

ORIGINAL ARTICLE

# Is the post-disturbance composition of a plant population determined by selection for outcrossed seedlings or by the composition of the seedbank?

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Seedbanks are expected to buffer populations against disturbances, such as fire, that could alter the genetic composition of smaller, ephemeral adult populations. However, seedling genotypes may be influenced by the spatially heterogeneous nature of both the seedbank and the disturbance (for example, germination may vary with local disturbance) and also by selection acting on germination and post-germination performance. We used microsatellite-DNA surveys of seedlings emerging from the soil-stored seedbanks of *Grevillea macleayana* after wildfire to compare diversity and spatial structure in seedlings and adults, and through resampling of the seedling data set, to determine whether the resultant adult population reflected the effects of selection or random seedling mortality. The large post-fire seedling cohorts captured the full allelic diversity of the pre-fire adult population. However, we found a mismatch in the genotypic structure of adults and seedlings. Seedlings displayed larger heterozygous deficits than adults; however, over the ensuing 11 years, seedling heterozygosity eventually matched values for the pre-fire adults. Increasing heterozygosity among adults has generally been attributed to heterosis and/or reduction in Wahlund effects via self-thinning. Resampling of early post-fire seedlings to generate samples of equivalent size to survivors at 11 years showed that increases in heterozygosity must be driven by selection favouring outcrossed seed. This finding is important in an evolutionary context but also has implications for the restoration of natural or managed populations where a seedbank is a viable source of recruits.

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## INTRODUCTION

Soil-stored seedbanks are expected to function as a reservoir of genetic diversity that may buffer populations of plants against changes in the genetic diversity and genotypic composition of the standing crop of adults that might result from disturbances such as fire (Cabin, 1996; McCue and Holtsford, 1998; Ayre *et al.*, 2009). However, paradoxically, fire is both the major source of adult mortality and the stimulus for the emergence and recruitment of seedlings for many so-called 'fire-sensitive' species worldwide (Whelan, 1995). Post-fire seedlings may, therefore, capture the diversity present in the current and recent generations of adults. This prediction has rarely been tested (Ayre *et al.*, 2009).

Predicting the effects of fire on seedling emergence and consequent population structure for fire-sensitive species with long-lived seedbanks is made difficult because the number of seedlings emerging after fire can be vastly greater than the pre-fire adult population, providing great potential for selection to operate before the post-fire generation reaches maturity. Moreover, seedbanks may be structured in a variety of ways; as a result of temporal variation in outcrossing and scales of pollen dispersal, short distance seed dispersal and spatial heterogeneity in selection pressures (Alvarez-Buylla *et al.*, 1996; Cabin *et al.*, 1998; Vitalis *et al.*, 2004). With few exceptions (for example,

Dolan *et al.*, 2008; Ayre *et al.*, 2009), we know little about the genetic composition of soil-stored seedbanks and their likely interaction with the potentially patchy effects of fire, or indeed, in general, the extent to which the genetic structure of standing adults reflects structure within groups of post-germination seedlings or the effects of natural selection (Honnay *et al.*, 2008). However, it is known that the likelihood of seedling emergence varies with factors such as the age of the seed, depth of seed burial, and the timing, intensity and duration of fire (Ooi *et al.*, 2004; Santana *et al.*, 2010; Anderson *et al.*, 2012) and for many species, seeds are likely to be aggregated in full and half-sib clusters representing the seed shadow of pre-fire adults (for example, Ayre *et al.*, 2009) or in seed caches (Chung *et al.*, 2003).

The Proteaceous shrub *Grevillea macleayana* is potentially an excellent species with which to compare both the genetic diversity present within adults and seedling cohorts, and to determine whether the genotypic structure of adult population is reflected in post-fire seedlings. *G. macleayana* is a fire-sensitive, perennial shrub whose pollination biology and seed biology are relatively well studied (Edwards and Whelan, 1995; Vaughton, 1996, 1998; Roberts *et al.*, 2007; Whelan *et al.*, 2009). Multilocus outcrossing rates,  $t_m$ , can vary dramatically in space and time, with estimates ranging from almost zero (implying complete self-fertilisation) to one (as expected for

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random mating) (Ayre *et al.*, 1994). However, even within populations that are currently highly inbred, samples of adult plants display evidence of only moderate levels of inbreeding (England *et al.*, 2001). This mismatch could reflect either altered patterns of mating over time, greater representation of seed from more highly outcrossed years in the seedbank or selection favouring outcrossed seed.

*G. macleayana* seedbanks have been inferred to be long-lived (> 30 years) (Whelan *et al.*, 2006; Roberts *et al.*, 2007), and seeds are typically clustered under maternal plants (Edwards and Whelan, 1995; Vaughton, 1998). An earlier genetic study has shown fine-scale genetic spatial autocorrelation (England *et al.*, 2003). Here we track the temporal changes in allele and genotype frequencies that occur during seedling maturation and compare seedlings emerging after the effects of fire. The study took advantage of a naturally occurring wildfire that consumed two neighbouring subpopulations for which we had pre-existing data on adult genotypes. Our overall aim was to determine the extent to which the allelic and genotypic structure of a post-fire adult population was predictable from the composition of the pre-fire adults, and the composition of the soil-stored seedbank.

Specifically, we used microsatellite-DNA surveys of adults and seedlings emerging from the soil-stored seedbanks of two subpopulations of *G. macleayana* after a wildfire to compare: (i) the genetic and genotypic diversity and (ii) spatial structure present within seedlings, and, through resampling of the seedling data set, to determine whether the genotypic diversity of the resultant adult population reflects the effects of natural selection or random effects of seedling mortality.

## MATERIALS AND METHODS

### Collection of pre- and post-fire adults and seedlings

*G. macleayana* is a fire-sensitive shrub that occurs principally on the margins of Jervis Bay, New South Wales, Australia. A detailed description of the biology of *G. macleayana* can be found in Roberts *et al.* (2007). The Wrights Beach population of *G. macleayana* occurs on nutrient-deficient sandy soils, within a fire-prone dry sclerophyll heath- or shrub-land. At this location, the population is effectively divided into several subpopulations (hereafter termed 'stands') separated by at least tens of metres. Prior to the wildfire in 2001, the two major stands (upper and lower) forming the basis for our study were naturally separated by a dense ~100 m wide stand of *Banksia* heath without *G. macleayana*. In November 2000, we collected leaf material from the 55 and 84 adults forming the lower and upper stands respectively. The total area sampled was approximately  $1.0 \times 0.1 \text{ km}^2$ .

In early 2001, a moderately intense (though spatially patchy) wildfire destroyed all adult plants for which we had genotype data, and stimulated the germination of the soil-stored seedbank. After fire, *G. macleayana* populations typically support orders of magnitude more seedlings than the number of pre-fire adults (Vaughton, 1998), with most seedlings expected to emerge within the seed shadows of pre-fire adults (England *et al.*, 2003). In 2001, we searched for seedlings along 18,100 m transects distributed evenly throughout the two stands. Within each stand, we haphazardly tagged ~300 of the seedlings detected in these searches, noted their geographic coordinates with a global positioning system and collected leaf material for genotyping. We estimated that the total number of seedlings initially emerging within these stands exceeded 2000. We revisited the site in 2007, 2009 and 2012 to monitor the post-fire survival of the tagged seedlings. Unsurprisingly, both cohorts of seedlings had experienced high levels of mortality, with numbers of the initially tagged seedlings declining from 271 to 35 in the upper stand and from 304 to 6 in the lower stand between 2001 and 2012, respectively (Table 1).

### DNA extraction, genetic markers and genotyping

All adults and seedlings were genotyped using 9 previously published microsatellite primer sets England *et al.*, 1999; Whelan *et al.*, 2006), and 1 unpublished locus (Gm D, Whelan *et al.*, unpublished), for a total of 10 loci.

DNA extraction and genotyping protocols are described in detail in England *et al.* (2002) and Whelan *et al.* (2006).

### Genetic differentiation between upper and lower sub-stands of pre-fire adults

The natural spatial subdivision of the Wrights Beach *G. macleayana* stands before the fire suggests that the lower and upper stands may be genetically subdivided. To test for genetic differentiation, and in effect determine whether the upper and lower stands could be considered independent sites in which to test our hypotheses concerning genetic diversity and fine-scale genetic structure of seedlings, we estimated Weir and Cockerham's (1984) formulation of Wright's (1969)  $F_{ST}$  for the samples of pre-fire adults using the program FSTAT 2.9.3 (Goudet, 1995). The estimate of  $F_{ST}$  implied moderate genetic differentiation of pre-fire adults ( $0.051 \pm 0.018$ ,  $0.011\text{--}0.092$ ) that was statistically significant (as judged by 99% confidence intervals (CIs)), indicating genetic subdivision over a spatial scale of ~100 m, consistent with the results of our earlier work on this species (England *et al.*, 2002; Roberts *et al.*, 2007).

### Genetic diversity and differentiation within and among life stages and sequential groupings of surviving seedlings

For each stand, we used the programs GENALEX 6.41 (Peakall and Smouse, 2006), POPGENE (Yeh *et al.*, 1999) and FSTAT 2.9.3 (Goudet, 1995) to estimate standard measures of genetic diversity separately for all life stages, including the average number of alleles per locus ( $A$ ), allelic richness corrected for sample size ( $A_R$ ), observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ), and the inbreeding coefficient ( $F_{IS}$ ).

Our sequential survey of seedling survival allowed a temporal comparison of allele frequency variation. Allele frequency differences between sequential groupings of seedlings could simply reflect random genetic drift during seedling thinning, but may also indicate that natural selection is operating. We therefore tested for allele frequency differences among the cohorts of surviving seedlings using pairwise  $F_{ST}$ . Weir and Cockerham's (1984) formulation of Wright's (1969)  $F_{ST}$  was used to estimate genetic differentiation, with the level of statistical significance determined by means of a permutation test (FSTAT 2.9.3; Goudet, 1995).

### Spatial genetic structure of seedlings

We tested for spatial clustering of individuals (within the 2001 post-fire seedlings) that are genetically similar using a spatial autocorrelation analysis implemented in GENALEX (Peakall and Smouse, 2006). In this analysis, the autocorrelation coefficient,  $r$ , provides a measure of relative genetic similarity between pairs of individuals whose spatial separation falls within predefined distance classes. Analyses were based upon matrices of geographical distance between individuals generated from the global positioning system coordinates of all plants, and the genetic distance calculation described in Smouse and Peakall (1999). Distance-class sizes were constructed using the 'advanced single population spatial structure' subroutine of GENALEX, using relatively even sample sizes for each of the distance classes. The statistical significance of  $r$  was determined using 9999 permutation and bootstrap replicates, randomizing genotypes among distance classes. We inferred statistically significant spatial genetic structure (that is,  $r \neq 0$ ) when the observed  $r$ -value fell outside of the 95% CI of the  $r$ -values generated through permutation and corresponding to the null hypothesis of no spatial genetic structure ( $r = 0$ ). Within a specific distance class,  $r$  was deemed statistically significant when the 95% bootstrap CI did not intersect  $r = 0$ .

### Resampling of the seedling data set

Because our approach of following the fate of seedling cohorts through time inevitably means that, as time passes, we are focussing on progressively smaller sets of seedlings, it is important to demonstrate that differences in the  $F_{IS}$ 's of progressively older plants do not simply reflect the effects of random mortality of seedlings. Such effects could be reflected by chance variation in single-locus genotype frequencies (reflected in the actual data set by decreasing values of  $F_{IS}$ ). Resampling of the 2001 seedling data set allowed us to test the hypothesis that changes are a random effect of decreased sample size, by generating means

**Table 1** Average number of alleles ( $A$ ) ( $\pm$  s.e.), allelic richness based on minimum sample size in each stand ( $A_R$ ), observed heterozygosity ( $H_o$ ), Nei's 1973 expected heterozygosity ( $H_e$ ), and the inbreeding coefficient ( $F_{IS}$ ), for *Grevillea macleayana* adults, and post-fire seedlings

	N	A	$A_R$	$H_o$	$H_e$	$F_{IS}$
<i>Lower—inter-fire</i>						
Adults	55	3.7 (0.5)	3.2 (0.4)	0.294 (0.047)	0.440 (0.063)	0.313 (0.064)
<i>Lower—post-fire</i>						
Seedlings—2001 <sup>a</sup>	304	4.1 (0.8)	3.1 (0.5)	0.171 (0.041)	0.383 (0.073)	0.574 (0.054)
Seedlings—2007	47	3.2 (0.5)	3.1 (0.5)	0.180 (0.050)	0.362 (0.075)	0.500 (0.071)
Seedlings—2009	20	3.0 (0.5)	3.0 (0.5)	0.200 (0.057)	0.342 (0.073)	0.335 (0.108)
Seedlings—2012 <sup>b</sup>	6	—	—	—	—	—
<i>Upper—inter-fire</i>						
Adults	84	3.7 (0.5)	3.1 (0.4)	0.339 (0.033)	0.492 (0.056)	0.280 (0.049)
<i>Upper—post-fire</i>						
Seedlings—2001	271	3.7 (0.7)	3.0 (0.5)	0.256 (0.034)	0.481 (0.060)	0.453 (0.039)
Seedlings—2007	93	3.6 (0.7)	3.0 (0.5)	0.323 (0.045)	0.490 (0.057)	0.326 (0.056)
Seedlings—2009	68	3.4 (0.5)	2.9 (0.4)	0.325 (0.053)	0.477 (0.053)	0.323 (0.055)
Seedlings—2012	35	3.3 (0.5)	3.0 (0.3)	0.342 (0.050)	0.486 (0.054)	0.278 (0.071)

Data are presented for two stands of plants (lower and upper).

<sup>a</sup>The survival of all 2001 post-fire seedlings was monitored in 2007, 2009 and 2012.

<sup>b</sup>Low survival precluded analysis.

and 95% CIs for  $F_{IS}$  at each census date. Here we generated 1000 samples of seedlings with sizes equivalent to the numbers surviving to that time (Table 1).

## RESULTS

### Genetic and genotypic diversity within life stages before and after fire

Neither the levels of genetic diversity present within the different age classes (reflected by allelic richness and expected heterozygosity) nor the patterns of underlying allele frequencies differed significantly between the adults of the lower and upper stands and their corresponding seedling cohorts (reflected by pairwise estimates of  $F_{ST}$ ). Mean  $A_R \pm$  s.e. per locus and  $H_e \pm$  s.e. were almost identical for adults from the lower ( $3.2 \pm 0.4$  and  $0.44 \pm 0.06$ ) and upper stands ( $3.1 \pm 0.4$  and  $0.49 \pm 0.06$ ), and perhaps surprisingly, this variation was captured by both seedling cohorts regardless of time of sampling (Table 1). A small number of rare alleles ( $n = 9$  and  $4$  in the lower and upper stands, respectively) were not detected in our sampling of pre-fire adults, but occurred at very low frequency ( $< 0.05$ ) in the much larger sample of newly emerged (2001) seedlings. Perhaps unsurprisingly, these rare alleles were lost as the seedlings matured. Pairwise  $F_{ST}$  estimates for adults and seedlings within the lower stand were  $0.019$ , whereas for the upper stand comparison of adults and seedlings revealed a value of just  $0.002$ .

In contrast to the similarity revealed by comparison of the allelic composition of stands of adult and recruits, we found that values for  $F_{IS}$  decreased significantly over time in both post-fire seedling cohorts, reflecting increases in levels of observed heterozygosity (Table 1). Mean  $F_{IS} \pm$  s.e. was  $0.31 \pm 0.06$  for the lower stand of adults and  $0.28 \pm 0.05$  for the upper stand (Table 1), whereas within the seedling cohorts initial values were  $0.57 \pm 0.05$  in the lower and  $0.45 \pm 0.04$  in the upper stand, that is, 1.6–1.8 times larger than the values for the adults (Table 1). Most interestingly,  $F_{IS}$  declined considerably and significantly with the age of the surviving post-fire seedlings and patterns were consistent across loci (data not shown). After 8 years in the lower stand and 11 years in the upper stand ( $n = 6$  surviving

seedlings at the time of sampling in 2012 precluded analysis in the lower stand),  $F_{IS}$  was a moderate  $0.34 \pm 0.11$  and  $0.28 \pm 0.07$  for the upper and lower seedlings (compared with  $0.57 \pm 0.05$  and  $0.45 \pm 0.04$  immediately after fire-stimulated germination), and did not differ significantly from that of the previous adults (lower adults,  $F_{IS} = 0.31 \pm 0.06$ ; upper adults,  $F_{IS} = 0.28 \pm 0.05$ ) (Table 1).

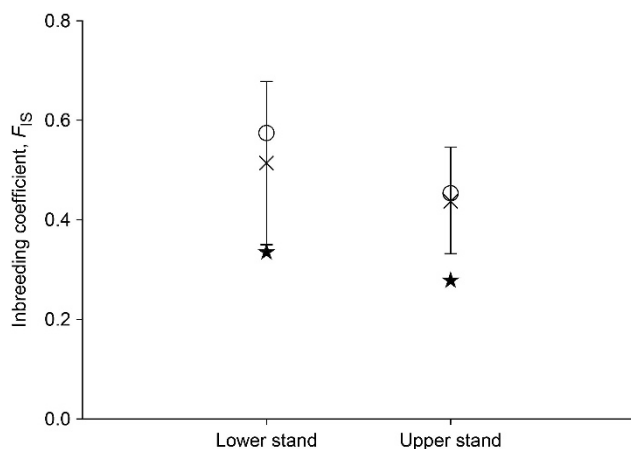
Resampling of the initial seedling data set to generate expected distributions of  $F_{IS}$  within 1000 randomly generated sets of seedlings with sample sizes equal to the groups of seedlings surviving to 2009 in the lower and 2102 in the upper stands revealed that the increased heterozygosity seen in the surviving seedlings was not an artefact of reduced sample size. In each case (as expected), the mean for the 1000 resampled sets of seedling cohorts was statistically indistinguishable from the initial 2001 seedling data sets, whereas the observed values of  $F_{IS}$  fell below the estimated lower 95% CI (Figure 1).

### Spatial genetic structure of seedlings

The spatial autocorrelation analyses on the seedlings present after fire in 2001 in both the lower and upper sub-stands demonstrate fine- to medium-scale spatial aggregation of genetically similar individuals. Estimates of  $r$  were strongly positive and statistically significant to 20 and 24 m, respectively, with  $r$ -values negative and generally statistically significant for all distance classes beyond these points (Figure 2).

## DISCUSSION

Our sequential surveys of the life stages of *G. macleayana*, together with our earlier work on its pollen and seed dispersal (for example, Edwards and Whelan, 1995; Roberts *et al.*, 2007), are one of the first investigations of the link between reproductive biology, soil-stored seedbanks and the genetic composition of a resultant population of adult plants following fire-stimulated germination (Honnay *et al.*, 2008; though see also Dolan *et al.*, 2008). The effects of restricted seed and pollen dispersal on seedling genotypes were apparent in the spatially heterogeneous nature of the pre- and post-fire adult populations and the seedlings that emerged immediately post-fire.



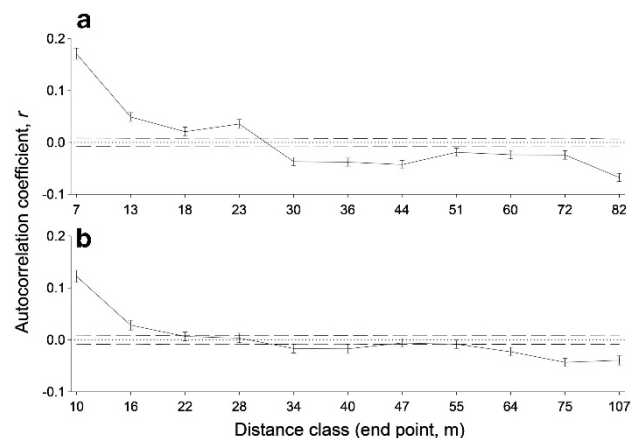
**Figure 1** Plot depicting the distribution of the average  $F_{IS}$  based on  $n=1000$  simulated data sets consisting of random samples of seedlings, from the seedlings that had emerged following fire-stimulated germination in 2001, and equal in size to the observed number of seedlings surviving to 2009 (lower stand) ( $n=20$ ) and 2012 (upper stand) ( $n=35$ ), respectively. The crosses are the mean values of  $F_{IS}$ , whereas the error bars depict the 95% CIs. The open black circles represent the values of  $F_{IS}$  for the initial 2001 post-fire seedlings, whereas the black stars show  $F_{IS}$  for the individuals surviving to the final sampling time (2009 and 2012 in the lower and upper stand, respectively). An insufficient number of survivors precluded analysis of the lower stand in 2012.

As predicted, the combined effects of inbreeding resulting from limited pollen and seed dispersal (Ayre *et al.*, 1994; Vaughton, 1996, 1998; Roberts *et al.*, 2007) were reflected by moderate-to-high positive values of  $F_{IS}$  in newly emerged seedlings and significant spatial autocorrelation over up to 24 m. Nevertheless, because the fire-stimulated seedling emergence was widespread throughout both stands, it was perhaps unsurprising that levels of genetic diversity within the post-fire seedlings and the pre-fire adult populations were very similar, as has been found in many comparisons of adult plants and their seedbanks (Honnay *et al.*, 2008). Most intriguingly, our results suggest that selection favouring highly heterozygous (presumably outcrossed) seed elevates levels of heterozygosity. Further exploration of the seedling data set using a resampling approach supports the hypothesis that selection explains the increasing heterozygosity of later stage seedlings.

#### Genetic diversity within life stages, and the dynamics of the population after fire

We detected similar levels of genetic diversity and allele frequencies within pre-fire adults and each sampling of post-fire seedlings. These findings support the results of a growing number of studies that have compared genetic diversity across plant life stages (see Honnay *et al.*, 2008 for review), and more specifically, the results of a very limited number of studies that have compared either the soil- or canopy-stored seedbanks of species occurring in fire-prone habitats (Barrett *et al.*, 2005; Premoli and Kitzberger, 2005; Ayre *et al.*, 2009, 2010). Our findings likely reflect the presence of long-lived, soil-stored seedbanks that, together with extended inter-fire periods, would allow the build-up of large persistent seedbanks that capture the progeny of a number of years of mating (England *et al.*, 2001), and potentially the contributions of multiple generations of adult plants (Ayre *et al.*, 2009).

Our longitudinal sampling revealed that a cohort of up to 2000 plus seedlings emerging after the fire generated just several 10s of



**Figure 2** Plots of multilocus spatial autocorrelation for the Wrights Beach (a) lower and (b) upper stands of *Grevillea macleayana* seedlings present after fire-stimulated germination. Analyses were based on 10 microsatellite loci. The autocorrelation coefficients,  $r$ , are represented by the unbroken black line. The broken long dashes represent the 95% CIs around  $r$ -values generated under the null hypothesis of no spatial genetic structure ( $r=0$ , dotted black line), whereas the error bars represent the 95% CIs around estimates of  $r$  for each distance class.

mature adult plants more than a decade later. It is important that threatened-species managers are conscious of these dramatic fluctuations in population sizes for *G. macleayana*, and a broad range of other long-lived fire-sensitive species (for example, Ayre *et al.*, 2009). Sampling soon after fire may provide grossly inflated estimates of the ultimate population sizes of adult plants. In the context of this study, however, the close match between the allelic compositions of the seedlings likely reflects the relatively uniform nature of the wildfire and consequent widespread seedling emergence. Indeed, anecdotal evidence suggests that *G. macleayana* seeds remain viable in the soil for decades (Roberts *et al.*, 2007).

#### Post-germination variation in levels of heterozygosity

Many earlier studies have invoked either selection favouring heterozygous genotypes at specific loci or genome-wide heterosis favouring outcrossed seed, to explain greater heterozygosity observed in adult *cf.* seedling cohorts (Cabin *et al.*, 1998; Asuka *et al.*, 2004; Caldiz and Premoli, 2005; Mandák *et al.*, 2006; Van Geert *et al.*, 2008; Fuchs and Hamrick, 2010). Importantly, majority of these earlier studies used allozyme markers that might have been directly subject to natural selection, although similar patterns have also been observed in later studies using microsatellite markers (for example, Jones and Hubbell, 2006). Wahlund effects may be particularly pronounced in seedling cohorts, such as those of *G. macleayana*, where high levels of selfing and bi-parental inbreeding are expected (England *et al.*, 2001). Here, we found that in the two stands,  $F_{IS}$  decreased markedly when using a set of 10 microsatellite loci and comparing seedlings germinating in response to the disturbance provided by fire with those surviving for a further 6, 8 and 11 years.

In our study, newly emergent seedlings displayed large and consistent heterozygous deficits and had a consequently large and positive value of  $F_{IS}$ . This is consistent with high levels of selfing or inbreeding, which contrast with the closer fit to random mating seen in these and other groups of adult *G. macleayana* (Table 1; England

*et al.*, 2001; Roberts *et al.*, 2007). Although previous studies of seedbanks stimulated both by fire and other cues have suggested various explanations for this pattern, our longitudinal survey of seedling cohorts provides the first clear evidence that this contrast reflects the effects of selection favouring seedlings resulting from outcrossing rather than other mechanisms, such as heterosis or temporal fluctuation in patterns of mating. We observed that  $F_{IS}$  values for the oldest surveyed seedlings in each of our lower and upper stands approached the level observed in the pre-fire adults. Our resampling of the initial seedling data sets demonstrates that these decreases in  $F_{IS}$ , which were consistent across loci, are not explicable as a simple consequence of variation in heterozygosity caused by random mortality. Moreover, consistent increases in heterozygosity for a set of unlinked loci would be unlikely to reflect similarly strong selection favouring heterozygosity at each locus (Hedrick, 2012).

### Spatial genetic structure of seedlings

As predicted for *G. macleayana*, which typically has very limited seed and pollen dispersal (Vaughton, 1996, 1998; though see Roberts *et al.*, 2007), we found significant fine-scale spatial structure (see also England *et al.*, 2003) when examining the 2001 seedling cohorts. Similar fine-scale spatial structure typically at distances of 5–10 m has been reported for other Proteaceae in fire-prone habitats (Ayre *et al.*, 2009, 2010; Forrest *et al.*, 2011; Ritchie and Krauss, 2012; Ayre and O'Brien, 2013) and has been argued to reflect the formation of clusters of closely related seedlings in seed shadows of pre-fire adults. Intriguingly, spatial clustering of seedlings is often reported over large spatial scales than is the case for adults (for example, Ayre *et al.*, 2009). Over the 11 years of our study, there was significant natural mortality of post-fire seedlings, but we are unable to comment on the extent to which this represented self-thinning within sib-ships because of the small numbers of older seedlings. In general, the contrast between the size of adult or older seedlings and newly germinated seedling stands ensures that the genetic structure of seedling cohorts is the better indication of seed-dispersal distances.

### CONCLUSION

Taken together, our results, and those of other studies of the genetic composition of pre- and post-disturbance plant populations (see Honnay *et al.*, 2008 for review), confirm that persistent seedbanks buffer plant populations against large fluctuations in genetic composition due to disturbances such as fire (for example, Premoli and Kitzberger, 2005; Dolan *et al.*, 2008; Ayre *et al.*, 2009) despite striking variation in the size of above-ground populations. Overall, patterns of genetic diversity and spatial structure were closely matched to the composition of the seedbank. Nevertheless, at least for *G. macleayana*, it is clear that post-germination selection favouring outcrossed individuals (detectable as a decrease in positive values of  $F_{IS}$  over 11 years) rather than temporal variation in mating systems explains the apparent mismatch of mating systems inferred from the examination of adult plants and the current mating systems (Ayre *et al.*, 1994; England *et al.*, 2001; Roberts *et al.*, 2007). The relatively intense selection for outcrossed seed implied by the present study may reflect *G. macleayana*'s apparent lack of preference for self or outcrossed pollen (Harris and Whelan 1993; Vaughton, 1995; Whelan *et al.*, 2006). Post-germination selection may compensate for a lack of mate choice with the potential for such selection and forming part of a bet-hedging strategy that allows even isolated plants to generate substantial seedbanks. Further longitudinal studies of genotype frequencies within seedling cohorts of species with varying degrees of mate choice/self compatibility are therefore needed to determine

whether this finding is typical. Nevertheless, the implication of our data is that, in the absence of such studies, those concerned with the management or restoration of plant populations that undergo intense self-thinning should, wherever possible, use natural seedbanks.

### DATA ARCHIVING

Data available from the Dryad Digital Repository: doi:10.5061/dryad.bc14v.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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