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Wild seedlings of a tree endemic on granite outcrops show no evidence of inbreeding depression

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Abstract. Plant communities on granite outcrops are characterised by high biodiversity and endemism, and strong population genetic structuring. The floristic diversity and structure of inselberg flora receives much attention in international conservation research, yet our understanding of how recruitment influences population genetic structure of plants endemic to granite outcrops is limited. To determine the immediate genetic consequences of recruitment and to quantify mating and dispersal patterns by parentage analysis, I conducted an opportunistic study following a wildfire in a stand of the lignotuberous tree *Eucalyptus caesia* Benth. (Myrtaceae) at Boyagin Nature Reserve. The entire adult stand ($n = 180$) plus seedlings ($n = 115$) were genotyped with 14 microsatellite loci. Compared with adults, seedlings had low heterozygosity and a high inbreeding co-efficient, but similar levels of allelic diversity. There was no evidence of post-germination selection against homozygous seedlings over the 20 month survey period. Seedlings that resulted from cross-pollination were more heterozygous than seedlings resulting from selfing, but could not be distinguished based on height measurements or survivorship. Parentage analysis in CERVUS revealed mostly limited seed dispersal, assuming that candidate parents closest to seedlings were the maternal parent. By comparison, pollen movement was more extensive, yet still restricted to plants within the stand. Recruitment has increased the population size, but appears insufficient to enhance genetic diversity in the Boyagin stand of *E. caesia*. Nonetheless, *E. caesia* appears adept at persisting as extremely small populations.

Additional keywords: genetic diversity, granite-outcrop endemism, parentage analysis, recruitment, seed and pollen dispersal.

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Introduction

Inselbergs are isolated rocky outcrops or mountains, often of granitic or gneissic origin, that support biota distinct from that of surrounding forest or grassland landscapes (Porembski and Barthlott 2012). The floristic diversity, high endemism and uniqueness of vegetation communities of inselberg habitats have been a focal point of international conservation research (Groger and Barthlott 1996; Porembski *et al.* 1996; 1997). Around the globe, inselberg habitats are severely threatened by quarrying, recreational destruction, urbanisation, livestock, and invasion of exotic plants (Porembski *et al.* 2016; Fitzsimons and Michael 2017).

Strong genetic differentiation amongst populations is widely reported for plants endemic to granite outcrops (Wyatt *et al.* 1992; Barará *et al.* 2007; Byrne and Hopper 2008; Tapper *et al.* 2014; Hmeljevski *et al.* 2017), although some populations do maintain connectivity (Gevaert *et al.* 2013; Gonçalves-Oliveira *et al.* 2017). Much less is known about the immediate effects of recruitment on the population genetics of granite-endemic flora, perhaps due to the sporadic and sometimes inefficacious nature of recruitment (Vlasáková 2013; Hmeljevski *et al.* 2014). Harsh

environmental conditions that characterise rocky habitats, such as high radiation, extreme temperatures, high runoff and low soil moisture may impede recruitment or cause high mortality of new recruits (Szarzynski 2000). Additional constraints such as lack of rock crevices suitable for seedling establishment may constrain population growth (Yates and Ladd 2004). Combining demographic studies with recruitment genetics is critical to develop appropriate genetic management strategies for small, fragmented populations.

In small plant populations, highly outcrossed mating systems and widespread pollen dispersal may ensure high levels of heterozygosity in canopy or soil-stored seed crops as they develop over time (Krauss *et al.* 2009; Ayre *et al.* 2010; Breed *et al.* 2015b). Pulse recruitment from genetically diverse seed crops thus may buffer genetic decline in isolated populations (Ayre *et al.* 2009). In the absence of cross-pollination, selection against homozygous progeny may enhance genetic diversity. Post-dispersal selection against homozygotes has been demonstrated in long-term field trials of selfed and outcrossed seedlings, and genetic surveys of recruits in wild populations (Hardner and Potts 1997; Roberts *et al.* 2014).

It is unclear whether harsh environmental conditions that exert strong selection pressure will lead to rapid mortality of inbred progeny that is observable over short time periods.

Pollination systems involving highly mobile animals, in particular nectar-feeding birds, are theorised to maintain genetic connectivity between fragmented populations (Byrne *et al.* 2007; Breed *et al.* 2015a; Krauss *et al.* 2017). However, low population densities, insularity and human disturbance may lead to reduced levels of pollen migration and restricted mating patterns, even in plants with bird pollinators (Llorens *et al.* 2012; Coates *et al.* 2013). Most studies that document mating patterns do so by sampling seeds held in canopy- or soil-stored seedbanks – few have focused on seedling recruits that form the next generation (Barrett *et al.* 2005; Jones *et al.* 2010; Roberts *et al.* 2014). Our understanding of genetic structure in the typically small proportion of seeds that germinate and survive to contribute to the standing population is therefore limited.

In south-west Australia, granite outcrops support hyper-diverse plant communities and provide a refuge for fire-sensitive biota in an otherwise seasonally dry and fire-prone landscape (Hopper *et al.* 1990, 1997). A large proportion of plants on granite outcrops are obligate seeders that may be killed by fire (Yates *et al.* 2003a) whereas other plants require disturbance, such as fire, to trigger recruitment (Burrows 2013). The present study opportunistically investigates recruitment genetics in *Eucalyptus caesia* Benth. (Myrtaceae), an endemic of granite outcrops that is known from 27 small, disjunct populations in the Wheatbelt Region of Western Australia (Bezemer *et al.* 2016) including two populations recently documented by N. Bezemer and S. D. Hopper. Recruitment from seed in *E. caesia* is rare, associated with the occurrence of fire, and seldom recorded in the wild – as observed in other mallee eucalypts (Wellington and Noble 1985a; Davies and Myerscough 1991; Yates *et al.* 2003b). Seed dispersal in eucalypts occurs predominantly by gravity and is considered to be highly restricted (Potts and Wiltshire 1997), yet studies that provide direct genetic confirmation of this assumption are lacking (Booth 2017). Genetic connectivity is usually maintained via long distance pollen movement in gravity-dispersed species. In several eucalypts, bird pollination has been linked to increased gene flow (Southerton *et al.* 2004; Hopper 2009; Breed *et al.* 2015b). However, in a population of the predominantly bird-pollinated *E. caesia*, mating appears spatially restricted, despite high levels of multiple paternity (Bezemer *et al.* 2016).

I used a field survey of adult plants and seedling growth and survivorship over 20 months, in combination with microsatellite genotyping and parentage analysis, to test two hypotheses: (i) that seedlings will predominantly result from mating between plants within the study population; and (ii) that levels of genetic diversity in 20 month old seedlings will reflect that of adults despite high levels of inbreeding, because of selection against inbred seedlings.

Materials and methods

Study area and species

The study was conducted on a natural stand of *Eucalyptus caesia* Benth. at Boyagin Nature Reserve in the Central Wheatbelt

region of Western Australia (32°28'21.0"S 116°52'23.5"E). *E. caesia* is monoecious, with a mean realised outcrossing rate of 0.72 reported for the Boyagin stand (Bezemer *et al.* 2016). Populations of *E. caesia* are characterised by low genetic diversity and high levels of genetic differentiation (Moran and Hopper 1983; Byrne and Hopper 2008). In the winter months, large pink flowers produce copious amounts of nectar that appear to favour pollination by nectar-feeding birds such as honeyeaters, although flowers are also visited by insects – including the European honeybee (Hopper 1981). Although recruitment has been rarely observed in *E. caesia*, asexual reproduction via lignotubers is common. Individual plants appear to have a capacity for extreme longevity based on the size of lignotubers, inferred population age structure, and presence of large clonal patches (Hopper *et al.* 1982; Bezemer *et al.* 2016).

Field data collection

Commencing 5 months after a wildfire, I conducted on-ground searches for *E. caesia* seedlings over seven intermittent surveys (1 October 2015, 28 October 2015, 6 December 2015, 14 January 2016, 17 March 2016, 25 August 2016 and 6 April 2017). The aim was to locate as many seedlings as possible, rather than conduct a transect-style survey. A total of 70 h was spent searching near all adult plants. When no seedlings were found in unburnt areas after the first two surveys, searches were concentrated in burnt areas. The global positioning system (GPS) co-ordinates (UTM easting and northing) of all seedlings were recorded with a Trimble GeoExplorer differential GPS (Digital Mapping Solutions, Perth, WA, Australia), and for seedlings with more than two expanded leaves, a sample collected for genetic analysis. This precautionary strategy was employed to reduce the risk of killing very small seedlings by sampling. Seedling height was measured as the length (cm) between the base of the stem and terminal node, and repeat measurements taken at subsequent surveys.

Microsatellite genotyping

DNA was extracted from seedling leaf samples following a CTAB method modified from Doyle and Doyle (1987). Fourteen microsatellite loci were amplified with a QIAGEN Multiplex PCR Kit (Qiagen, Venio, Limburg, Netherlands), following the manufacturer's instructions. Loci included EGM30, EGM47, EGM14, EGM12, EGM34, EGM35, EMBRA10, EMBRA7, EMBRA20, EMBRA18, EMCRC11, EV22, EV23, EV28 and ES140 (Brondani *et al.* 1998; Glaubitz *et al.* 2001; Steane *et al.* 2001; Bradbury *et al.* 2013; Nevill *et al.* 2013). Multiplex mixes and PCR conditions follow Bezemer *et al.* (2016), except for modified annealing temperatures of 53°C for EMBRA18, EMCRC11 and EV22, and 65°C for EV23, EV28 and ES140. To optimise amplification peaks, the concentrations of several primer pairs were increased or reduced from a 2 µM concentration.

Amplified loci were visualised with an automated 3500 Genetic Analyser with 0.2 µl 500 LIZ size standard/10 µl Hi-Di Formamide (Applied Biosystems, Thermo Fischer Scientific, Melbourne, Vic., Australia). Alleles were scored manually with Geneious 7.1.4 (Drummond *et al.* 2011). Positive and negative controls were included in each 96 well plate and electropherogram profiles double-checked for all

samples. For some primers, changing primer manufacturer from Applied Biosystems to Alpha DNA (Montreal, Canada) resulted in a consistent shift in allele size; for example alleles were always four base pairs larger for EMBRA10 primers manufactured by AB than Alpha DNA. To confirm Mendelian inheritance (i.e. all progeny should have at least one maternally-inherited allele), all primers were screened with different trees and a sample of their progeny.

Genetic diversity and structure

Genetic diversity parameters including N_a (number of alleles), number of alleles with a frequency of $\geq 5\%$ (N_a freq. $\geq 5\%$), N_e (effective number of alleles), H_E (expected heterozygosity), H_O (observed heterozygosity), and F (fixation index, also called the inbreeding co-efficient) were calculated in GenAlEx ver. 6.5 (Peakall and Smouse 2012) for adults and seedlings, and for seedling subsets that were present at repeat surveys. To test for genetic differentiation of adults and seedlings, genetic distances between all multi-locus genotypes were calculated and a principle co-ordinates analysis (PCoA) performed, and genetic differentiation measures including G_{ST} , G'_{ST} , N , G'_{ST} , H , G'_{ST} , D_{EST} and P (G_{ST}) calculated. Fewer seedlings were included in the genetic analysis than reported in the on-ground survey results because some seedlings were too small to sample and died before the next survey.

Parentage assignment

Parentage assignment was conducted by categorical allocation in CERVUS ver.3.0.3 with strict confidence levels set at 95% and relaxed confidence levels of 80%, and critical Logarithm of Odds (LOD) scores were used to determine confidence of assignments (Kalinowski *et al.* 2007). Two analyses were performed – first, with all reproductive adults as candidates and second, with duplicate genotypes of all candidates (to allow detection of selfing). Candidate parents were grouped according to Euclidean distance from seedlings. For each parent-pair, the individual nearest/furthest to the seedling was assumed the maternal parent/pollen donor respectively (Guidugli *et al.* 2016). I used these results to infer realised pollen and seed dispersal distances.

To test for differences in growth rate or survivorship of seedlings resulting from self- versus cross-pollination, the minimum number of days alive, growth (taken as height at the last measurement) and growth (cm)/(minimum days alive) were calculated. Seedlings were assumed to be at least seven days old upon initial sampling (based on the presence of at least two expanded leaves), and the minimum days alive calculated as the number of days from the initial to the final observation (at which point seedlings were either still alive or recorded as dead or missing) plus seven. For example, a seedling first recorded on 28 October 2015 and noted as dead on 25 August 2016 may have died anywhere between 17 March 2016 to 25 August 2016 and was therefore alive for a minimum of 145 days.

Results

Seedling survey

A total of 118 seedlings were found; these were easily distinguishable from coppice growth from adult plants by the

presence of dissected cotyledons or glaucous leaves, and by differences in leaf shape and stem thickness (see Fig. S1, available as Supplementary Material to this paper). Seedlings were located only underneath the canopies of burnt adult plants, except for two seedlings which were ~2 m downslope from the canopy edge of the nearest adult (Fig. 1).

Genetic diversity

There were some differences in genetic variation between the adult and seedling cohorts, with a 20% reduction in observed heterozygosity (H_O adults = 0.582 and H_O seedlings = 0.464) and fixation indices of -0.005 in adults and 0.181 in seedlings (Table 1). Allelic diversity (N_a , N_a freq. $< 5\%$, and N_e) was similar for adults and seedlings, but nine rare alleles detected in adults were absent in the sampled seedlings. Despite an increase in seedling number from 31 individuals in the initial survey (1 October 2015) to 92 in the third survey (6 December 2015), and subsequent reduction to 32 seedlings in the final survey (6 April 2017), observed heterozygosity and fixation index of seedling samples did not change to match that of adults. There was little genetic differentiation of adult and seedling cohorts (G_{ST} = 0.006 \pm 0.002 (s.e.), Fig. S2).

Parentage assignment

Out of 115 seedlings analysed, 34 candidate parent matches had critical logarithm of odd (LOD) scores meeting the strict confidence threshold and a further 49 matches met the relaxed confidence threshold. Most likely parents were assigned for an additional 22 seedlings. Parents were not assigned for 10 seedlings for which more than two candidate parents were identified (but a pool of potential parents from within the study site was identified). Overall, an absence of non-local alleles suggested no evidence of gene flow from outside the study population that was represented in new recruits. The mean

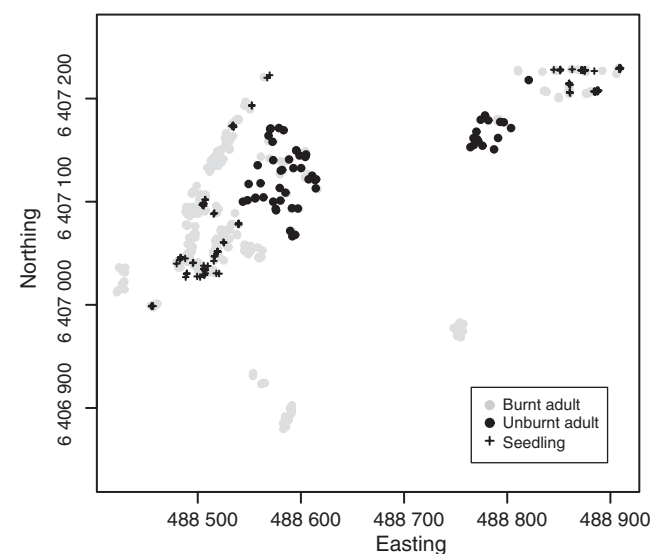


Fig. 1. Distribution of *Eucalyptus caesia* at Boyagin Nature Reserve, Western Australia, showing burnt and unburnt adults, and seedlings located after a 2015 wildfire.

Table 1. Genetic variation across 14 microsatellite loci (mean and standard error) for the entire adult population, all sampled seedlings, and the seedling cohort over seven intermittent surveys for *Eucalyptus caesia* at Boyagin Nature Reserve, Western AustraliaAbbreviations: *n*, sample size; *N_a*, number of alleles; *N_a* freq. $\geq 5\%$, number of alleles with a frequency of $\geq 5\%$; *N_e*, effective number of alleles; *H_O*, observed heterozygosity; *H_E*, expected heterozygosity; *F*, fixation index

	<i>n</i>	<i>N_a</i>	<i>N_a</i> frequency $\geq 5\%$	<i>N_e</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>
Adults	180	4.70 (0.54)	3.29 (0.37)	2.74 (0.32)	0.580 (0.041)	0.578 (0.040)	-0.004 (0.024)
All seedlings sampled	115	4.43 (0.54)	3.29 (0.32)	2.65 (0.29)	0.464 (0.050)	0.558 (0.050)	0.178 (0.037)
1 October 2015 seedlings	38	4.14 (0.45)	3.50 (0.29)	2.59 (0.25)	0.489 (0.053)	0.563 (0.043)	0.138 (0.058)
28 October 2015 seedlings	79	4.21 (0.49)	3.36 (0.29)	2.64 (0.27)	0.481 (0.051)	0.561 (0.049)	0.154 (0.041)
6 December 2015 seedlings	102	4.29 (0.52)	3.21 (0.30)	2.63 (0.28)	0.460 (0.049)	0.555 (0.040)	0.181 (0.039)
14 January 2016 seedlings	81	4.21 (0.49)	3.36 (0.31)	2.63 (0.28)	0.463 (0.049)	0.552 (0.051)	0.168 (0.032)
17 March 2016 seedlings	67	4.21 (0.49)	3.29 (0.29)	2.57 (0.27)	0.453 (0.048)	0.548 (0.050)	0.181 (0.036)
25 August 2016 seedlings	48	4.14 (0.45)	3.07 (0.27)	2.55 (0.27)	0.458 (0.048)	0.541 (0.051)	0.155 (0.041)
6 April 2017 seedlings	32	4.00 (0.44)	3.21 (0.26)	2.53 (0.28)	0.453 (0.045)	0.534 (0.053)	0.138 (0.036)

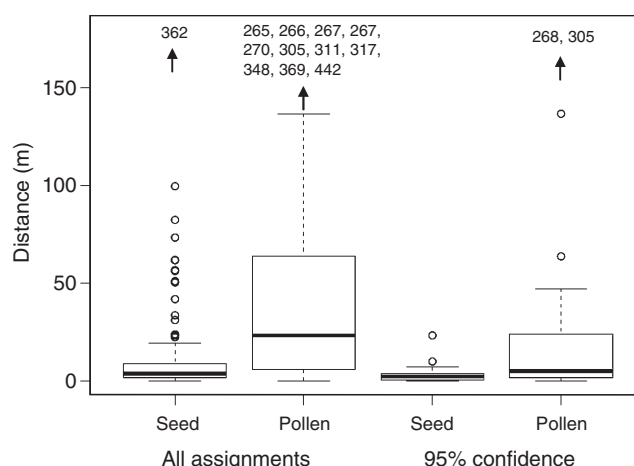
LOD score of all assignments was 12.9 ± 0.5 (s.e.), whereas that for presumed selfs was higher at 18.1 ± 1.2 (s.e., Fig. S3).

A total of 19 seedlings (16% of the total analysed) were identified as products of self-pollination. Considering all assignments, mean distance to the nearest parent was significantly lower than mean distance to the furthest parent (14.6 ± 3.8 (s.e.) vs 67.8 ± 9.4 (s.e.) m, $P < 0.001$). The same was true when only assignments that met the 95% confidence threshold were considered (6.6 ± 1.8 (s.e.) vs 39.9 ± 12.9 (s.e.) m, $P = 0.021$; Fig. 2). Maximum recorded dispersal was 362 m (seed) and 442 m (pollen) for all assignments, 82 m (seed) and 353 m (pollen) at the relaxed confidence threshold, and 23 m (seed) and 305 m (pollen) for assignments meeting the 95% confidence threshold. The mean distance between all adults (and mean pollen dispersal expected under random mating) was 158 m, while the mean distance between unburnt adults was significantly lower at 105 m ($P < 0.001$).

Outcrossed seedlings neither outlived nor outgrew seedlings resulting from selfing (Table 2; Fig. 3), despite being more heterozygous as a group (H_O selfs = 0.29 ± 0.04 (s.e.) and H_O outcrosses = 0.53 ± 0.06 (s.e.)). Mean growth rates and survivorship of selfed and outcrossed seedlings did not vary significantly ($P = 0.723$ for growth rate and $P = 0.302$ for survivorship), although selfs performed slightly better. Of 32 seedlings still alive at the last survey, nine seedlings were selfs, amongst which was the tallest seedling (185 cm).

Discussion

A recent wildfire in a stand of *Eucalyptus caesia* in Boyagin Nature Reserve triggered a large-scale recruitment episode that was the first for at least 40 years, but probably much longer based on the size distribution of adults (Hopper *et al.* 1982; Bezemer *et al.* 2016). Recruitment in the wild has only ever been recently observed in one other population, also following fire (Yates *et al.* 2003b). As predicted, parentage analysis revealed that mating is spatially restricted within the study population, with mostly limited seed dispersal but more extensive pollen movement. Contrary to expectations, the inbreeding co-efficient of seedlings remained high even with rapid seedling mortality, and relatively inbred offspring did not have reduced growth rates or survivorship. Population genetic diversity of *E. caesia* at Boyagin was not enhanced following the documented recruitment episode.

**Fig. 2.** Euclidean distance (m) between *Eucalyptus caesia* seedlings and candidate seed (maternal) and pollen (paternal) parents at Boyagin Nature Reserve, Western Australia.**Table 2. Mean growth, survivorship and heterozygosity (s.e.) of *Eucalyptus caesia* seedlings resulting from cross-pollination and selfing**

	Outcross (<i>n</i> = 69)	Self (<i>n</i> = 19)
<i>Field data</i>		
Minimum days alive	252.5 (23.4)	306.8 (52.9)
Growth (cm)	32.0 (4.3)	36.0 (10.2)
Growth/days alive	0.10 (0.01)	0.09 (0.02)
<i>Genetic data</i>		
Observed heterozygosity	0.53 (0.06)	0.29 (0.04)
Fixation index	0.06 (0.05)	0.42 (0.07)

Post-fire recruitment genetics

Post-germination selection against homozygous progeny was not evident over the 20 month survey period. Although there was low heterozygosity and high fixation in seedlings compared with adults, no increasing/decreasing trends in these measures over the survey period were evident. Seedlings that resulted from cross-pollination had greater heterozygosity compared with the products of selfing, but these groups could not be distinguished based on height measurements or survivorship.

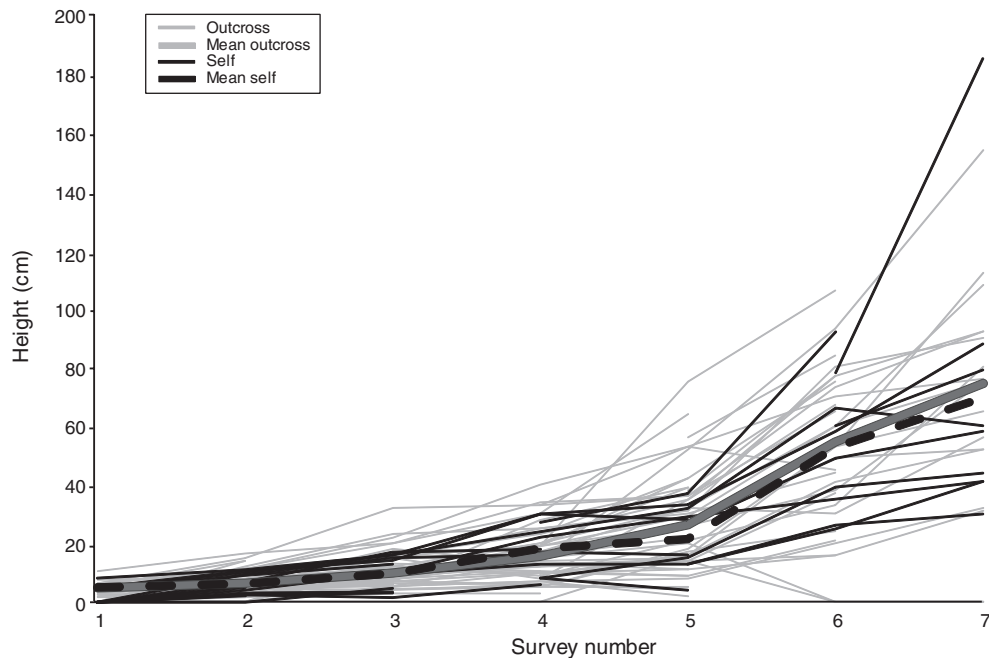


Fig. 3. Height (cm) of *Eucalyptus caesia* seedlings at Boyagin Nature Reserve, Western Australia, recorded over seven intermittent surveys after an April 2015 wildfire (survey numbers: 1, 1 October 2015; 2, 28 October 2015; 3, 6 December 2015; 4, 14 January 2016; 5, 17 March 2016; 6, 25 August 2016; and 7, 6 April 2017).

In contrast, strong patterns of selection have been documented in other plants with mixed mating systems, including *Eucalyptus grandis* and *Grevillea macleayana* (Hardner and Potts 1997; Roberts *et al.* 2014). In the *E. grandis* common garden experiment, selection was evident after 4 years, corresponding with canopy closure and increased competition. In *E. caesia*, low seedling densities may render intra-specific competition leading to self-thinning negligible (Westoby 1984). However, inbreeding depression may be more pronounced and more readily detectable with age (Hardner and Potts 1995), with the close-to-zero inbreeding co-efficient of adults at Boyagin suggesting that inbred individuals are eventually selected against. Environmental variability may have confounded selection patterns in the study population (Yates and Ladd 2004; Bailey *et al.* 2012), although in wild subpopulations of *G. macleayana*, selection favouring outcrossed progeny was evident despite variability in seedling microsites (Roberts *et al.* 2014). *E. caesia* has a root morphology specialised to exploit water resources held in fissures in the underlying rock (Poot *et al.* 2012), which may explain the distribution of surviving seedlings at the study site.

Selection against homozygotes may have occurred before commencement of the survey. The proportion of outcrossed progeny in canopy-stored fruit of six families from the same population of *E. caesia* was less than that of recruits in the present study (0.72 vs 0.84; Bezemer *et al.* 2016), although this could be an artefact of fewer maternal plants included in the Bezemer *et al.* (2016) study. However, mean pollen dispersal distance in the present study was nearly twice that previously recorded in an open-pollinated seed crop (67.8 ± 9.4 (s.e.) vs 39.4 ± 3.9 (s.e.) m, see Bezemer *et al.* 2016). These results may indicate selection against progeny resulting from near-neighbour mating during early- or late-stage fruit

development, preferential germination of heterozygous seeds (Kenington and James 1997), or, undocumented death of inbred offspring soon after germination. It is also possible that the study population may have undergone purging of deleterious recessive alleles (Byers and Waller 1999; Crnokrak and Barrett 2002). Extant populations of *E. caesia* appear to have a long history of isolation, over the course of which purging, in combination with genetic drift and limited gene flow, may have contributed to low levels of genetic variation (Byrne and Hopper 2008), but empirical testing of purging is difficult in *E. caesia* given the longevity of individuals.

Seed and pollen dispersal

Seedlings were generally only found beneath the canopies of adult plants, supporting the prediction of predominant seed dispersal by gravity in eucalypts (House 1997). There was a tendency for seedlings to occur downslope from maternal plants. Previous studies in mallee eucalypts such as *Eucalyptus incrassata* and *Eucalyptus luehmanniana* have assumed that seedlings found beneath the canopy of adult conspecifics originated from those plants (Wellington and Noble 1985b; Davies and Myerscough 1991). The present study is among the first to provide direct genetic confirmation of restricted seed dispersal patterns. The assumption that the closest candidate parent to the seedling is the maternal parent is valid because the alternative of seed dispersal towards the pollen parent is implausible and biologically inexplicable (Guidugli *et al.* 2016). Occasional seed dispersal over relatively large distances was recorded in the present study, perhaps as a consequence of transport by parrots as they leave feeding sites, by water transport along drainage lines, or by convective

updrafts during a fire (Kirkpatrick 1977; He *et al.* 2004; Southerton *et al.* 2004). Although rare, such movement may be critical for local population expansion (Vivian *et al.* 2008).

Mean pollen dispersal distance exceeded that of seed dispersal, and mating occurred frequently between non-neighbours in the study population. Nonetheless, mating was restricted to individuals within the study site, as previously reported for open-pollinated progeny in the same population (Bezemer *et al.* 2016). The limitation of both studies to a single flowering season may have resulted in failure to detect pollen migration, and so the possibility of mating between populations cannot be discounted. In contrast to the apparently restricted mating documented at Boyagin, frequent pollen exchange has been reported for similarly small or fragmented plant populations (Byrne *et al.* 2007, 2008; Lander *et al.* 2010; Breed *et al.* 2015a; Guidugli *et al.* 2016). Highly mobile pollinators such as nectar-feeding birds are thought to maintain genetic connectivity between populations (Krauss *et al.* 2017), yet gene flow appears rare or lacking in the apparently bird-adapted *E. caesia* (Byrne and Hopper 2008). *E. caesia* may have other mechanisms in place to cope with genetic and geographic insularity, such as extreme longevity of individuals, biological specialisation or enhanced resilience, or cytogenetic processes conserving genetic variation despite inbreeding (Hopper 2009). Conservation management strategies such as assisted migration or genetic rescue are gaining popularity (Frankham 2015; Whiteley *et al.* 2015), but may be inappropriate for species such as *E. caesia* that have intrinsically low genetic diversity and a naturally fragmented and strongly genetically differentiated population structure (Weeks *et al.* 2011).

Population response to fire

The Boyagin population has a large canopy-stored seed bank, and sixty-eight percent of the standing population was burnt, yet intensive on-ground searches yielded only 118 seedlings. The observed density of less than 10 seedlings per hectare at the study site is considerably lower than post-fire seedling densities reported for other eucalypts, for instance, 7000 seedlings ha⁻¹ in *E. incrassata* (Wellington and Noble 1985a), 6000 seedlings ha⁻¹ in *Eucalyptus tetradonta* (Bowman and Panton 1993) and up to 60 seedlings m² in *Eucalyptus salmonophloia* (Yates *et al.* 1994). Similarly divergent recruitment densities occur between the woody shrub *Clusia blattopila*, a granite inselberg specialist, and its less restricted congener *Clusia nemorosa* (Vlasáková 2013). Interestingly, seedling density at the study site was less than recorded for *E. caesia* at Chidaarcooping Nature Reserve, where nearly 5 seedlings m² were recorded 10 months after a fire (Yates *et al.* 2003b).

At Boyagin, low levels of recruitment may be a consequence of predation of seeds by ants, preventing the accumulation of seeds in the soil over long inter-fire intervals (Yates *et al.* 1995; Bezemer *et al.* 2013), or a combination of low soil deposition/high water runoff that washes seed away (Szarzynski 2000). Nonetheless, seedling survivorship was high (with 30% of seedlings still alive at the final survey) compared with other granite outcrop specialists (Yates *et al.* 2003b, 2007; Vlasáková 2013). At Boyagin, it is interesting to note that seedlings were absent from some burnt patches (see Fig. 1),

perhaps as a result of the wildfire reaching a high intensity that killed canopy or soil-stored seed (Auld and Denham 2006; Bezemer *et al.* 2013).

Large-scale disturbance such as wildfire may be critical to stimulate recruitment in *E. caesia* (Yates *et al.* 2003b), but may also have adverse consequences in small plant populations. A reduction in the number of reproductive adult plants may result in loss of attractiveness to pollinators and lower reproductive potential (Menz *et al.* 2011; Phillips *et al.* 2014). High intensity fires may contribute to population contraction by killing plants or increasing mortality rates (Wellington and Noble 1985a; Moreno and Oechel 1991). At Boyagin, some ramets have not resprouted, warranting further investigation of the impact of fire.

Conclusions

Wildfire represents a rare but potentially important opportunity for large-scale recruitment in granite-outcrop endemics such as *Eucalyptus caesia*. The addition of 32 individuals at Boyagin (if all survive to adulthood) represents a substantial expansion of the population. Similar recruitment events are unlikely to occur frequently. It is unclear whether failure to detect selection against homozygous progeny over the survey period is a consequence of the survey duration, environmental variability of microsites, or purging of genetic loads. Further experimentation through common garden trials may provide clarification. Genetic mixing through frequent non-neighbour mating between plants within the study site may delay inbreeding, but appears insufficient to enhance genetic diversity in the Boyagin stand of *E. caesia*. Here, mating system and pollen dispersal analyses in additional populations would be useful to shed light on the generality of this conclusion. Nonetheless, *E. caesia* appears adept at persisting as extremely small populations.

Conflicts of interest

The author declares no conflicts of interest.

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