Sex allocation, pollen limitation and masting in whitebark pine

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Summary

1. Masting, the synchronous and episodic production of seed crops, is thought to benefit plant reproductive success through positive density-dependent effects on pollination, dispersal and seed survival. Of these, only increased pollination efficiency in mast years can be a proximate mechanism for masting by synchronizing reproductive effort across individuals through pollen coupling.

2. Increased pollination efficiency requires synchronous investment in male and female function during mast years. Sex allocation theory, however, predicts a trade-off in investment between male and female reproductive allocation dependent on total resources invested in reproduction.

3. We describe patterns of sex allocation in *Pinus albicaulis* (whitebark pine), using data on pollen and seed cone counts over 5 years for 29 trees across 7 sites in Montana, U.S.A.

4. Whitebark pine seed cone maturation increased with site pollen cone production, indicating pollen limitation, and pollen and seed cone production were positively correlated across years.

5. Simulating mature seed cone production from these empirical relationships resulted in greater average mature seed cone production than alternative scenarios of (i) no synchrony between pollen cone production and pollen cone initiation, (ii) negative correlation (trade-off) between seed cone initiation and pollen cone production or (iii) no masting.

6. *Synthesis*. Our data support a role for pollination efficiency in both increasing long-term seed production and as a proximate mechanism for synchronizing masting in *Pinus albicaulis*. Increased pollination efficiency joins greater seed dispersal and survival in mast years seen in other studies, as an additional positive density-dependent benefit of masting. Positive density-dependent fitness benefits may therefore influence patterns of sex allocation in relation to total resources invested in reproduction. The pollen limitation found here combined with stand isolation and reduced tree density due to mortality from forest pests and other environmental stressors may lead to reduced seed cone maturation and changes in masting patterns.

Key-words: mast seeding, *Pinus albicaulis*, pollen coupling, pollination efficiency, reproductive ecology, reproductive synchrony, seed production, sex ratio

Introduction

Mast seeding is a phenomenon in which individual plants produce seeds synchronously at super-annual intervals, leading to wide fluctuations in seed production at the population level. In general, evolutionary ecologists have hypothesized that mast seeding occurs because synchronous reproduction increases reproductive success through positive density dependence, such as increased pollination, increased dispersal and/or reduced seed predation in mast years (Silvertown 1980; Kelly 1994; Kelly & Sork 2002). Of these three, increased

pollination in mast years is especially intriguing because it can be a proximate mechanism for masting (Isagi *et al.* 1997; Satake & Iwasa 2000), in addition to improving reproductive success. In brief, this proximate mechanism, known as the 'resource budget' model (Isagi *et al.* 1997), is based on the assumption that seed production depletes stored resources, so that individual plants cannot set seed in successive years. If plants that flower asynchronously are not pollinated, they would not set seed or deplete stored resources, and they would continue to flower until the next mast year, when they would be pollinated, produce seeds, deplete resources and become synchronous with other individuals in the population. This mechanism is hypothesized to be especially important for wind-pollinated species (Kelly, Hart & Allen 2001),

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but has also been demonstrated in a bee-pollinated perennial herb (Crone, Polansky & Lesica 2005; Crone, Miller & Sala 2009).

This hypothesis depends on the assumption that male and female gametes are produced in the same years, that is, years in which plants produce more female flowers are also those in which they produce more pollen (Smith, Hamrick & Kramer 1990). However, many conspicuously mast-seeding plants, including oaks, beeches and pines, are monoecious. In other words, single plants produce separate male and female flowers. There are at least some reasons to believe that a plant's resource status would affect its relative allocation to male vs. female function, as well as its total allocation to reproduction. Sex allocation theory (Charnov 1982; Klinkhamer, deJong & Metz 1997) has a long history of evaluating the effect of resource status on relative allocation to male vs. female function, but this body of theory has not been well integrated with studies of mast seeding. For species in which pollen production is not correlated with female gamete production, mast seeding is unlikely to increase pollination efficiency, and the resource budget model is not a plausible proximate mechanism of synchrony. At the same time, most sex allocation theory assumes the resource status of plants is uncorrelated with that of neighbouring plants, which would not be true if stored resources fluctuate synchronously among individuals, in concert with mast seeding.

Sex allocation models are based on gain curves that relate investment in male or female function to the gain in fitness realized by the organism (Charnov 1982; Klinkhamer, deJong & Metz 1997; Campbell 2000; Zhang 2006). Gain curves are typically described as power functions, with the value of the exponent determining the shape of the curve (Klinkhamer, deJong & Metz 1997). When both male and female gain curves are linear and parallel (exponent = 1), sex allocation is independent of absolute investment in reproduction, similar to the assumptions of the resource budget model of mast seeding. If the exponents of the gain curves differ, sex allocation is expected to change with absolute investment, with relative investment becoming greater in the sex with the larger exponent. For example, animal pollinated plants have a decelerating gain curve for male function because pollination becomes less effective with increasing numbers of flowers on an individual plant (Dejong & Klinkhamer 1989). For wind-pollinated plants, the male gain curve is usually considered linear because air does not saturate with pollen (Burd & Allen 1988; Bickel & Freeman 1993; Friedman & Barrett 2009), but the female gain curve may be decelerating because of negative density dependence in seed or seedling survival (Janzen 1970; Connell 1971; Lloyd & Bawa 1984). This leads to sex allocation becoming more male with increasing overall investment in reproduction. However, because masting species are hypothesized to benefit from positive density dependence of pollination, seed dispersal and seed survival (Kelly 1994), female fitness gains may not be a decelerating function of allocation (Masaka & Takada 2006). Finally, female function might bear a higher cost of reproduction than male function. Some organisms switch entirely from male to female

function as more resources are available for reproduction (Bierzychudek 1984), directly in contrast to the assumptions of the resource budget model.

In this study, we explore patterns of sex allocation in whitebark pine (*Pinus albicaulis*), a wind-pollinated, masting tree species of high-elevation ecosystems in western North America. We address five questions about the role of sex allocation in mast seeding: (i) Do individual trees tend to be consistently more male or female? (ii) Are years in which trees produce more female cones also years in which they produce more male cones? (iii) Is male and female cone production synchronous at the site level? (iv) Is female cone maturation higher in high male cone years? (v) Is among-tree synchrony of cone production greater for matured than initiated seed cones? Finally, we use the empirically estimated relationships determined by answering these questions to explore whether reproductive synchrony increases total seed production in this species, via increased pollination efficiency.

Materials and methods

STUDY SPECIES

Pinus albicaulis Englem. (whitebark pine) is a long-lived, stress-tolerant tree found in high-elevation forests in western North America. It often forms monospecific stands near the tree line but can be found in mixed stands with other conifer species at lower elevations. Large cone crops are produced by the species at irregular intervals. Clark's nutcracker (*Nucifraga columbiana*) is the dominant seed disperser (Hutchins & Lanner 1982; Lanner 1982; Tomback 1982), and *P. albicaulis* cones are also an important resource for many other wildlife species, including red squirrels (*Tamiasciurus hudsonicus*) and grizzly bears (*Ursos arctos*). *P. albicaulis* was recently added to the list of species under consideration for endangered species protection in the United States (US Fish and Wildlife Service 2011) because it is threatened by an introduced pathogen, white pine blister rust (*Cronartium ribicola*) and a native insect pest, mountain pine beetle (*Dendroctonus ponderosae*).

P. albicaulis trees are capable of initiating cone production every year, and while seed cones are pollinated in the first year of development, they take two full summers to mature. Cones leave visible scars on branches after dispersal, which remain for many years and can be used to estimate cone production through time (Morgan & Bunting 1992). Trees produce new needles every year, but old needles remain on the tree for 5–8 years (Richardson & Rundel 1998). Between the needles and growth constrictions that indicate where twig growth ceased each year, pollen cone scars are visible on pollen cone-producing branches, which can be distinguished for at least as long as the needles remain on the twigs.

FIELD METHODS

In 2011, we visited 7 *P. albicaulis* stands in western Montana; these are a subset of the 36 stands described by Crone, McIntire & Brodie (2011). Across these sites, we evaluated seed and pollen cone production for 29 trees (3–7 trees per site). In *P. albicaulis*, seed cones are produced on thick upright branches in the upper crown, while pollen cones are produced in clusters on smaller spur branchlets on branches originating from lower portions of the crown. We counted the number

of seed cone-bearing branches on each tree and sampled 3–5 (mean = 4.2) of these branches per tree. Seed cone production over time was estimated by counting the number of cone scars between each annual growth constriction (Morgan & Bunting 1992). We scored whether the cone scar was full or partial. Full cone scars were larger, indicating a mature seed cone, while we interpreted partial scars as being where a seed cone was initiated but did not mature. Therefore, the total number of cone scars (full scars plus partial scars) is an estimate of the total number of seed cones initiated in each year, and the number of full cone scars is an estimate of the number of cones that reached maturation. We sampled 8–12 pollen cone-bearing branchlets per tree and counted the number of cone scars between each annual growth constriction. Dating and cone counts were performed by two independent workers, and comparisons showed strong repeatability of counts (Crone, McIntire & Brodie 2011). For each tree, we calculated the mean number of seed cones per branch and pollen cones per branchlet for each year we could reconstruct cone production history (9–22 years for seed cones; 3–9 years for pollen cones).

DATA ANALYSIS

We examined relationships between seed cone and pollen cone production over five years (2006–2010), within and across trees and sites. Specifically, we analysed the average number of seed cones per branch and pollen cones per branchlet (hereafter 'cones per branch' when referring to either) for each tree. We used cones per branch as a metric of year to year changes in reproductive output, because the number of reproductive branches would not have changed for this slow-growing tree over the relatively short (5–8 years) period of our study. Because the observed variation in cones per branch among trees and years is a function of the true variation plus sampling error (variation in the sampled mean cones per branch), we calculated the standard error of the mean cone production for each individual tree from the raw counts (within-tree variation) and compared these with the standard deviations of the mean cones per branch in each tree among years (among-year variation) and of each year among trees (among-tree variation) prior to further analyses. For pollen cones, seed cones initiated, and seed cones matured, the mean within-tree variation was roughly half the magnitude of the among-year variation and the among-tree variation (Table S1), indicating it was possible to distinguish differences in cone production among trees and years. In the presence of variation in cone production between branches on the same tree, analysing mean cones per branch should make our tests more conservative, because there is no reason to expect the branches collected were biased. We also verified that neither absolute reproductive output per branch nor sex allocation depended on tree size (see Appendix S1 in Supporting Information), so we did not include basal area in these analyses.

We tested four relationships between seed cone and pollen cone production, described below with numbering matching the questions posited at the end of the introduction. (i) We analysed whether the number of pollen cones was associated with seed cones initiated for each tree averaged over years, including site and the interaction between site and seed cones initiated as random effects. We then analysed the number of pollen cones as a function of seed cones initiated across years both (ii) within individuals (data: mean cones per branch for each tree in each year) and (iii) within sites (data: mean cones per branch for each site in each year). Both analyses included site and the interaction between site and seed cones initiated as random effects, and the first also included random effects for tree and

the interaction between tree and seed cones initiated. A Gaussian distribution with a log link was used in all three analyses (log-normal model) because the response was the mean of several counts. Finally, we analysed (iv) seed cone maturation rate for each tree as a function of the site-mean number of pollen cones per branch for each year with pollen cones as a fixed effect, and tree, tree × pollen cones, site and site × pollen cones as random effects. A binomial distribution with a logit link was used in this final analysis.

Analyses were carried out using generalized linear mixed effects models (GLMMs) implemented with the lme4 package version 0.999375-42/r1414 (Bates, Maechler & Bolker 2011) in R version 2.14.0 (R Development Core Team 2012). We rescaled predictor variables by subtracting the mean from each observation and dividing by two times the standard deviation to improve model convergence (Gelman 2008). We used two types of random effects: (i) we included tree as a structural random effect to avoid pseudo-replication and account for the time-series nature of the data; and (ii) we included site-level effects (random intercept, random slope or both) because we hypothesized that cone production and pollination might be influenced by unmeasured site characteristics. We did not assess the significance of the first type of random effect because it was a required part of the model. Because we were interested in evaluating whether the response differed among sites, and to account for site if it did, we evaluated the second type of random effect using likelihood ratio tests, and non-significant effects were removed from the final model.

Likelihood ratio tests are not recommended for use with fixed effects in mixed effects models (Pinheiro & Bates 2000; Bolker *et al.* 2009), so we evaluated fixed effects using the approach recommended by Gelman & Hill (2007). Using the R package arm version 1.4/14 (Gelman *et al.* 2011) with code modified from Bagchi *et al.* (2011), we simulated the posterior distribution 10000 times to calculate 95% confidence intervals and approximate *P*-values for the fixed effects. In cases where the full model included only site as a random effect and both intercept and slope effects were non-significant at $\alpha = 0.05$, we analysed the fixed effects using generalized linear models (GLMs) and tested for significance using Wald statistics. Summary tables of the final (reduced) model parameterizations (Table S2) and full results (Table S3) of all analyses are included as a supplement.

Finally, (v) we calculated synchrony of cones initiated and matured between individuals within sites as $\bar{r}$, the mean Pearson correlation of cone production between individuals in each population (Buonaccorsi *et al.* 2003). We calculated jackknifed confidence intervals for each site by sequentially removing one observation and recalculating $\bar{r}$.

EMPIRICAL MODELS OF SEED CONE PRODUCTION

We used the fitted tree- and year-specific regression coefficients from the above GLMMs to evaluate whether the observed patterns of pollen limitation and correlated production of pollen and seed cones would lead to increased overall seed cone production. First, we calculated the predicted number of pollen cones per branch by tree for each year in the period 2003–2010 as:

$$\text{cones}_{\male,i,j} = e^{(A_{2,i}+\beta_{2,i}*\text{cones}_{\female,\text{init},i,j})}, \qquad \text{eqn 1}$$

where $\text{cones}_{\female,\text{init},i,j}$ is the average number of seed cones initiated each year by tree i in year j, and $A_{2,i}$ and $\beta_{2,i}$ are the tree i specific intercept and slope from analysis (ii) above. We took the mean predicted pollen cones of all the trees at each site in each year to calculate the predicted site average pollen cones per branch for each year. We then

calculated the predicted number of mature seed cones per branch by tree for each year in the period 2003–2010 as:

$$\text{cones}_{\female,\text{mat},i,j} = \text{cones}_{\female,\text{init},i,j} \times \frac{e^{(A_{4,i} + \beta_{4,i}*\text{cones}_{\male,k,j})}}{1 + e^{(A_{4,i} + \beta_{4,i}*\text{cones}_{\male,k,j})}}, \qquad \text{eqn 2}$$

where $\text{cones}_{\male,k,j}$ is the average number of pollen cones per branch produced at site k in year j, where tree i exists, and $A_{4,i}$ and $\beta_{4,i}$ are the tree i specific intercept and slope from analysis (iv) above. We calculated the average annual expected cone production from 2003–2010 by averaging the expected number of mature seed cones over all trees and all years. We calculated confidence limits for expected cone production by parametric bootstrapping, implemented using the sim() function in R.

To explore the impact of synchrony on long-term seed production, we compared the expected production of mature seed cones given the observed relationships to three alternative scenarios of pollen cone production: (i) no correlation between pollen cones and seed cones initiated per site, implemented by calculating the expected number of seed cones each year, given the number of initiated seed cones and average pollen production in every year:

$$\text{cones}_{\male,i,j} = e^{(A_{2,i})} \qquad \text{eqn 3}$$

(ii) negative correlation between pollen cones and seed cones initiated per site, implemented by replacing the observed positive relationship between pollen and seed cones initiated (see *Results*) with a symmetrical negative relationship:

$$\text{cones}_{\male,i,j} = e^{(A_{2,i} - \beta 2,i*\text{cones}_{\female,\text{init},i,j})}, \qquad \text{eqn 4}$$

and (iii) average seed cone initiation in each year (no masting):

$$\text{cones}_{\male,i} = e^{(A_{2,i} + \beta 2,i*\text{cones}_{\female,\text{init},i})}, \qquad \text{eqn 5}$$

where $\text{cones}_{\female,\text{init},i}$ is the average number of seed cones initiated across years for each individual i. For each scenario, we calculated seed cone maturation as in eqn 2 above. We calculated confidence limits for expected cone production in each of these scenarios using parametric bootstrapping.

Results

Individual trees differed in sex allocation, with some tending to be more male and others more female; across individuals, the average number of seed cones initiated was negatively associated with average number of pollen cones produced [Fig. 1a; parameter estimate for the effect of the average seed cones initiated per branch on the average pollen cones per branch $\beta_{\text{tree}} = -0.34$, 95% CI = −0.54 to −0.14, $P = 0.0026$; this answers question (i)]. Within individuals, however, the numbers of initiated seed cones and pollen cones were positively associated through time, indicating that relative sex allocation by individuals does not differ substantially between years in which they produced many or few cones [$\beta_{\text{tree, year}} = 0.22$, 95% CI = 0.09 to 0.34, $P = 0.0004$; this answers question (ii)].
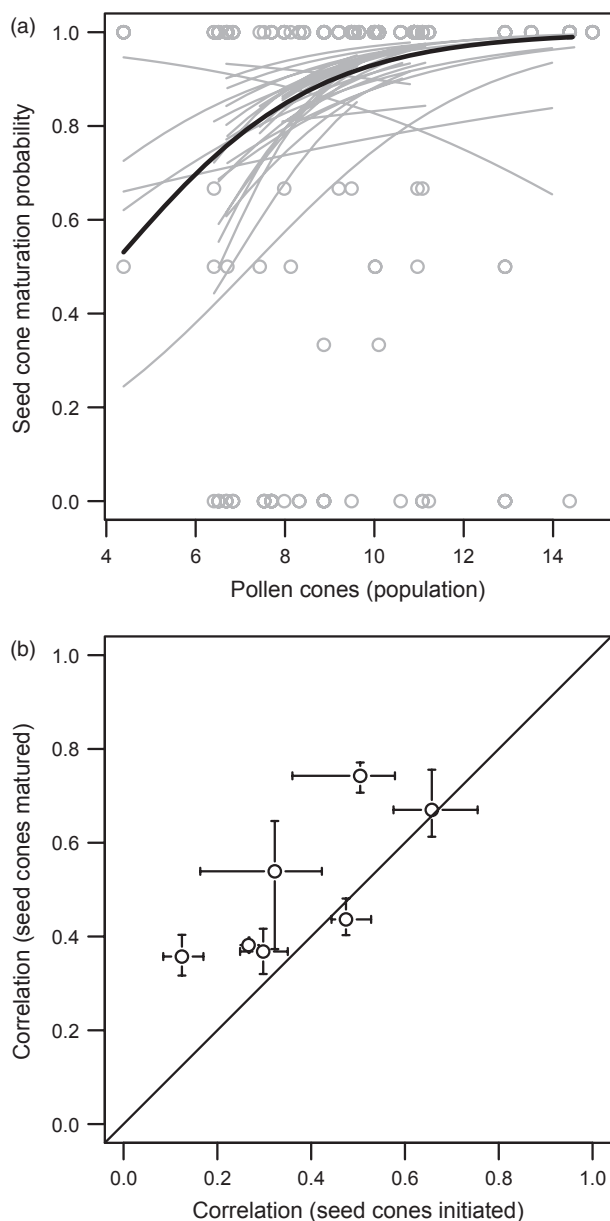
Seed cone initiation was synchronous among trees within populations ($\bar{r} = 0.38$; SE = 0.18; range = 0.12 to 0.66). The positive relationship within individuals between the numbers of initiated seed cones and pollen cones, coupled with the synchrony of cone production among individuals, meant initiated seed cones and pollen cones were also positively associ-
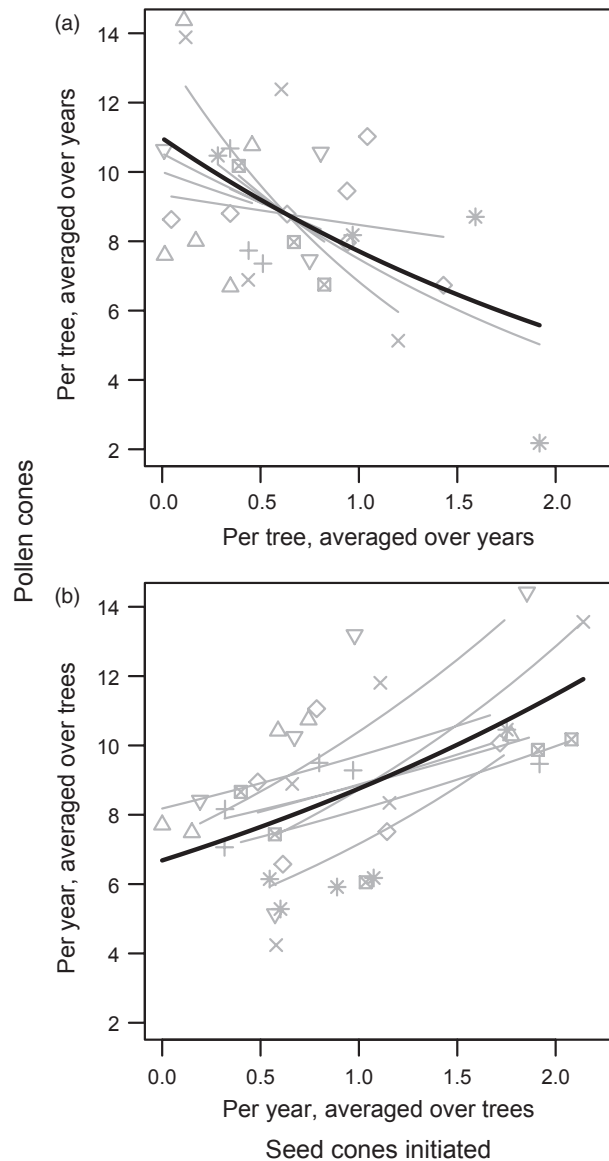
Fig. 1. Pollen cone production as a function of seed cone initiation for (a) individual trees averaged over years; and (b) among years averaged over trees. Grey lines show relationships for individual sites, while the black lines show relationships across sites; each point represents a different tree, while symbols indicate different sites.

ated at the site level [Fig. 1b; $\beta_{\text{site,year}} = 0.32$, 95% CI = 0.18 to 0.46, $P < 0.0001$; this answers question (iii)]. Seed cone maturation of individual trees increased with pollen availability, consistent with the idea of density-dependent pollen limitation [Fig. 2a; $\beta_{\text{maturation}} = 2.02$, 95% CI = 1.08 to 2.94, $P < 0.0001$; this answers question (iv)]. Therefore, synchrony of the number of mature seed cones was higher than that of initiated seed cones [Fig. 2b; this answers question (v)].

We used the empirical relationships described above to model average number of mature cones produced across all trees during the study period. Predictions from these models matched observations of mature cones (Fig. 3). These empirical relationships led to production of more mature seed cones than hypothetical alternative scenarios of no masting, no syn-
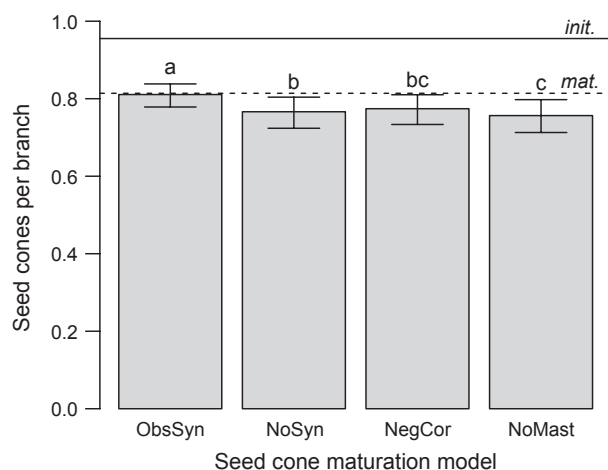
Fig. 3. Results of scenario modelling. Predicted number of mature seed cones produced per branch per year over the eight-year period for each of the modelling scenarios (ObsSyn – observed synchrony of pollen and seed cone initiation; NoSyn – constant average pollen cone production by each tree in each year; NegCor – negative correlation between pollen and seed cone initiation; NoMast – average seed cone initiation for each tree in each year). Error bars depict boot-strapped 95% confidence intervals of the means; bars with the same letter above them were not statistically different at alpha = 0.05. Horizontal lines show the observed mean number of seed cones initiated and matured.

(Isagi *et al.* 1997; Satake & Iwasa 2000), as a mechanism of synchronous mast seeding, and decreased pollen limitation as a fitness benefit of mast seeding. While sex allocation varied among individuals (Fig. 1a), pollen and seed cones were positively associated within individuals over years (Fig. 1b). This led to greater pollen production in mast years, improved pollination efficiency (Fig. 2a) and greater among-tree synchrony in seed cone maturation (Fig. 2b). Synchrony in the production of pollen and seed cones increased the mature seed cone crop in comparison with scenarios of no synchrony, a negative correlation between pollen and seed cone production, or constant reproductive effort (Fig. 3). Enhanced pollination is not the only benefit of mast seeding in *P. albicaulis*; mast years enhance seed dispersal by the tree's avian mutualist, Clark's nutcracker (McKinney, Fiedler & Tomback 2009; Barringer *et al.* 2012) and increase seed survival (Siepielski & Benkman 2007; McKinney, Fiedler & Tomback 2009) in mast years. However, unlike the previously demonstrated advantages of masting in *P. albicaulis*, pollen coupling may also be a physiological mechanism for enhancing synchrony in *P. albicaulis*.

These results have implications for research on both masting and sex allocation. Theories from both areas of research make predictions about how individual plants should allocate resources to reproduction, but these predictions are contradictory. The pollen coupling hypothesis for synchronizing masting depends on investment in male and female reproductive function being positively correlated across years and changing resource status (Smith, Hamrick & Kramer 1990), while sex allocation theory predicts that sex expression should change in response to resource availability to maximize fitness (Charnov 1982).

Fig. 2. (a) Seed cone maturation rate as a function of the site average mean pollen cones per branch. Grey points show values for individual trees in each year; grey lines show the modelled relationship for each tree; thick black line shows the overall modelled relationship across trees. (b) Mean pairwise correlations between trees within each site for seed cones initiated and matured. Bars show the range of jack-knifed values for each.

chrony or negative correlations (Fig. 3). The no masting scenario produced the fewest mature seed cones, but only slightly fewer than the no synchrony scenario (*P* = 0.048) and statistically the same as the negative correlation scenario (*P* = 0.202).

Discussion

In *P. albicaulis*, patterns of seed and pollen cone production support the resource budget and pollen coupling hypothesis

In many plant species, sex expression varies within individuals in relation to resource status, although such variation is not ubiquitous (reviewed in Korpelainen 1998). For masting species, if seed dispersal and survival are higher in mast years, fitness is an accelerating function of investment in female reproductive function (Charnov 1979). Male fitness gains with increasing reproductive investment are expected to be linear in wind-pollinated species (Charnov 1979; Burd & Allen 1988). Therefore, trees should become more female in mast years, with investment in female function increasing at a greater rate than investment in male function compared with non-mast years. This tendency would be even stronger if there was a trade-off between male and female allocation such that higher female allocation in mast years led to decreasing male function. In *P. albicaulis,* however, pollen and seed cones were positively correlated across mast and non-mast years (Fig. 1b). This occurred even though mast years deplete stored phosphorous and nitrogen in *P. albicaulis* (Sala *et al.* 2012), consistent with the idea that mobile resources available for reproduction fluctuate through time in masting species (Isagi *et al.* 1997). Our results suggest that increased reproductive success due to pollen coupling could outweigh benefits of changing resource allocation to male and female function in response to changing resource availability in masting species, although this remains to be tested for other species.

In contrast to consistent sex expression within individuals over time, average sex allocation varied among individuals in *P. albicaulis* (Fig. 1a). A similar negative relationship between male and female reproductive allocation among individuals in three species of masting California oaks was linked to the proportion of resources invested in female reproductive function being positively correlated with average annual above-ground net productivity (Knops & Koenig 2012). Instead of responding to annual variation in resource availability, a tree's sex allocation could reflect long-term average available resources. This could explain why sex allocation is often related to plant size (Klinkhamer, deJong & Metz 1997), because larger individuals will have higher productivity, other factors being equal.

In addition to a consistent sex allocation within individuals, synchronous flowering across individuals in a population and pollen limitation are necessary requirements for pollination efficiency to drive masting. Site-level interannual variation in pollen production is high in several genera of temperate trees (Geburek *et al.* 2012), suggesting synchrony in pollen production within populations of trees. Likewise, pollen limitation is widely observed, with self-incompatible species, woody plants and tropical plants thought to be more pollen-limited than self-compatible, herbaceous and temperate ones (reviewed in Ghazoul 2005). Density-dependent pollen limitation has also been shown in several temperate wind-pollinated species, both herbaceous and woody (e.g. Knapp, Goedde & Rice 2001; Davis *et al.* 2004; Hesse & Pannell 2011; Koenig *et al.* 2012), in addition to this current study (Fig. 2a). Density-dependent pollen limitation therefore seems common and likely to favour mast seeding. Our results demonstrate this relationship quantitatively.

Pollen coupling enhances synchrony of seed production when seed maturation increases in high pollen production years (Fig. 2). While pollen coupling can synchronize masting in the absence of external forcing, environmental variation can also lead to synchrony through the 'Moran effect' (Royama 1992; Liebhold, Koenig & Bjornstad 2004). The environment can influence reproductive synchrony at several stages of seed production, including flower initiation, pollination and seed maturation. For instance, weather cues trigger multi-species mass flowering events in South-East Asia (Sakai *et al.* 2006). Environmental variability affects pollination in California oaks in that pollen limitation in individual trees is determined by how well they track the population mean budburst date, selecting for trees to flower synchronously within years (Koenig *et al.* 2012). In stressful environments, environmental conditions can influence seed maturation, synchronizing seed crops after pollination occurs (Fernandez-Martinez, Belmonte & Maria Espelta 2012; Montesinos, Garcia-Fayos & Verdu 2012). The relative importance of pollen coupling and environmental variation in synchronizing masting is probably dependent on both biological factors such as population size and pollination system, and the environmental factors, both average conditions and extreme events, that affect resource availability during reproduction.

Consistent sex allocation within individuals and pollen limitation contribute to higher long-term reproduction under masting in *P. albicaulis*. The role of pollen limitation is especially intriguing because this species is in decline. Throughout its range, stands are being impacted by blister rust and pine beetles (Zeglen 2002; Smith *et al.* 2008; Jewett *et al.* 2011; Larson 2011; Millar *et al.* 2012). These pests isolate remnant stands and create lower tree densities within stands. Such changes could affect cone production qualitatively [by changing patterns of masting (i.e. Crone, McIntire & Brodie 2011)] as well as quantitatively (by reducing pollination of female cones). Bark beetles are epidemic on several conifer species in western North America (Meddens, Hicke & Ferguson 2012), and globally high plant mortality is linked to biotic attack and climate stressors (McDowell *et al.* 2011). High tree mortality reduces stand density and isolates populations, while fragmented populations experience increased pollination limitation (O'Connell, Mosseler & Rajora 2006) and reduced gene flow (Jump & Penuelas 2006; Provan *et al.* 2008). Masting is a common plant reproductive strategy, especially among wind-pollinated trees (Herrera *et al.* 1998). Therefore, changes in pollen limitation and subsequent seed production may decrease viability of populations that are fragmented, experience pests or diseases, or are affected by environmental changes in general.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Size-dependent sex allocation.

Figure S1. Proportion of male cones out of total cones (seed cones plus pollen cones) per tree as a function of tree basal area.

Table S1. Sources of variation in numbers of cones per branch.

Table S2. Final model parameterizations of GLMMs.

Table S3. Full results of GLMMs.