraction of individuals identifiable as immigrants) (Campbell *et al.*, 2003). In addition, LDD events are typically much rarer than local dispersal events (He *et al.*, 2004; Nathan, 2006). Therefore, we applied a stringency level of  $\delta = 1.0$  to accept an individual as an immigrant; thus, a multilocus genotype had to be at least 10 times more likely to belong to one (source) population (other than the (sink) population from which it was collected) than any other (i.e. respective probabilities of 0.91

and 0.09 in a two population scenario; Duchesne & Bernatchez, 2002; Campbell *et al.*, 2003). When  $\delta$  between the populations with the highest and the second highest likelihood was greater than 1.0, an immigrant with known source population was accepted and assigned to the population with the highest likelihood. In the situation with multiple potential source populations, if  $\delta$  between the populations with the highest and the second highest likelihood was not  $> 1.0$ , but  $\delta$  of the source population with the least likelihood and the home population was  $> 1.0$ , an immigrant with unknown source population was accepted. Individuals not accepted as immigrants were regarded as residents.

## Results

### Seed dispersal by ants

One ant species, *Rhytidoponera violacea*, was observed carrying *D. triflora* seeds in the cafeteria experiment. Five seed-removing events were tracked, with an average distance 1.95 m, and maximum of 4.7 m. The distances observed for *D. triflora* fell within the range observed for the carriage of *A. blakelyi* seeds within the metapopulation study area by the two major seed dispersers, *R. violacea* and *Rhytidoponera metallica*, with an



**Fig. 2** Frequency distribution of the dispersal distances of *Acacia blakelyi* (open bars) and *Daviesia triflora* (tinted segment) by ants (*Rhytidoponera violacea* and *Rhytidoponera metallica*).

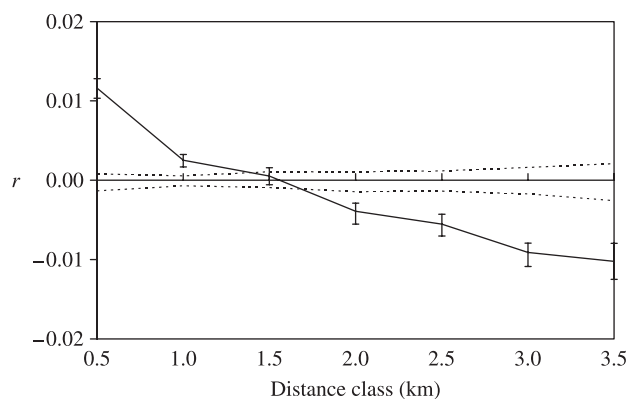
average distance of 3.3 m, and maximum of 8.1 m (Fig. 2). For these 37 dispersal events, 50% were < 3.2 m, and 95% were < 6.9 m.

### Genetic variation and differentiation among populations of *D. triflora*

Forty dunes were identified within the 10-km<sup>2</sup> study area, of which *D. triflora* occupied 23. *D. triflora* population size varied from 100 to over 10 000 individuals, averaging 2700 (Table 1). Eleven microsatellite primer pairs specifically developed for *D. triflora* successfully amplified 183 alleles (5–31 from individual primer pairs, averaging 16.6 per locus, SD = 8.9) among 764 individuals. High levels of genetic variation were revealed (Table 1). The mean number of alleles (*N<sub>a</sub>*) amplified per locus in each population ranged from 7.5 to 10.2, averaging 9.2, while the expected heterozygosity varied from 0.70 to 0.75. The low overall mean inbreeding coefficient of 0.09 (Table 1) suggests that *D. triflora* is predominantly outcrossing. Genetic differentiation among populations was low but significantly greater than zero, with overall  $F_{ST} = 0.03$  ( $P < 0.05$ ), and AMOVA partitioning 98% of the total genetic variation within populations and 2% among populations. Genetic distance was correlated with geographic distance ( $P = 0.01$ ). The standardized measure of population differentiation indicated that some population pairs were well differentiated, with standardized  $F_{ST}$  averaging 0.06, ranging from almost zero to 0.14. Spatial autocorrelation analysis revealed a strong isolation by distance effect within the metapopulation, with significant positive spatial genetic structure at the two shortest distance classes (up to 1.4 km), and increasingly large negative correlation coefficients with increasing distance classes beyond 2 km (Fig. 3).

### Assignment tests

For each individual tested, there was at least one population that could not be excluded as a source (at the 0.001 confidence



**Fig. 3** Correlogram showing the genetic correlation coefficient as a function of distance class for the metapopulation of *Daviesia triflora*. Broken lines show the 95% confidence envelope about the null hypothesis of no spatial structure.

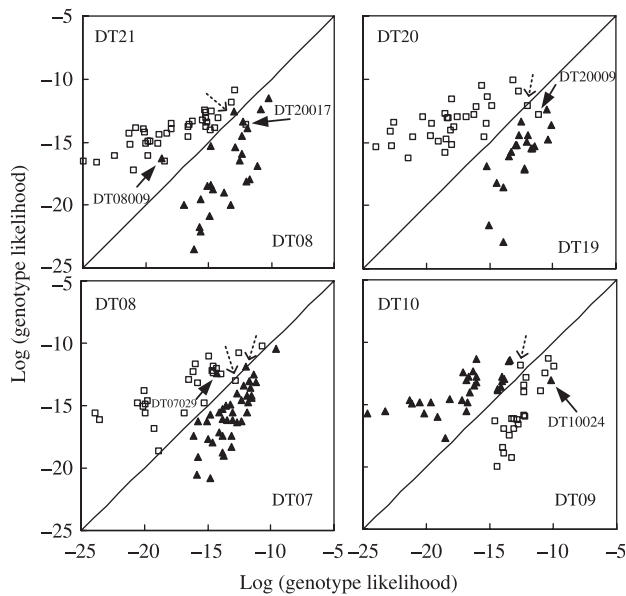
**Table 2** Immigrants identified by assignment test and their source populations

Immigrant	Source	Distance (km)	$\delta^1$	$\delta^2$
DT02006	DT01	0.41	1.5	–
DT04036	DT11	1.33	1.4	–
DT05003	DT15	1.64	1.1	2.2
DT05006	DT02	2.17	1.4	–
DT07029	DT08	0.69	1.7	1.9
DT08009	DT21	1.28	2.0	2.4
DT10024	DT09	0.87	1.6	2.8
DT16021	DT01	1.45	1.3	–
DT20009	DT19	0.51	1.2	1.7
DT21017	DT08	1.30	1.1	1.3
DT24023	DT04	2.35	1.2	1.4
DT12005	DT19, DT04, DT03	1.37 <sup>a</sup>	0.3	1.3
DT14002	DT16, DT08	1.42 <sup>a</sup>	0.2	1.3

The first two numbers of the immigrants refer to the sink population. When multiple potential source populations were identified and it was not possible to further identify a unique source by the designated criteria, all potential source populations were listed.  $\delta^1$  is the difference of the log likelihood between the most likely source population and the second most likely source population.  $\delta^2$  is difference of the log likelihood between the most likely source population and home population when the home population was not the second most likely source population.

<sup>a</sup>Average of distance between potential source populations and sink population.

level), suggesting that all individuals originated from one of the sampled populations. Genetic analysis indicated that the majority of individuals originated within the population from which they were sampled. Eleven of the 764 individuals tested (in 10 of the 22 populations tested) were identified as having immigrated from another population based on the criteria used ( $\delta > 1$ ). Two further individuals were identified as immigrants but with multiple potential source populations (Table 2). The  $\delta$  values (between source and sink population)

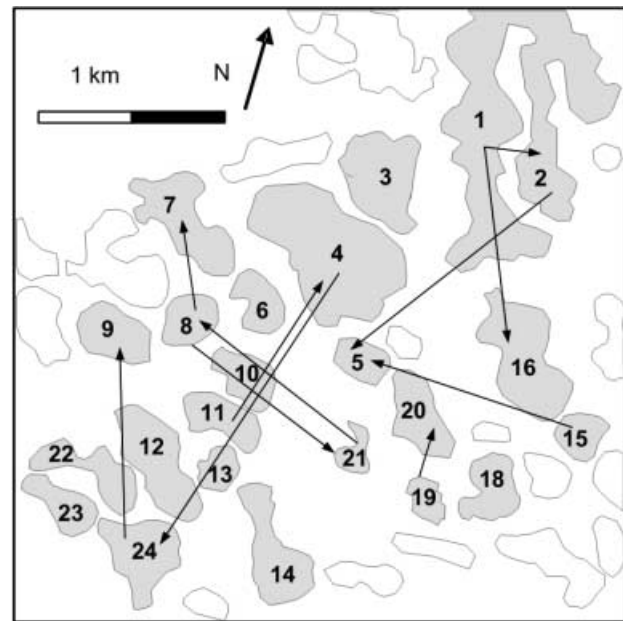


**Fig. 4** Assignment plots for four pairwise *Daviesia triflora* population comparisons. Identified immigrant is indicated with a solid arrow. Dotted arrow indicates an individual for which the most likely source population was not the population from which it was sampled, but with a  $\delta$  that was under the assignment threshold, and therefore not accepted as an immigrant.

for these 13 immigrants varied from 1.1 (92.05% confidence) to 2.8 (99.98% confidence). Plots of assignment likelihoods for individuals from four pairwise population comparisons graphically illustrates residents, immigrants (as genetic outliers to their home population falling within the cluster of the source population) and outliers that were not accepted as immigrants because of  $\delta$  falling below the predefined threshold (Fig. 4). In total, this represents an immigration rate of 1.7%. The populations assigned as source populations were not exceptionally large, although the source populations tended (but not significantly) to have higher densities of *D. triflora* than those not assigned as source populations ( $470 \pm 280 \text{ ha}^{-1}$  vs  $380 \pm 180 \text{ ha}^{-1}$ ,  $P = 0.19$  for 1-tailed  $t$ -test). For the 11 immigrants with identified source populations, the geographic distance between source and sink averaged 1260 m (SD = 630), with a range of 410–2350 m (Fig. 5). No predominant direction of seed movement was evident.

## Discussion

Our results show that the arillate seeds of *Daviesia triflora*, considered to be dispersed primarily by ants, may reach distant habitats. Indeed, population assignment tests revealed that 1.7% of 764 adult individuals of *D. triflora* were genetically assignable to a population other than that in which they occurred. Since *D. triflora* is a resprouting species whose seeds germinate from a soil seed bank following fire, this rate represents both interpopulation seed dispersal, and recruitment



**Fig. 5** Identified immigrants with unambiguous source population for 764 individuals sampled from within the *Daviesia triflora* metapopulation showing the source and sink populations of dispersal events inferred from population allocation analysis. Each arrow indicates an inferred long-distance dispersal (LDD) by seed event.

from the seed bank over many fire cycles. From this we infer that these plants arose from seeds dispersed to a mean distance of 1260 m and as far as 2350 m within the metapopulation of 23 populations. Such distances are comparable to those reported for the co-occurring *B. bookeriana* (He *et al.*, 2004), and *B. attenuata* (He *et al.*, 2008b) whose seeds are held higher above the ground in a canopy seed bank and are adapted for dispersal by wind. While the rate of LDD for *D. triflora* (1.7%) is lower than those for these *Banksia* species (5–7%), it is of the same order of magnitude, although the use of different genetic markers and assignment tests make direct comparison difficult.

Ants are usually considered the primary dispersers of arillate seeds (Berg, 1975). For *D. triflora* seeds carried by *Rhytidoponera* species, we observed a mean distance of 2.0 m and a maximum of 4.7 m, and for our study area, a mean of 3.8 m and a maximum of 8.1 m for seeds of *A. blakeyi*, which has similar attributes to those of *D. triflora*. Thus we can confirm that this arillate species is indeed a myrmecochore. The findings that most individuals originated from the population within which they were sampled, and the strong spatial genetic structure observed, are also consistent with restricted ant dispersal. However, the rare long-distance dispersal events detected by genetic analysis were two to three orders of magnitude greater than expected by ants, indicating that long-distance dispersal must be achieved by other processes.

The presence of LDD vectors is crucial for the successful colonization of newly formed suitable habitat patches as well

as for the recolonization of lost habitat patches. In south-western Australia, wind is probably a major dispersal vector in dune landscapes. Wind vortices have been proposed as the most likely agent of the observed LDD for species with winged-seeds, since the tail produced by the processes responsible for short-distance transport (wind gusts) cannot account for the observed LDD (He *et al.*, 2004; He *et al.*, 2008b). For species without obvious adaptations for LDD, such as species with arillate seeds, we must invoke other dispersal agents to account for LDD. Previous investigations in our study area showed that the dung of one large herbivore, the emu (*D. novaehollandiae*), contains seeds of many arillate species, including three *Daviesia* spp. (Calviño-Cancela *et al.*, 2006, 2008). Emus are large flightless birds distributed over much of Australia, and they feed on a great variety of fruits, seeds, flowers, insects and green herbage of annual and perennial plants (Davies, 1978). Their feeding habits, together with their mobility, make them particularly strong candidates as long-distance dispersers of seeds of species with otherwise limited dispersal capability (Davies *et al.*, 1971; Rogers *et al.*, 1993; McGrath & Bass, 1999; Calviño-Cancela *et al.*, 2006).

The effectiveness of LDD of seeds by emus will depend on their movement patterns and on the time that the seeds are retained in their gut. Emus have been reported to retain diaspores for from as little as 3–4 h to > 100 d (Davies, 1978; Willson, 1989). Calviño-Cancela *et al.* (2008) reported maximum retention times of 3 d for *Daviesia* spp, although 85% of the seeds were retrieved within the first day in an emu-feeding experiment. Emus may sporadically migrate over very large distances, but even when settled, the home range of emu pairs is comparable to the area of the study site (8–16 km<sup>2</sup>, Davies, 1963), so there is no question of their capability of dispersing ingested seeds these distances.

Few studies have reported seed dispersal by secondary (nonstandard) mechanisms, and the different methodologies employed by those that do make comparisons difficult. Vellend *et al.* (2003) inferred white-tailed deer dispersal of seeds of the primarily ant-dispersed species *Trillium grandiflorum* from gut retention time and movement data to a maximum distance of 3730 m. Using a similar approach, Couvreur *et al.* (2005) estimated that seeds of some ant-dispersed species could reach a distance of 10 km in the faeces of free-ranging donkeys. Our results are the first quantification of realized LDD in a myrmecochorous species.

More generally, our results contribute data to the growing number of studies that apply molecular markers and novel approaches to accurately quantify long-distance seed dispersal (Cain *et al.*, 2000). For example, using microsatellites for the assignment of maternal plant to individual seeds in *Prunus mahaleb*, Godoy & Jordano (2001) showed that up to 18% of sampled seeds were immigrants from surrounding populations 3–5 km away. We have previously used population assignment procedures on amplified fragment length polymorphism (AFLP) data to show that 7% of sampled individuals

were first generation immigrants in a metapopulation of *Banksia hookeriana* (He *et al.*, 2004). Using microsatellites, Isagi *et al.* (2000) estimated that 36 of 91 (40%) saplings of *Magnolia obovata* were the results of seed dispersal from outside of a 69-ha population. Together, these studies provide critical empirical data for developing general theory and modelling of LDD of seeds, which is particularly important in predicting community dynamics in the face of rapid global climate change (Cain *et al.*, 2000).

Within the metapopulation studied here, *D. triflora* exhibited weak overall population genetic differentiation ( $F_{ST} = 0.03$ ), probably a result of the limited spatial scale, but a strong isolation by distance effect with marked clinal variation. High rates of microsatellite polymorphism could compromise the ability to quantify population differentiation through normal  $F$ -statistics (Hedrick, 2005). The pairwise standardized  $F_{ST}$  values varied by an order of magnitude, and enabled population assignment with sufficient statistical power. The strong autocorrelation observed suggests that allele frequencies of neighbouring populations are correlated, and implies the need for high stringency levels in identifying immigrants to avoid false conclusions (type I errors). Such strict criteria could negatively affect assignment performance (type II errors), so that our estimate of interpopulation seed dispersal rate must be considered conservative.

The strong spatial structure of genetic variation revealed in *D. triflora* is expected for species with limited dispersal capacity. Although there is little detailed knowledge about its pollination and reproductive biology, *D. triflora* has small yellow and red flowers, apparently specialized for pollination by small bees (Crisp, 1995), and we have observed native bees and thrips visiting flowers of *D. triflora* (unpublished). These bees and thrips have little capacity to carry pollen to distant individuals, exhibiting typically leptokurtic pollen dispersal between nearest neighbours, as shown by Krauss (2000) for other species. In addition, *D. triflora* is primarily adapted for ant dispersal. As a result, both seed and pollen dispersal must occur mainly within populations. Seed dispersal among populations, although of great ecological and evolutionary significance, will therefore have had little impact in shaping spatial genetic structure of the metapopulation because of its rarity.

## Implications

For plants without obvious adaptations for LDD, such as those with ant-dispersed seeds, their capacity for dispersal, estimated from their primary adaptations, often cannot match the distance required to explain their current pattern of distribution (Reid's paradox; Clark *et al.*, 1998). Such dispersal limitation also raises the question of their survival in the face of increasing climate change. As demonstrated in our study, species with severe primary dispersal limitations still have a secondary capacity to reach distant habitats possibly via nonstandard vectors including free-ranging generalist herbivores.

Consequently, along with other studies (Vellend *et al.*, 2003), our results provide crucial empirical evidence to support the resolution of Reid's paradox. They also enable ecological modellers to predict floristic changes and responses in the face of human impacts on ecosystems, including fragmentation, loss of large herbivore species and climate change (Esther *et al.*, 2008; Fitzpatrick *et al.*, 2008; Keith *et al.*, 2008).

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