LETTER

Contributions of long-distance dispersal to population growth in colonising *Pinus ponderosa* populations

Mark R. Lesser[1][†][*] and Stephen T. Jackson[1][‡]

Abstract

Long-distance dispersal is an integral part of plant species migration and population development. We aged and genotyped 1125 individuals in four disjunct populations of *Pinus ponderosa* that were initially established by long-distance dispersal in the 16th and 17th centuries. Parentage analysis was used to determine if individuals were the product of local reproductive events (two parents present), long-distance pollen dispersal (one parent present) or long-distance seed dispersal (no parents present). All individuals established in the first century at each site were the result of long-distance dispersal. Individuals reproduced at younger ages with increasing age of the overall population. These results suggest Allee effects, where populations were initially unable to expand on their own, and were dependent on long-distance dispersal to overcome a minimum-size threshold. Our results demonstrate that long-distance dispersal was not only necessary for initial colonisation but also to sustain subsequent population growth during early phases of expansion.

Keywords

Allee effects, intrinsic population growth, long-distance dispersal, parentage, *Pinus ponderosa*.

Ecology Letters (2013) **16**: 380–389

INTRODUCTION

The establishment and expansion of new populations are fundamental processes in population ecology and conservation biology (Silvertown & Charlesworth 2001). The mechanisms by which species shift their ranges and expand into new territory are essential for understanding invasion biology (Shigesada & Kawasaki 1997), post-disturbance re-colonisation (Swanson *et al.* 2011) and migration into new habitat in response to changing climate (Higgins *et al.* 2003; Nathan *et al.* 2011). Documenting these mechanisms, however, can be challenging, because for many species they unfold over the course of decades to centuries.

Paleoecology records demonstrate broad-scale patterns of expansion over long-time periods (*e.g.* Jackson & Overpeck 2000). However, paleoecological proxies, such as pollen and plant macrofossils, are limited in the inferences that can be made below the scales of local to regional populations (Davis *et al.* 1991). To fully understand the mechanisms of how populations are initiated and expand, it is necessary to study them at the level of individuals.

The success of individuals in newly founded populations largely depends on the availability of suitable habitat and the timing and frequency of dispersal events (Drake & Lodge 2006). Stochastic demographic events, such as long-distance dispersal, can have strong affects on small populations, and may rescue colonising populations from extirpation (Taylor & Hastings 2005; Drury *et al.* 2007). This is especially important for species where there are Allee effects, defined as reductions in individual fitness caused by small population size or density (Allee 1931; Stephens *et al.* 1999). Under Allee effects, small populations do not exhibit positive intrinsic growth rates, but may nonetheless persist, or even expand through immigration (Keitt *et al.* 2001; Taylor & Hastings 2005).

Many studies have been concerned with the frequency of long-distance dispersal events and much modelling effort has been made in this area (*e.g.* Clark *et al.* 1999; Nathan *et al.* 2008, 2011). Documented distances of effective wind-dispersed pollen and seed range up to 100 and < 10 km respectively. However, long-distance dispersal can be difficult to measure quantitatively for both experimental and biological reasons (Kremer *et al.* 2012).

Here, we attempt to overcome this difficulty by studying small disjunct populations of a colonising species using microsatellite-based parentage analysis to discriminate between long-distance dispersal and local parentage. We studied four populations of *Pinus ponderosa* (ponderosa pine) where the history of population development over the past 4–5 centuries is almost completely known, from the initial coloniser through to the present (Lesser & Jackson 2012).

Pinus ponderosa is a long-lived conifer that is distributed over much of western North America. It often occurs in small isolated populations that, especially at drier sites, is related to available soil moisture (Oliver & Ryker 1990). Masting occurs on average every 8 years with medium cone crops every 2–5 years. Seeds are winged; however, dissemination also occurs via small mammals and birds. Although it is capable of self-fertilisation (Sorenson & Miles 1970), *P. ponderosa* is almost completely outcrossed (Mitton 1992) which may constrain population growth in small populations.

Nearly, every individual tree in the study populations has been aged and genotyped, providing a unique data set to support parentage analysis to discern local establishment events vs. establishment

[1]*Program in Ecology, Department of Botany, University of Wyoming, 1000 E. University Ave., Laramie, WY, 82071, USA*
[†]*Current Address: Department of Biology, Syracuse University, 114 Life Sciences Complex, Syracuse NY, 13244, USA*

[‡]*Current Address: USGS Southwest Climate Science Center, 1955 E. Sixth Street, Tucson, AZ 85719 USA*
[*]*Correspondence: E-mail: mrlesser@syr.edu*

events where only one, or neither, of the parents was present in the population. The objectives of this study were to: (1) assess contributions of long-distance dispersal to population development and (2) determine how long-distance dispersal eventually overcame initial lags in growth. Results show the importance of long-distance dispersal events in population development, and the timeframes necessary for populations to become self-sufficient.

METHODS

Study sites and data collection

We studied four populations of *P. ponderosa* in the Bighorn Basin of north-central Wyoming (Fig. 1). The Bighorn Basin is largely composed of fine-textured and alkaline soils and vegetated by desert shrubland and *Artemisia* steppe (Knight 1994). Within this landscape, the study populations are restricted to sandstone outcrops and escarpments, where they co-occur with *Pinus flexilis* and *Juniperus* spp. The study populations were separated from each other, and any other *P. ponderosa*, by distances > 10 km. The Castle Garden population is situated on the eastern side of the Bighorn Basin and is only removed from other *P. ponderosa* on the western flank of the Bighorn Mountains by approx. 20 km (Fig. 1) (Driese *et al.* 1997). The other three populations are located on the western side of the Bighorn Basin. No other *P. ponderosa* have been observed in the intervening space between the Castle Garden site and the other three sites. However, given the remote and complex terrain, the existence of other small populations of *P. ponderosa* in the Bighorn Basin cannot be completely ruled out.

All four study populations had identifiable boundaries and were small enough that every individual present in each population was mapped. A small percentage of mapped trees (15%) were not included in the analysis due to trees having heart rot, and thus not being aged, or DNA not amplifying at > 4 loci (Table 1).

Trees that did not amplify and heart-rot trees, based on size and growth form, were randomly distributed throughout the population's history. Lesser & Jackson (2012) provide additional details on site descriptions.

Needle tissue was collected from all sampled trees in each population for DNA extraction. All individuals were typed at nine microsatellite loci, and alleles were scored using the Genemapper software package (Applied Biosystems, Carlsbad, CA. USA). Detailed descriptions of extraction and polymerase chain reaction protocols are given in Appendix S1.

Data analysis

Genotype data were paired with tree age data taken from Lesser & Jackson (2012). For each population, individuals were binned by decade of establishment. The candidate parents for a given cohort of establishing individuals were all of the trees already present in that population that were > 10-years old. This age was selected based on reported sexual maturity in *P. ponderosa* occurring as early as age 7 (Curtis & Lynch 1965), and on observation at the study sites of young trees producing both seed and pollen. No upper age limit was placed on potential parents. *P. ponderosa* trees produce viable seed for at least 350 years (Oliver & Ryker 1990), and mature trees at each study site produce ovulate cones.

The software package Cervus (Marshall *et al.* 1998) was used to carry out parentage analysis. Cervus calculates the log-likelihood of each potential parent being the true parent relative to random individuals. Parent-pairs and single parents were assigned based on LOD (logarithm of odds) critical values obtained through simulations using the genotype data (Marshall *et al.* 1998). For each population, 100 000 simulations runs were performed at decadal intervals to calculate critical LOD values at 80 and 50% confidence levels. The initial number of candidate parents was set to 100 to
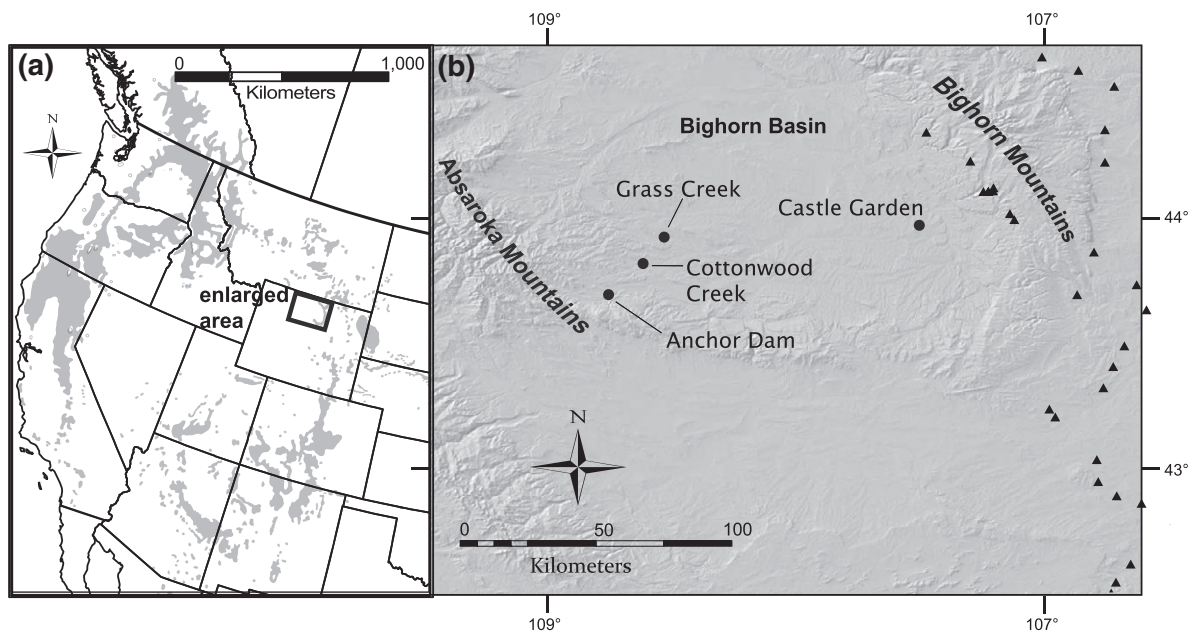

Figure 1 (a) Map of full distribution of ponderosa pine (grey shading) (U.S. Geological Survey 1999); box shows area of study sites in Wyoming, USA. (b) Enlarged map of study sites and surrounding area. Solid triangles designate ponderosa pine occurrence based on Rocky Mountain Herbarium Specimen Database records (2011). Study sites are shown with solid circles. Locations are overlaid on a hillshade surface. Figure is reproduced from Lesser & Jackson 2012 with permission of the publisher.

Table 1 Number of individuals at each study site placed into three categories: Two local parents (inferred to represent intrinsic-based establishment, One local parent (inferred to represent long-distance pollen dispersal), or no local parents (inferred to represent long-distance seed dispersal). Values are shown for 80 and 50% assignment confidence along with compatible, but not assigned, parents (*i.e.* LOD threshold value> LOD score> 0). Decade of initial intrinsic-based establishment along with the offset from population initiation are given for the same three groups

Site	Total # of indiv.	# of indiv. aged and genotyped	# indiv. with two local parents			# indiv. with one local parent			# indiv. with no local parents			Prop. of pop. with no local parents			Decade of initial intrinsic based establishment			Offset from population initiation (years)		
			80%	50%	Compat.	80%	50%	Compat.	80%	50%	Compat.	80%	50%	Compat.	80%	50%	Compat.	80%	50%	Compat.
Castle Garden	177	145	0	2	82	7	41	82	138	102	63	0.95	0.70	0.43	\	1870	1710	\	280	120
Grass Creek	82	62	0	1	28	2	13	28	60	48	34	0.97	0.77	0.55	\	1940	1770	\	340	170
Cottonwood Creek	927	798	43	370	739	264	196	739	491	232	59	0.62	0.29	0.07	1820	1770	1650	290	240	120
Anchor Dam	143	122	1	4	64	14	37	64	107	81	58	0.88	0.66	0.48	1950	1900	1810	300	250	160

reflect un-sampled non-local parents, or previously present local trees that were not sampled because they have died and disappeared. The number of candidate parents and the proportion of sampled parents were increased over time as the population grew (Appendix S2).

Mismatches at individual loci are a common problem in parentage analysis and can be caused by genotyping errors, null alleles or mutations. Allowing mismatches increases the overall success of parentage analysis (Kalinowski *et al.* 2007). For our analysis, mismatches were not rejected if LOD scores were positive. We calculated estimated frequency of null alleles using Micro-Checker (van Oosterhout *et al.* 2004) for each population. Unlike Cervus, Micro-checker can discriminate between deviations caused by null alleles vs. other factors that can affect Hardy–Weinberg equilibrium (van Oosterhout *et al.* 2004).

Parentage results were used to place individuals into one of three categories: (1) individuals with two local parents, (2) individuals with one local parent and (3) individuals with no local parents. Trees were placed into each of these categories with 80 and 50% confidence based on LOD critical values. In addition, we placed individuals into these categories based on compatible parents being present. Compatible parents were those that had a positive LOD value, indicating greater than equal odds of being the true parent, yet were not significant at 80 or 50% confidence. These three levels of confidence provide upper and lower bounds for actual immigration estimates. The 50% confidence level and, to an even larger degree, the compatible parents, increase false assignments and thus decrease immigration estimates, while the 80% confidence level decreases false local assignments and provides an upper estimate of immigration.

We equate each tree with two compatible parents as an intrinsic establishment event, meaning that it was the product of within-population reproduction. Trees with only one compatible parent are inferred to be the product of a local seed parent and an extra-local pollen parent. Finally, trees with no compatible parents are inferred to be the product of long-distance seed dispersal events, where neither parent is present in the population. For each population, we calculated the cumulative number of individuals in each of these categories through time.

To examine long-distance dispersal between the study populations, we took all individuals that were assigned only one or no local parents with 80% confidence and re-ran the parentage analysis with candidate parents from the other three populations included. To minimise wrong assignments, parentage was based on LOD critical values at 95% confidence. LOD critical values were calculated for each decade from 100 000 simulation runs using parameters for the combined populations and a high level of un-sampled parents (95%).

For all individuals that were assigned parentage at 80 or 50% confidence, we determined age at the time of their first successful reproductive event and modelled this against time of establishment. Time of establishment was calculated as the number of years elapsed between the parent trees establishment and the initial colonising event at the site. We used the survival package in R (R Development Core Team 2012) to fit a model using a weibull distribution to the data (Appendix S7).

Finally, total and intrinsic per capita population growth were calculated for each population as the number of total new establishments, or intrinsic establishments, respectively, divided by total

population size at the beginning of each decade. Intrinsic growth was calculated separately for individuals assigned at 80 and 50% confidence and the compatible parent category. Per capita population growth was modelled against population size using breakpoint linear regression. Final models (i.e. number of breakpoints) for each growth rate in each population were selected based on AIC (Akaike information criterion) scores.

RESULTS

A total of 1125 trees were genotyped and aged (Table 1). Across the nine microsatellite loci the number of alleles ranged from 9 to 16 (Appendix S3), giving a total of 149 alleles. Average and individ-

ual polymorphic information content scores (Appendix S4, Appendix S5) indicated that the nine markers were informative (Botstein et al. 1980). Null allele frequencies for the nine loci ranged from 0.05 to 0.30 (Appendix S6). Micro-Checker designated seven of the nine loci as having null alleles present in two of the populations. The other two populations each had eight loci designated as having null alleles.

The percentage of trees with no assigned local parents ranged from 97 to 62%, and 77 to 29% based on 80 and 50% assignment confidence respectively (Table 1, Fig. 2). Most of the individual's assigned parentage were from the Cottonwood Creek population, where even at 80% confidence only 62% of trees had no parents assigned. In the other three populations, > 85% of trees had no
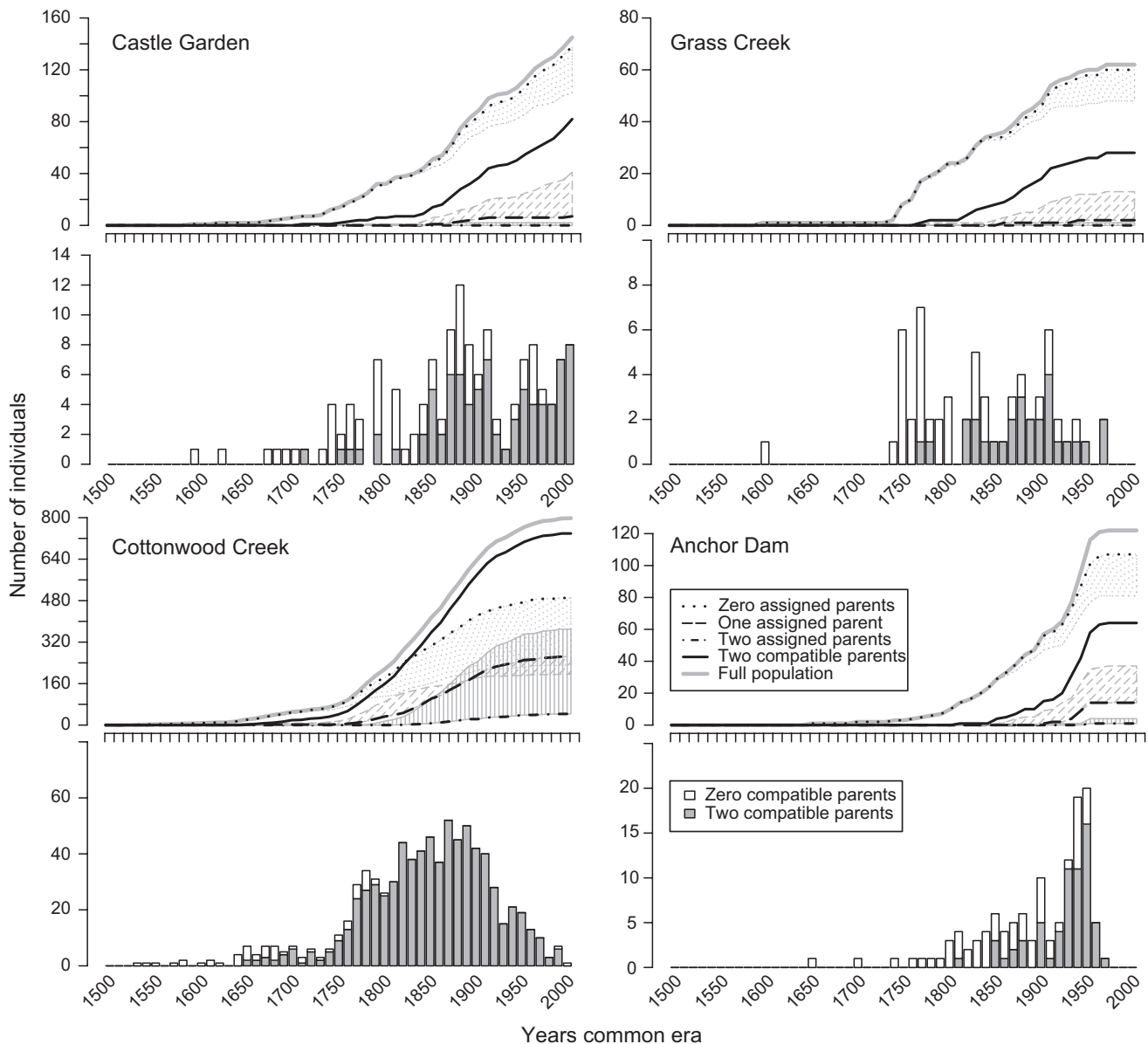

Figure 2 Demographic history of the four study populations. In the upper panel for each population cumulative establishment is shown through time for the full population along with individuals with compatible but unassigned local parents (0 < LOD score < LOD critical value). Individuals with no local assigned parents, one local assigned parent and two local assigned parents are also shown at 80 and 50% confidence. The thick line of each envelope designates assignments at the 80% confidence level. Bottom panels for each population show the number of individuals establishing per decade with and without compatible local parents.

assigned parents with 80% confidence, and at 50% confidence in assignment > 50% of trees still had no local parent (Table 1). All individuals that had one compatible parent were also assigned a compatible parent-pair (Table 1). At Cottonwood Creek 7% of trees had no compatible parents. The other three populations had > 40% no compatible parents (Table 1, Fig. 2).

Temporally, all four populations showed a similar trend in the establishment type. With 80% confidence assignments, there were never any intrinsic establishments at Castle Garden or Grass Creek, while the time-spans at Cottonwood Creek and Anchor dam are 290 and 300 years following population initiation respectively (Table 1). At 50% confidence levels, the first intrinsic-based establishment still does not occur for time-spans ranging from 280 to 340 years. With compatible parents time-frames were shorter, but still exceeded a century in all four populations (Fig. 2, Table 1).

Over time, establishment resulting from one local parent and intrinsic-based events made up increasing proportions of each population (Fig. 2). However, at Castle Garden and Anchor Dam establishment events resulting from these two categories only just exceeded 50% of the total population even when only compatible parents were considered (Fig. 2). Grass Creek showed a similar pattern where not even 50% of the population had compatible parents. A different pattern occurs at Cottonwood Creek where establishments with compatible local parents comprised 93% of the current population (Table 1, Fig. 2).

For trees that were assigned parentage at 80 and 50% confidence the time of establishment from population initiation was significant in predicting the age at which they first reproduced in the Castle Garden, Cottonwood Creek and Anchor Dam populations (Appendix S7, Fig. 3). Individuals establishing during the early stages of population development were significantly older when they first successfully reproduced than individuals which established later in the course of the population (Fig. 3). At Grass Creek, this trend was only significant at 50% confidence (Appendix S7); however, there were only two parents assigned with 80% confidence.

Per capita population growth decreased over time in all four populations when total establishment events were included (Fig. 4, Appendix S8). However, when only establishments with local compatible parents or parents assigned at 50 and 80% confidence were included per capita growth was initially positive in all four populations. In almost every case, a breakpoint was identified following which the slope became negative (Fig. 4, Appendix S8).

The percentage of establishments in each population with at least one parent from one of the other sampled populations ranged from 0.6% at Cottonwood Creek to 10% at Castle Garden (Table 2). The number of establishments at Anchor Dam, Cottonwood Creek and Grass Creek was fairly similar (5–8). Across all four populations, 50% of individuals assigned an extra-local single parent also had a compatible local second parent indicating the importance of pollen flow to dispersal.
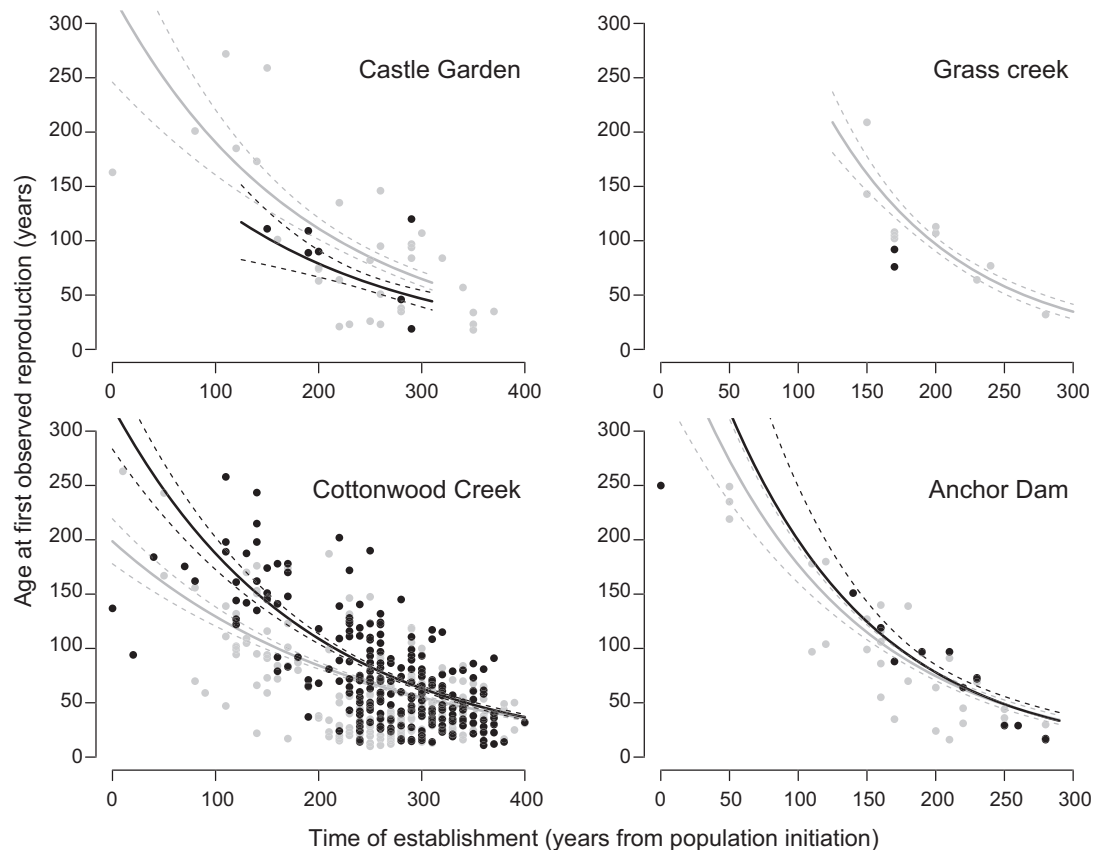

Figure 3 Age of first observed successful reproduction for an individual plotted against the time of establishment from population initiation of that same individual. Black and grey circles represent parents assigned at 80 and 50% confidence respectively. Solid lines are the fitted values using a weibull distribution survival model. Dashed lines show the modelled standard error around the fitted values.
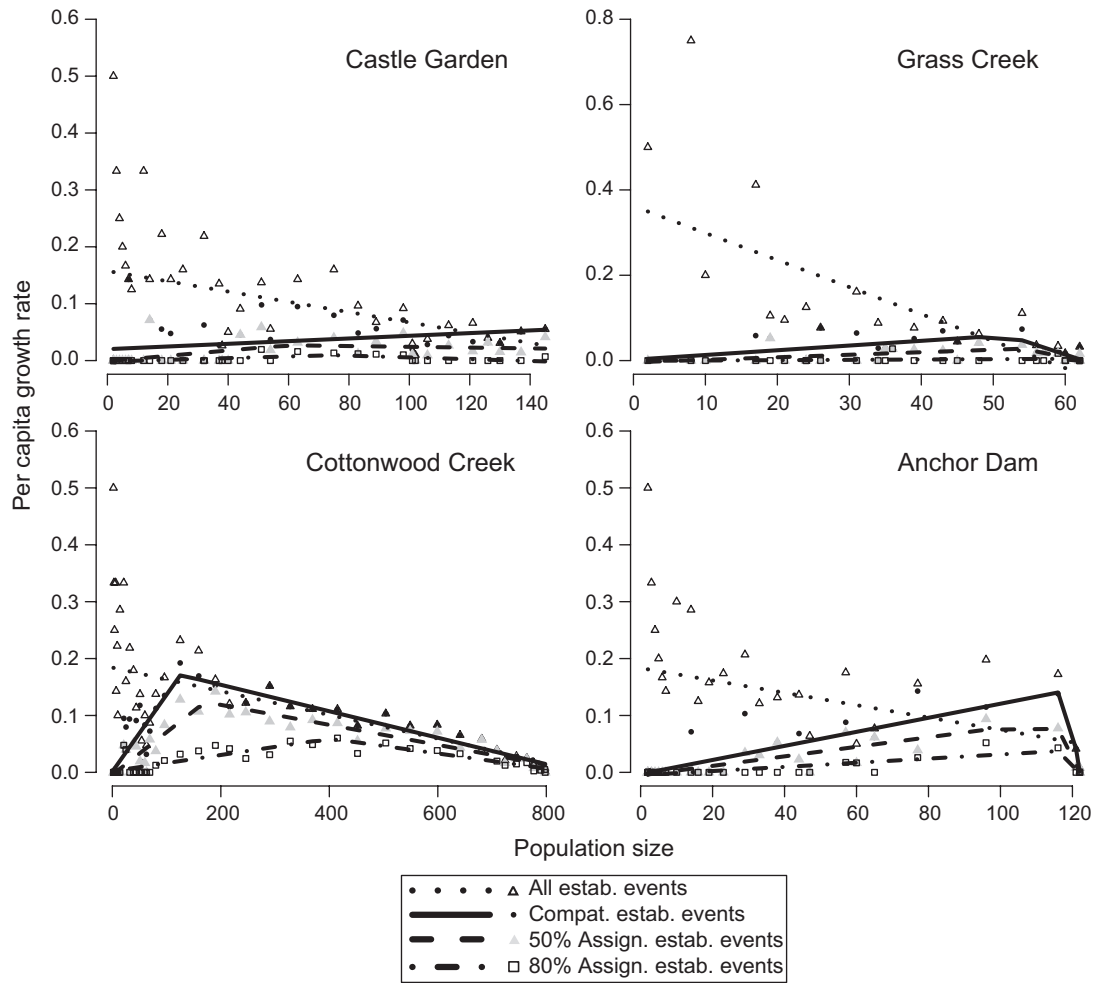
Figure 4 Per capita growth plotted against population size for the four study populations. Results are shown for four scenarios: (a): total establishments, (b) establishments with compatible local parents, (c) establishments with local parents assigned with 50% confidence and (d) establishments with local parents assigned with 80% confidence. Trend lines represent fitted values from linear (breakpoint) regression. Slopes and breakpoints are given in Appendix S8.

Table 2 Parentage assignments for establishment events at the four study sites with one or two parents assigned from the other three populations (all assignments were made with 95% confidence based on LOD critical value calculated for each decade of establishment). The total number of establishments resulting from at least one parent in one of the other sampled populations is given along with the proportion of the population that this represents

	Site of parent origin									
	Castle Garden		Grass Creek		Cottonwood Creek		Anchor Dam			
Site	Two parents	One parent	Two parents	One parent	Two parents	One parent	Two parents	One parent	Total	Proportion of pop.
Castle Garden			0	7	0	5	0	2	14	0.097
Grass Creek	1	4			0	0	0	0	5	0.081
Cottonwood Creek	0	4	0	1			0	0	5	0.006
Anchor Dam	0	3	0	1	0	4			8	0.066

Fifty per cent of assigned extra-local parents at Anchor Dam originated from Cottonwood Creek, the closest population to Anchor Dam (18 km). However, extra-local parents for Grass Creek and Cottonwood Creek were almost entirely from Castle Garden, and even at Anchor Dam almost 50% of extra-local parents were from Castle Garden, suggesting Castle Garden may be an intermediary location between the Bighorn Mountains and western side of the Bighorn Basin. The only assigned extra-local two-parent establishment occurred at Grass Creek and originated from Castle Garden. The earliest extra-local parent was assigned in 1770 at Grass Creek. Following that point in time, extra-local parent establishments from sampled populations occurred at low rates up until present (Appendix S9).

DISCUSSION

This study represents a unique examination of population dynamics in a long-lived tree species. By combining dendroecological and genotypic data from populations where nearly every individual has been inventoried and aged (Lesser & Jackson 2012), we were able to perform a comprehensive and detailed parentage analysis for each population. Our results show that long-distance dispersal was essential for sustaining population growth during early phases of expansion.

Parentage

The nine microsatellite loci used in the study provide a high level of confidence that correct parents were assigned. Simulation models have shown that the probability of detecting the true parent with this many variable loci is high, even if mistyping rates due to genotyping error or null alleles are high (Slavov *et al.* 2005). However, Slavov *et al.* (2005) only simulated mistyping rates of up to 10% and for the frequency of null alleles at our loci (Appendix S6), Dakin & Avise (2004) have shown that the effects on parentage assignment may be significant. If null alleles affect parentage assignments, it would mean that true parents had not been assigned based on observed homozygotes actually being heterozygotes at a particular locus. If this is the case, then intrinsic population growth may be underestimated in the study.

Even though these issues exist, we retained all loci for the analysis for several reasons. First, the observed null allele frequencies may be artificially high due to family structure or other barriers to gene flow within populations. Second, assignment of parents with only 50% confidence includes parents with high numbers of mismatches at multiple loci and greatly alleviates the issue of false exclusion (Dakin & Avise 2004). Finally, the nine loci used in this study represent the full complement of available microsatellite resources for *P. ponderosa*, and confidence in results will decrease substantially with fewer loci.

Issues with unassigned parentage when the true parent was present may also result from incomplete sampling. We have attempted to sample every individual present in the four populations. A small number of existing trees (15% of total number of trees) were not included in the analysis because they could either not be aged or successfully genotyped. This proportion of un-sampled trees has been accounted for in the calculation of critical values used to assign parentage (Appendix S2).

A more serious issue is that trees may have once existed in a population but have now disappeared from the record. However, there is no evidence of past *P. ponderosa* mortality at any of the sites. The small number of dead trees observed at the study sites (24) all died within the past 10 years (Lesser & Jackson 2012). Dead wood persists for centuries in semi-arid environments like the Bighorn Basin (Gray *et al.* 2004; Millar *et al.* 2007). Dead wood and snags of *P. flexilis* and *Juniperus* spp. occurred, often abundantly, at all of the sites, while none of *P. ponderosa* was observed. Furthermore, a complete absence of fire-scarred trees, absence of surface or soil charcoal, and the scarcity of surface fuels all indicate that fire has not destroyed evidence of past establishment events. This evidence pertains only to adult trees and gives no indication of past seedling or sapling mortality. However, it is unlikely that trees small enough to have been completely erased from the record were reproductively mature.

We cannot, however, rule out the possibility that local parents have died and disappeared. The oldest trees in our populations may be close to the expected life-span of ponderosa pine (Oliver & Ryker 1990). Therefore, it is possible that some individuals were established before our oldest living trees and are now gone. However, evidence from *Neotoma* (woodrat) middens shows that the nearest source populations, in the western Bighorn Mountains, were not established until 1500 years ago (Norris 2006), which places an absolute constraint on age of our populations. The centuries required to build extensive populations in the Bighorns also suggests that initial establishment at our sites could not have predated our oldest individuals very much.

Estimates of long-distance dispersal may be inflated if trees present early in population development, but now absent, produced offspring. This possibility has been accounted for as much as possible in the parentage analysis, where we have included a high number of un-sampled trees. Furthermore, the very low levels of local reproduction during the first two centuries following population establishment observed in existing trees (Fig. 2, Fig. 3) suggest that it is unlikely that there were significant numbers of now-absent individuals. Unassigned individuals in Cervus can result from both un-sampled parent trees and from sampled, yet unassigned local parents. It is likely therefore, that true levels of immigration lie somewhere between the compatible parent category and the assignments made with 80% confidence.

A final potential cause for false parentage assignments is cryptic immigration events. Cryptic immigration can be a problem when there are large numbers of un-sampled potential parents (Sork *et al.* 1999) as is the case in our study. However, the lack of any local assigned parents during the early stages of population development and the overall low levels of intrinsic establishment events at all of the study sites except Cottonwood Creek suggest that our estimates of parentage are not adversely affected by cryptic immigration.

Long-Distance dispersal

Pinus ponderosa seed is morphologically suited for wind transport, but seeds rarely travel further than 30 metres (Oliver & Ryker 1990). Clark *et al.* (2003) suggest that pine seed may travel long distances more often than previously assumed, and Nathan *et al.* (2002) show that long-distance seed dispersal (10 of kilometres) can be appreciable (1–5% of seed produced by a tree), for many tree species. However, seeds dispersing long distances by wind are commonly lighter than seeds falling close to the tree, making them less likely to germinate and survive (Nathan *et al.* 2002).

Morphological dispersal adaptations may only determine local dispersal capabilities, with long-distance dispersal being primarily a result of non-standard dispersal mechanisms (Higgins *et al.* 2003; Nathan *et al.* 2008). Studies suggest that bird dispersal may be a common mechanism for long-distance colonisation (Willson & Traveset 2000). Two bird species, *Nucifraga columbiana* (Clark's nutcrackers) and *Gymnorhinus cyanocephalus* (pinyon jays) are primarily responsible for seed dispersal in pines of western North America (Tomback & Linhart 1990). Both species have been documented by the North American Breeding Bird Survey in the Bighorn Basin. We have observed *G. cyanocephalus* at the Castle Garden site and in *P. ponderosa* stands on the west flanks of the Bighorn Mountains. In the Bighorn Basin, where food supply may be limited, *P. ponderosa* may constitute an important part of their diet. *N.*

columbiana have been shown to cache *P. ponderosa* seed (Giuntoli & Mewaldt 1978), and *G. cyanocephalus* will eat and cache *P. ponderosa* seed, especially when *Pinus edulis* seed is not available (Bateman & Balda 1973). Thus, while wind-dispersed seed may have contributed to these populations, bird dispersal is a more likely mechanism. Birds preferentially revisit sites that provide favourable roosting and foraging conditions. Thus, once trees have colonised a site, the probability of additional bird dispersal of seed to that site can increase (Willson & Traveset 2000). In the Bighorn Basin, where tree populations are sparse, this may be the primary source of continued long-distance dispersal to the sites following initial colonisation.

The Castle Garden site represents a different scenario than the other three populations. Castle Garden is situated much closer to other *P. ponderosa* in the western foothills of the Bighorn Mountains, than it is to the other study populations (Fig. 1). Although our analysis found the highest number of immigrants originating from the other three populations for Castle Garden, we interpret this result with caution, due to the large number of un-sampled candidate parents that are in relatively close proximity (20–30 km) to Castle Garden (Fig. 1). It is probable that many immigrants into the Castle Garden population originated from these closer sources. Furthermore, Castle Garden is the source of the majority of assigned extra-local parentage in the other study populations. This suggests that Castle Garden may be an intermediary step in long-distance dispersal between the western Bighorn Mountains and the other study populations.

Allee effects

Lags in population growth are inherent during the early stages of logistic growth (Crooks 2005). However, during early stages of population growth, the study populations show lags greater than expected from logistic growth (Lesser & Jackson 2012), indicating other factors are retarding initial growth (Crooks & Soulé 1999). Our findings, that population growth appears reliant on long-distance dispersal for over a century following initial colonisation and continued to be primarily supported by long-distance dispersal for over two centuries, suggest that Allee effects may have played a role in this lag period. Allee effects have not been well studied in tree species. In a review of documented Allee effects in natural plant populations, Kramer *et al.* (2009) identified 28 studies on Allee effects. However, only one of these studies dealt with a temperate forest tree (*Sorbus torminalis*), and it is an insect, not wind-pollinated species.

Allee effects are decreases in fitness caused by small population size (Allee 1931; Stephens *et al.* 1999). At small population sizes per capita growth rate will initially show a positive association with population size when there is an Allee effect (Berec *et al.* 2006). When we removed establishments with no local parents from the analysis of per capita growth rate an initially positive trend was observed in all four populations (Fig. 4). Under our assumption of negligible past mortality in the populations the per capita growth rate is never negative, indicating a weak Allee effect with no extinction threshold (Berec *et al.* 2006).

Allee effects may manifest themselves in tree populations in several ways. First, at low population size, probability of successfully finding a mate is lowered (Drake & Lodge 2006). For anemophilous trees this is a function of the probability of viable ovules intercepting pollen dropping as population size decreases.

Pollen limitation issues are compounded for *P. ponderosa*, which is an almost completely outcrossed species and poor at selfing (Farris & Mitton 1984; Mitton 1992). However, *P. ponderosa* is capable of selfing and in controlled pollination trials Sorenson & Miles (1970) fond that selfing rates in *P. ponderosa* could be appreciable (as high as 96%, mean 37%). Selfing has been shown to be more prevalent in low density populations (Mitton 1992) such as those studied here, however, even at low densities outcrossing rates were still at 80%. We examined genotypes of offspring and potential parents and found no evidence of selfing in the study populations. However, this result does not rule out the possibility that selfing has occurred in the populations and that self-pollinated seeds have failed to survive to maturity due to decreased fitness.

A second way in which Allee effects may affect population fitness is that small populations may not support secondary dispersers. Secondary dispersal, or the movement of seed following primary dispersal, can play a significant role in the establishment of pine species (Vander Wall 2003). Secondary dispersers (*i.e.* birds, small rodents) decrease seed loss from seed predators and often displace seed to better microsites which may increase germination success (Vander Wall & Longland 2004). Sparse stands, however, may not support disperser populations, and therefore, the tree species will not benefit from secondary dispersal until a threshold population size has been reached.

Under Allee effects, demographic stochasticity (*e.g.* long-distance dispersal) may increase the probability of successful establishment and facilitate surmounting of population-size thresholds (Taylor & Hastings 2005). Furthermore, Keitt *et al.* (2001) show that for species with Allee effects, populations can become trapped when some individuals successfully disperse into new territory but are then unable to generate a net positive growth rate. Our study populations appear to demonstrate both of these phenomena, whereby individuals are successfully established via long-distance dispersal, but further population growth requires additional long-distance dispersal events, until a threshold population size is surpassed.

Initial lags in population growth may result from factors other than Allee effects. Changing climate following initial colonisation may have created more favourable establishment conditions for seedlings. However, Lesser & Jackson (2012) showed that climate conditions alone were not responsible for population growth, although climate may have been a factor in conjunction with Allee effects at specific time points in population development.

Although climate change may not have directly governed population development, the inherent climate of the region may still restrict population growth and cause the observed lags in establishment. The populations are located near a range-margin, formed by much of the Great Basin (Fig. 1). Oliver & Ryker (1990) suggest that *P. ponderosa* is absent from this large area due to summer rainfall limitations. Populations occurring near the edge of climatic tolerances often exhibit suboptimal growth and development (Kremer *et al.* 2012). The slow rate of expansion and associated lags in intrinsic-based establishment seen in the study populations may be a factor of the population's location and the near insupportable environmental conditions that exist so close to the range-margin.

Another alternative to Allee effects causing lags in population growth is that individuals in the early stages of population development do not produce enough seed, or are not reproductively active until they reach a certain age. Tree size has been shown to be positively correlated to seed production (Thomas 2011). During the early stages of population development few trees are reproductively

mature, and they are all relatively young. However, our results demonstrate that individuals establishing in the first century following population initiation do not successfully reproduce until much later in their life-span compared with individuals establishing after the first century of population growth (Fig. 3). This result suggests that age, and/or size, is not inhibiting reproductive success, and that if mates are available young trees are capable of successfully reproducing. However, this result may be biased if older trees have died and disappeared from the populations. It is possible that older trees produced offspring at younger ages but those offspring, due to their increased age, have since died and were not sampled.

Regardless of the mechanism, for most species the centuries required to overcome threshold population sizes would likely result in extirpation of the population. However, for species like *P. ponderosa*, where individuals are capable of living for hundreds of years, the population may persist on the landscape while the population size is built up through low-frequency long-distance dispersal events.

In conclusion, our study shows that there can be considerable lags in population growth, with colonising populations being saved from extirpation by continued long-distance dispersal events. Our results demonstrate that it may take over a century before populations begin to contribute to their own growth, and even longer before intrinsic contributions are substantial. Understanding these timeframes and the associated number of individuals for populations to be self-sufficient is crucial, especially given predictions of species range shifts over the coming decades and centuries (Williams *et al.* 2007), and the increasing fragmentation of the landscape that species will have to migrate across (Bacles & Jump 2010). Further study is necessary to determine if the patterns shown here are applicable to other tree species and at what distances long-distance dispersal is no longer capable of sustaining new populations. These data are essential for conservation biology and for discussion on the utility and appropriate use of assisted migration.

ACKNOWLEDGEMENTS

AUTHORSHIP

MRL and STJ designed the study. MRL carried out field work, laboratory work and analysis, with input from STJ. The manuscript was written by MRL with editing by STJ.

REFERENCES

Allee, W.C. (1931). *Animal Aggregations, a Study in General Sociology*. The University of Chicago Press, Chicago, Il.

Bacles, C.F.E. & Jump, A.S. (2010). Taking a tree's perspective on forest fragmentation genetics. *Trends Plant Sci.*, 16(1), 13–18.

Bateman, G.C. & Balda, R.P. (1973). Growth, development, and food habits of young pin on jays. *Auk*, 90, 39–61.

Berec, L., Angulo, E. & Courchamp, F. (2006). Multiple Allee effects and population management. *Trends Ecol. Evol.*, 22(4), 185–191.

Botstein, D., White, R.L., Skolnick, M. & Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.*, 32, 314–331.

Clark, J.S., Silman, M., Kern, R., Macklin, E. & HilleRisLambers, J. (1999). Seed dispersal near and far: patterns across temperate and tropical forests. *Ecology*, 80(5), 1475–1494.

Clark, J.S., Lewis, M., McLachlan, J.S. & HilleRisLambers, J. (2003). Estimating population spread: what can we forecast and how well? *Ecology*, 84(8), 1979–1988.

Crooks, J.A. (2005). Lag times and exotic species: the ecology and management of biological invasions in slow-motion. *Ecoscience*, 12(3), 316–329.

Crooks, J.A. & Soulé, M.E. (1999). Lag times in population explosions of invasive species: Causes and implications. In *Invasive species and biodiversity management*. (eds Sandlund, O.T., Schei, P.J., Viken, A.). Kluwer Acedemic Publishers, Boston, pp. 103–112.

Curtis, J.D. & Lynch, D.W. (1965). Ponderosa pine (*Pinus ponderosa* Laws.). In: *Silvics of Forest Trees of the United States*. (ed. Fowells, H.A.). U.S. Dept. of Agriculture, Agriculture Handbook 271. Washington, D.C., pp. 417–431.

Dakin, E.E. & Avise, J.C. (2004). Microsatellite null alleles in parentage analysis. *Heredity*, 93, 504–509.

Davis, M.B., Schwartz, M.W. & Woods, K. (1991). Detecting a species limit from pollen in sediments. *J. Biogeogr.*, 18, 653–668.

Drake, J.M. & Lodge, D.M. (2006). Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasion. *Biol. Invasions*, 8, 365–375.

Driese, K.L., Reiners, W.A., Merrill, E.H. & Gerow, K.G. (1997). A digital land cover map of Wyoming, USA: a tool for vegetation analysis. *J. Veg. Sci.*, 8, 133–146.

Drury, K.L.S., Drake, J.M., Lodge, D.M. & Dwyer, G. (2007). Immigration events dispersed in space and time: Factors affecting invasion success. *Ecol. Model.*, 206(1), 63–78.

Farris, M.A. & Mitton, J.B. (1984). Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. *Evolution*, 38(5), 1151–1154.

Giuntoli, M. & Mewaldt, L.R. (1978). Stomach contents of Clark's nutcrackers collected in western Montana. *Auk*, 95(3), 595–598.

Gray, S.T., Fastie, C.L., Jackson, S.T. & Betancourt, J.L. (2004). Tree-ring based reconstructions of precipitation in the Bighorn Basin, Wyoming since 1260 A.D. *J. Clim.*, 17, 3855–3865.

Higgins, S.I., Nathan, R. & Cain, M.L. (2003). Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? *Ecology*, 84(8), 1945–1956.

Jackson, S.T. & Overpeck, J.T. (2000). Responses of plant populations and communities to environmental changes of the Late Quaternary. *Paleobiology*, 26 (Supplement), 194–220.

Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.*, 16, 1099–1106.

Keitt, T.H., Lewis, M.A. & Holt, R.D. (2001). Allee effects, invasion pinning, and species' borders. *Am. Nat.*, 157(2), 203–216.

Knight, D.H. (1994). *Mountains and plains: the ecology of Wyoming landscapes*. Yale University Press, New Haven. 338 pp.

Kramer, A.M., Dennis, B., Liebhold, A.M. & Drake, J.M. (2009). The evidence for Allee effects. *Popul. Ecol.*, 51, 341–354.

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R. *et al.* (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecol. Lett.*, 15, 378–392.

Lesser, M.L. & Jackson, S.T. (2012). Making a stand: five centuries of population growth in colonizing stands of Pinus ponderosa. *Ecology*, 93(5), 1071–1081.

Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.*, 7, 639–655.

Millar, C.I., Westfall, R.D. & Delany, D. (2007). Response of high-elevation limber pine (*Pinus flexilis*) to multiyear drought and 20th century warming, Sierra Nevada, California, USA. *Can. J. For. Res.*, 37, 2508–2520.

Mitton, J.B. (1992). The dynamic mating system of conifers. *New Forest.*, 6, 197–216.

Nathan, R., Katul, G.G., Horn, H.S., Thomas, S.M., Oren, R., Avissar, R. et al. (2002). Mechanisms of long-distance dispersal of seeds by wind. *Nature*, 418, 409–413.

Nathan, R., Schurr, F.M., Spiegel, O., Steinitz, O., Trakhtenbrot, A. & Tsoar, A. (2008). Mechanisms of long-distance seed dispersal. *Trends Ecol. Evol.*, 23(11), 638–647.

Nathan, R., Horvitz, N., He, Y., Kuparinen, A., Schurr, F.M. & Katul, G.G. (2011). Spread of North American wind-dispersed trees in future environments. *Ecol. Lett.*, 14, 211–219.

Norris, J.R. (2006). *Influence of climate on the modern and late Holocene biogeography of ponderosa pine in the Central Rockies*. Dissertation. University of Wyoming, Laramie, Wyoming, USA.

Oliver, W.W. & Ryker, R.A. (1990). *Pinus ponderosa* Dougl. Ex Laws. In: *Silvics of North America*. (eds. Burns, R.M. & Honkala, B.H.). U.S. Dept. of Agriculture, Agriculture Handbook 654. Washington, DC, pp 413–424.

van Oosterhout, C., Hutchinson, W.F., Wills, P.M. & Shipley, P. (2004). Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*, 4, 535–538.

R Development Core Team (2012). *R: a language and environment for statistical computing*. R foundation for statistical computing, Vienna Austria.

Shigesada, N. & Kawasaki, K. (1997). *Biological Invasions: Theory and Practice*. Oxford University Press, New York, New York. 205 pp.

Silvertown, J. & Charlesworth, D. (2001). *Introduction to Plant Population Biology*, 4th edn.. Blackwell Publishing, Ames Iowa. 347 pp.

Slavov, G.T., Howe, G.T., Gyaourova, A.V., Birkes, D.S. & Adams, W.T. (2005). Estimating pollen flow using SSR markers and paternity exclusion: accounting for mistyping. *Mol. Ecol.*, 14, 3109–3121.

Sorenson, F. & Miles, R.S. (1970). Self-pollination effects on Douglas-fir and ponderosa pine seeds and seedlings. *Silvae Genetica*, 23(5), 135–138.

Sork, V.L., Nason, J., Campbell, D.R. & Fernandez, J.F. (1999). Landscape approaches to historical and contemporary gene flow in plants. *Trends Ecol. Evol.*, 14(6), 219–224.

Stephens, P.A., Sutherland, W.J. & Freckleton, R.P. (1999). What is the Allee effect? *Oikos*, 87(1), 185–190.

Swanson, M.E., Franklin, J.F., Beschta, R.L., Crisafulli, C.M., DellaSala, D.A., Hutto, R.L. et al. (2011). The forgotten stage of forest succession: early-successional ecosystems on forest sites. *Front. Ecol. Environ.*, 9(2), 117–125.

Taylor, C.M. & Hastings, A. (2005). Allee effects in biological invasions. *Ecol. Lett.*, 8, 895–908.

Thomas, S.C. (2011). Age-related changes in tree growth and functional biology: the role of reproduction In: Size- and Age-Related Changes in Tree Structure and Function. *Tree Physiol.* 4(2): 33–64. DOI: 10.1007/978-94-007-1242-3_2

Tomback, D.F. & Linhart, Y.B. (1990). The evolution of bird-dispersed pines. *Evol. Ecol.*, 4, 185–219.

Vander Wall, S.B. (2003). Effects of seed size of wind-dispersed pines (Pinus) on secondary seed dispersal and the caching behavior of rodents. *Oikos*, 100, 25–34.

Vander Wall, S.B. & Longland, W.S. (2004). Diplochory: are two seed dispersers better than one? *Trends Ecol. Evol.*, 19(3), 155–161.

Williams, J.W., Jackson, S.T. & Kutzbach, J.E. (2007). Projected distributions of novel and disappearing climates by 2100 AD. *Proc. Natl Acad. Sci. USA*, 104, 5738–5742.

Willson, M.F. & Traveset, A. (2000). The ecology of seed dispersal. In: *Seeds: the ecology of regeneration in plant communities* (ed. Fenner, M.). CABI Publishing, New York. Pp 85–109.

SUPPORTING INFORMATION

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com).