Geographical variation in cone traits of *Pinus nigra* as result of crossbill predation: implications for assisted migration

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The interaction between Spanish Black pine (*Pinus nigra* subsp. *salzmannii*) and common crossbill (*Loxia curvirostra*) could have resulted in a geographic mosaic of coevolution in the Iberian Peninsula. In turn, this coevolutionary interaction might limit the future applicability of assisted migration as a tool to adapt forests of Spanish Black pine to ongoing climate change. Several cone and seed traits were measured in 740 cones collected at six stands distributed across the range of Spanish Black pine. Data were analysed by means of linear-mixed effects models, canonical analysis of principal coordinates and random-forest analysis. Results showed that cones from the northern pine populations exhibited more defensive traits against crossbill predation than cones from the central and southern populations, resulting in a geographic mosaic of selection. Assisted migration of *P. nigra* would involve moving southern populations toward the north of the species' range and would result in introducing the genotypes of pines with poorly defended cones into areas where crossbills are comparatively more abundant. The resulting risks for the regeneration prospects of the translocated trees are discussed.

Introduction

Increased temperatures and altered rainfall patterns resulting from ongoing climate change are expected to reduce suitable forest habitat of many tree species (Dyderski *et al.*, 2018). Other climate changes have occurred in the past, forcing the migration of tree species upwards or polewards as they tracked the climate to which they were adapted (Davis and Shaw, 2001). However, natural migration in response to current climate change is unlikely to occur, because expected climate change will be more rapid than trees are able to disperse. Additionally, natural migration would be almost impossible because of forest fragmentation and existing barriers to seed dispersal (Aitken *et al.*, 2008). Considering these limitations, assisted migration has been proposed as an operational tool to adapt forests to climate change (Pedlar *et al.*, 2012; Benito-Garzón *et al.*, 2013).

Assisted migration is the intentional movement of propagules, individuals, or populations to compensate for observed or future climate change (Benito-Garzón *et al.*, 2013). Although such artificial movement of plant genotypes would be justified because of the unprecedented rate of current climate change, there are a number of limitations or the potential for failure of poorly conceived assisted migration. As an example, interspecific interactions often are not considered when contemplating the assisted migration of a single species (Ricciardi and Simberloff, 2009).

Pre-dispersal seed predation is an interspecific interaction that is particularly important for the evolution of traits in plant species. The plant-seed predator interaction frequently shows considerable variation in space and time, resulting in a shifting mosaic of interactions (Kolb *et al.*, 2007). A good example of such a mosaic is that of crossbills and conifers. Crossbills are songbirds (*Fringillidae*) that have evolved crossed bills to separate the cone scales of conifer trees and feed on the underlying seeds. In turn, conifers have evolved better defended cones, resulting in a coevolutionary arms race (Parchman and Benkman, 2008). However, traits and their respective counter-traits do not frequently coevolve as a unit, because of geographical differences in the intensity of the coevolving interaction. This results in geographic mosaics of selection (Thompson, 2005; Benkman and Parchman, 2009; Edelaar *et al.*, 2012).

Here, I examine the probable existence of such a geographic mosaic in the interaction between Spanish Black pine (*Pinus nigra* subsp. *salzmannii*: *P. nigra* hereafter) and common crossbill (*Loxia curvirostra*) inhabiting the Iberian Peninsula, and discuss its implications for the assisted migration of the pine species. Two geographic mosaics of coevolution involving common crossbill and *P. nigra*, subspecies *laricio* and *pallasiana*, in the Mediterranean region have already been described (Benkman and Parchman, 2009, 2013). Based on results reported in these two previous studies, I predicted that cones of *P. nigra* should be comparatively larger (longer, wider and heavier) and attain thicker, more numerous scales, heavier seeds and more numerous empty seeds in the northern forests of the species distribution area, where the *P. nigra*-crossbill interaction would have been stronger over evolutionary time. This circumstance could have generated a

geographic mosaic of selection involving *P. nigra* and common crossbill.

I address two major questions in this study. First, I test for the existence of variability in cone traits among *P. nigra* populations across the species distribution. I assume that geographical variability in cone traits should be the prime and necessary evidence for describing a pine-crossbill geographic mosaic of selection. Second, I investigate the traits selected by common crossbills foraging in the southern forests of *P. nigra*. Crossbills forage in the same stereotypic manner everywhere (Benkman and Mezquida, 2015). Nevertheless, I introduced this question to better support my prediction that, compared with northern cones, southern cones would attain less defenses, because predation rates have been historically lower and not because crossbills forage differently (Mezquida and Benkman, 2010). Then, I discuss how these questions may influence decisions to implement assisted migration of *P. nigra*. Assisted migration of *P. nigra* would involve translocating southern populations, which are presumably better adapted to drier, warmer conditions, but potentially not as well defended against crossbill predation (Benito-Garzón *et al.*, 2008).

Material and methods

Long-term geographical differences in the P. nigra-*crossbill interaction*

My predictions assume that over evolutionary time the *P. nigra*-crossbill interaction has been stronger in the northern portion of the pine distribution. Common crossbill exhibits a Holarctic distribution, whereas *P. nigra* is native to southeast France, eastern Spain and northern Morocco. French and Moroccan populations cover no more than 4000 ha, but *P. nigra* is the dominant tree species in 544 286 ha of Spanish forests (Alejano and Martínez, 1996).

In Spain, *P. nigra* frequently occurs in the vicinity of *Pinus uncinata*, *Pinus sylvestris* and *Pinus halepensis*, which are foraged by common crossbill as well. The size and stability of crossbill populations are largely determined in the Iberian Peninsula by the presence of the aforementioned pine species (Senar *et al.*, 1993; Borrás and Senar, 2003). Crossbills are notably abundant in the extensive *P. uncinata* and *P. sylvestris* pinewoods of the northeast, which provide good cone crops every year. Towards the south, crossbill abundance decreases as forests of *P. uncinata* disappear and the areas occupied by *P. sylvestris* become progressively smaller. *Pinus halepensis* thus become the main pine species occurring in the proximity of the southern forests of *P. nigra* (Figure 1). Forests of *P. halepensis* yield cone crops annually, although most cones may be too large to be handled by crossbills (Mezquida and Benkman, 2005). As a result, these forests harbour crossbill populations that are comparatively smaller than those found in the *P. uncinata* and *P. sylvestris* pinewoods (Borrás and Senar, 2003; Mezquida and Benkman, 2010). For their part, forests of *P. nigra* produce good cone crops irregularly every few years and they are consequently foraged by fluctuating populations of mainly nomadic crossbills that may be large during mast-seeding years only (Senar *et al.*, 1993; Borrás and Senar, 2003). However, as crossbill abundance decreases north-to-south along the *P. nigra* range, the probability of being foraged by nomadic crossbills during mast-seeding years is higher for the northern than for the southern forests of this pine species (Borrás *et al.*, 2008).

Paleodistribution studies have shown that the Iberian Peninsula acted as a refuge for *P. uncinata*, *P. sylvestris* and *P. nigra* during the last glacial maximum (21 000 years BP). In contrast, *P. halepensis* appears to be a recent addition to the Iberian flora that entered the Peninsula from the northeast after the last glaciation (for a review see Rodríguez-Sánchez *et al.*, 2010). Moreover, the large area currently occupied by *P. halepensis* (Figure 1) is mainly due to recent reforestation work carried out at the expense of the old domains of broadleaf tree species (Pausas *et al.*, 2004). Consequently, the possibility that southern forests of *P. nigra* could be invaded by nomadic flocks of crossbill coming from nearby forests of *P. halepensis* is recent in the evolutionary environment of *P. nigra* (Benkman and Mezquida, 2015).

Morphological and genetic studies indicate that crossbill populations preferably forage the pine species for which they might be specialized (Borrás *et al.*, 2008; Arizaga *et al.*, 2015). Foraging specialization is associated with differences in bill shape. Thus, crossbills foraging cones of *P. halepensis* attain longer and narrower bills than crossbills foraging cones of *P. sylvestris* and *P. uncinata* (Edelaar *et al.*, 2012). The cones of these latter species are smaller than those of *P. halepensis*, being similar in size to those produced by *P. nigra* (Ruiz de la Torre, 2006). Crossbills specialized in *P. sylvestris* and *P. uncinata* could then forage in *P. nigra* forests more readily than crossbills specialized in *P. halepensis*.

Geographic seed and cone variation

P. nigra inhabits the calcareous mid- to high-altitude eastern mountains of Spain. Its range is consequently fragmented, forming extensive forests separated by unsuitable lowland terrain at the northern, central and southern areas of its range. I analysed the geographic variation of cone and seed traits among the three most extensive natural provenances found along the north-to-south *P. nigra* distribution (Figure 1). Specifically, *Pre-pirineo Catalán* (northern forest hereafter), *Sistema Ibérico Meridonal* (central forest hereafter) and *Cordilleras Béticas* (southern forest hereafter) (Alía *et al.*, 2009). Gene flow between these three forests seems to have remained interrupted for more than 30 000 years (Afzal-Rafii and Dodd, 2007).

In December 2010, cones were collected in two sites of the northern forest: *Sant Esteve* (01° 03′E, 42° 07′N; 650 m a.s.l.) and *Serra Seca* (01° 33′E, 42° 00′N; 1200 m a.s.l.), two sites of the central forest: *Dehesa de*
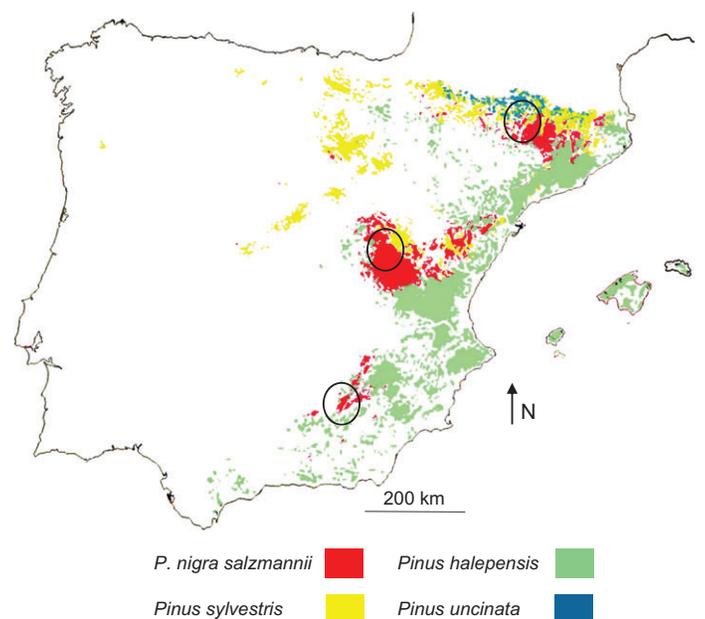

Figure 1 Distribution in Spain of the four pine species on which crossbills feed. Circles indicate the location of the northern, central and southern study forests.

Fuentelespino (02° 03′W, 39° 54′N; 1100 m a.s.l.) and *La Fuenseca* (01° 47′W, 40° 20′N; 1630 m a.s.l.), and two sites of the southern forest: *Las Navillas* (2° 53′W–37° 56′N, 1220 m a.s.l) and *Sierra del Pozo* (02° 57′W, 37° 48′N; 1850 m a.s.l.). Specifically, 10 mature cones were arbitrarily collected from 10 randomly selected trees (diameter at breast height, DBH ≥ 18 cm) at each of the first four aforementioned sites, while 6 and 28 trees were selected in *Las Navillas* and *Sierra del Pozo* sites, respectively. This design allowed me both to sample as many or more trees than those sampled in the previous research on which I built my predictions (Benkman and Parchman, 2009, 2013) and to account for potential within-tree variability of cone traits (Table A1). Diameters of the sampled trees ranged from 18 to 114 cm and mean diameters differed between forests (mean ± SE: 62.3 ± 4.2 > 36.1 ± 2.6 = 29.9 ± 1.6 cm for the southern, central and northern forest, respectively; $P < 0.001$ post hoc comparisons using the unequal Tukey HSD test).

Cones were picked from around the pine crowns, using a 7-m long pruning tool. I measured the following traits for each cone: maximum length and maximum width of the closed cone, number of scales crossed by a transect along the central axis of the cone, cone mass without seeds, number of full seeds, number of empty seeds, total mass of the full seeds without their wings, and the thickness of two scales from the middle portion of the distal third and the proximal third of the cone. Length measurements were made to the nearest 0.01-mm with a caliper. Mass measurements were made to the nearest 0.1-mg with a digital scale. Mean seed mass was calculated by dividing total mass of the full seeds by the number of full seeds. Cones were oven-dried to constant weight before weighing them. Seeds were weighed fresh. Since gene flow is possible within forests but not among them, data from sites within the same forest were pooled together in the analysis.

Due to the hierarchical structure of the data, I tested for differences in seed and cone traits among forests, incorporating the factors tree and cone as random effects (cone nested within-tree) in linear mixed-effect models fitted with the function *lme* of the *nlme* library in R (R Development Core Team 2014). Residuals were examined to test the assumptions of normality and homogeneity of variances. Two traits: number of full and number of empty seeds, were log-transformed to meet these assumptions.

I also performed a canonical analysis of principal coordinates (CAP), based on Euclidean distances, to test the hypothesis of no significant differences in multivariate location among the northern, central and southern forests for the cone traits maximum length and width of the closed cone, number of scales, cone mass, and the thickness of two scales from the middle portion of the distal third and the proximal third of the cone. The number of empty seeds depends on the success of pollination (Tíscar and Linares, 2011) and is expected to vary across large spatial scales within the same year. Additionally, the heritability of cone traits can double that of seed traits in *P. nigra* (Sivacioglu and Ayan, 2010). For these reasons, I did not include seed traits in the CAP analysis. The first two principal coordinate axes from the CAP were used to draw an unconstrained metric multidimensional scaling plot of the variables included in the ordination. I used the Spearmen correlation coefficient to correlate the axis scores with the original measures; the variables with the highest absolute correlations will likely determine the differences among study populations. The analysis of principal coordinates was performed with the computer program CAP (Anderson, 2004), using data standardized to *z*-scores and 9999 permutations.

Abiotic factors potentially influencing seed and cone traits

In order to test whether climate variability is correlated with seed and cone traits, I computed tests of Kendall's tau between these traits and the first two principal components extracted from a PCA of 13 climatic parameters as predictors. Namely: annual, spring, summer, autumn, and winter mean temperature (°C); annual, spring, summer, autumn,

and winter precipitation (mm); mean temperature of the coldest month and mean temperature of the warmest month; and annual potential evapotranspiration. Climatic data were obtained from Ninyerola *et al.* (2005) for the six collection sites. A climatic gradient was expected across these sites because of their altitudinal and latitudinal differences. I did not include altitude as an abiotic factor, because it is correlated with temperature and rainfall in mountainous areas, or bedrock type, because it was limestone in the six study sites. Edaphic variables were not available.

Cone and seed traits selected by foraging crossbills

Cone and seed traits selected by foraging crossbills were examined in the 28 trees sampled from the *Sierra del Pozo* (southern forest). These trees were at least 3 m away from the nearest neighbour, thus, I avoided sampling trees that could show overlapping crowns.

I estimated crop size by regularly collecting all the cones found within four 1 × 1-m quadrats, placed at the cardinal points beneath the crown edge of the sampled trees. Cone development and seed ripening take nearly two years in *P. nigra*. Pollination occurs in spring and cones are fully developed in the second summer after pollination, but they do not open to release the mature seeds until the next winter or early spring. In the study site, crossbills feed on the second-year cones from mid-summer onwards until seed dispersal is complete. After seed dispersal, the open cones of *P. nigra* are naturally shed within a few months. Considering this phenology, quadrats were sampled from August 2010 to September 2011. A small number of cones predated by crossbills were collected in the quadrats during the summer of 2011, but they were not counted as they belonged to a different cohort of cones. Cones coming from different cohorts are easily differentiated in pine species. I used the overall number of cones collected in the quadrats until September 2011 as an estimation of crop size, i.e. the number of cones available to foraging crossbills in each study tree. Collected cones were classified in two groups: predated by crossbills and unpredated. Cones preyed on by crossbills were easy to enumerate because foraging crossbills distinctively shred and peel apart scales to access seeds. Phenotypic selection exerted by crossbills on a given conifer can be accurately determined from studies carried out at one site during a single year (Mezquida and Benkman, 2010).

I first explored relationships among data, computing a Spearman correlation matrix with seed and cone traits, and crop size as variables. Then, assuming tree fitness is proportional to per cent of cones not preyed on by crossbills, relative fitness at the predispersal stage was estimated for each tree by dividing the percentage of unpreyed cones by the population mean (Benkman and Parchman, 2009). A random-forest analysis (Breiman, 2001) was conducted to identify the seed and cone traits that were relevant to the estimated values of relative fitness. The more traditional parametric approaches provided poor model fit, and random-forest analysis is well-suited for regression analysis with small sample sizes (28 in this study) and a large number of predictor variables (7 in this study).

After removing highly correlated variables (Table A2), the random forest was fitted with the predictor variables crop size, mean seed mass, number of full and empty seeds, cone mass, and proximal and distal scale thickness (VIF scores were ≤ 3.07 for all the variables included). I conducted the analysis with the *randomForest* package in R. Random forest is an ensemble tree-based method that extends standard Classification and Regression Tree (CART) methods by creating a collection of classification trees (the forest). The classification uncertainty of each tree is assessed using randomly selected cases which are retained during its construction (the out-of-bag or OOB cases). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy when those variables are permuted. This decrease is averaged over all trees to produce the final measure of importance.

Based on stability of the 'out-of-bag' error rate, 700 trees were assessed to be sufficient. The number of input variables randomly chosen at each split was set to two (the total number of predictor variables divided by three) (Cutler *et al.*, 2007). The significance of the importance measures was assessed using the *rfPermute* library in R.

Results

Geographic seed and cone variation

All seed and cone traits differed among the three study forests. Cones collected at the northern forest attained the greatest defenses against crossbills, since they were the heaviest, the longest and exhibited the most numerous scales. Additionally, northern cones were wider than cones from the central forest and attained thicker scales than cones collected at the southern forest. Northern cones also attained more empty seeds, although their mean seed mass was not the greatest as predicted (Table 1). No individual seed or cone trait was correlated with DBH (−0.220 < Spearman rho < 0.176, $n = 74$, $P > 0.05$ in all cases), indicating that geographical differences in seed and cone traits were unlikely to be the result of ontogenetic changes during tree growth (it must be remembered that mean DBH was significantly higher for trees sampled in the southern forest).

CAP analysis detected differences among the three study forests (trace statistic = 0.279, first squared canonical correlation = 0.226; *P*-value = 0.0001 for both tests). The first two PCoA axes from CAP explained 71.74 per cent of the variation observed in the data. All the variables considered were correlated with the two PCoA axes. Thus, the first axis of this ordination showed a strong negative correlation with cone length, cone width and cone mass, while the second axis showed a moderate negative correlation with distal and proximal scale thickness. These results placed larger cones at the left side of the graph and cones with the thickest scales at the bottom (Figure 2).

Relationships between abiotic factors and seed and cone traits

The first principal component (PC1) extracted from the PCA of 13 climatic parameters accounted for 93.6 per cent of the variation and loaded positively with annual precipitation (loading = 0.80), while the second principal component (PC2) accounted for 5.3 per cent of the variation and loaded positively with potential evapotranspiration (loading = 0.51). Mean cone and seed traits from every study population did not correlate with PC1 (Kendall's tau coefficients = [−0.2, 0.33]; $P > 0.10$), but PC2 was negatively correlated with the number of empty seeds (Kendall's tau coefficient = −0.73; $P = 0.04$). This latter result suggests that meteorological dryness reduces the production of empty seeds, probably because rainy weather during pollination leads to lower pollination success.

I also computed tests of Kendall's tau to analyse the relationship between latitude and cone and seed traits. All the tests were non-significant (Kendall's tau coefficients ≤ 0.60; $P > 0.05$).

Cone traits selected by foraging crossbills

Random forest analysis found that predictor variables explained 13.56 per cent of the observed variance in the relative fitness of *P. nigra* individuals. Crop size, mean seed mass, the number of full seeds, the proximal scale thickness and cone mass were identified as important variables explaining cone selection by crossbills, but only the first two contributed significantly to the regression (Figure 3). Crop size was not correlated with any cone or seed trait with the exception of seed mass (Spearmen rho = 0.401, $n = 28$, $P < 0.05$) (Table A2). The sign of parameter estimates of the linear regressions indicated that crossbills preferentially foraged on pine trees displaying larger crop sizes with heavier and more numerous seeds. On the contrary, pine trees attaining thicker scales and heavier cones increased their relative fitness.

Discussion

Geographic variation in pine traits and phenotypic selection exerted by crossbills

Results showed evidence for the existence of a geographic mosaic of selection in the pine-crossbill study system. Thus, the mixed-effect models and the CAP analysis provided evidence

Table 1 Mean cone measurements ± standard errors. Lowercase letters indicate differences among forests in seed and cone traits, based on Tukey tests ($P < 0.05$).

| Measurement	Forest			Among forests	
	Northern	Central	Southern	$F_{2,398}$	P
No. full seeds	30.92 ± 1.27a	21.35 ± 0.80b	27.19 ± 0.94a	25.97	<0.001
No. empty seeds	14.90 ± 0.82a	6.76 ± 0.45b	6.93 ± 0.40b	69.66	<0.001
Mean seed mass (mg)	19.68 ± 0.25b	22.02 ± 0.31a	19.81 ± 0.37b	29.63	<0.001
Cone mass (g)	11.61 ± 0.21a	10.37 ± 0.20b	10.59 ± 0.14b	18.43	<0.001
Cone length (mm)	60.60 ± 0.50a	55.25 ± 0.49c	57.48 ± 0.35b	49.19	<0.001
Cone width (mm)	29.65 ± 0.19a	28.49 ± 0.18b	29.21 ± 0.14a	12.11	<0.001
Proximal scale thickness (mm)	3.13 ± 0.03a	3.20 ± 0.02a	2.92 ± 0.02b	50.44	<0.001
Distal scale thickness (mm)	3.12 ± 0.03a	3.19 ± 0.03a	3.00 ± 0.02b	29.32	<0.001
No. scales	11.04 ± 0.12a	9.21 ± 0.07c	10.49 ± 0.08b	93.65	<0.001
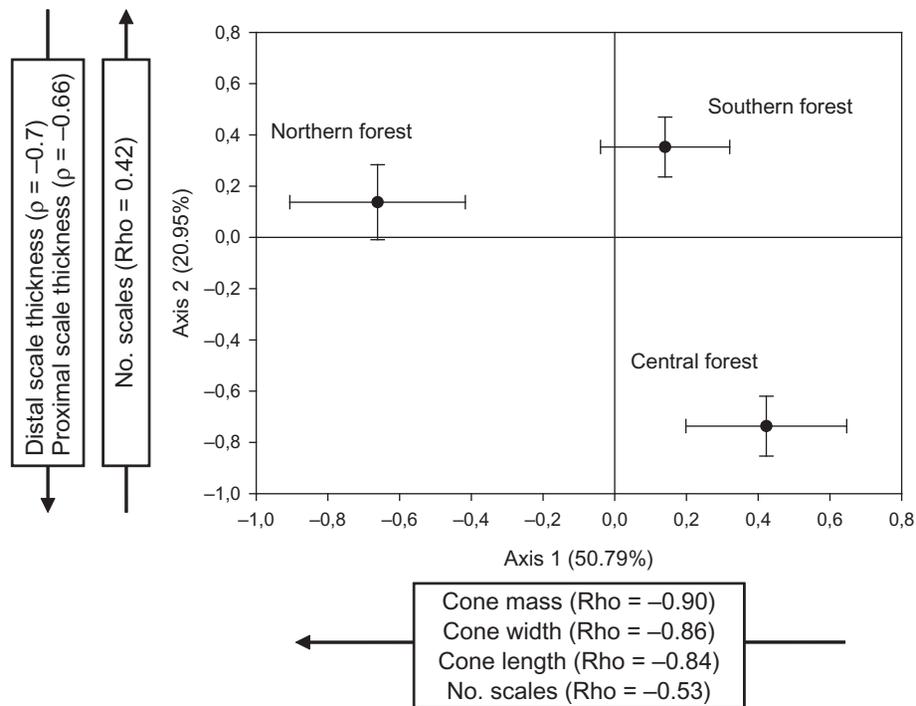
Figure 2 Canonical discriminant analysis of principal coordinates of six cone traits characterizing the study forests (northern, central and southern). Rho is the Spearman correlation coefficient. Data are mean scores ± 95 per cent confident intervals (*N* = 740).
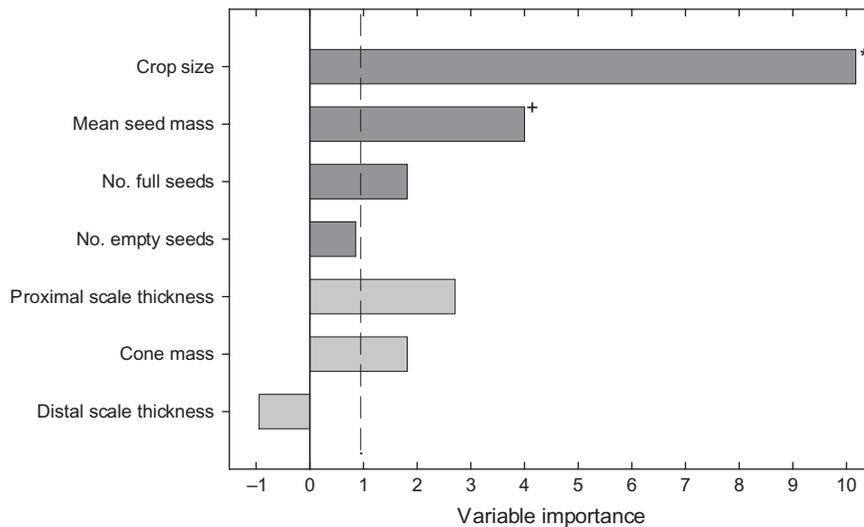

Figure 3 Random-forest analysis showing the relative importance of seed and cone traits in the relative fitness of *Pinus nigra* subsp. *salzmannii*. Variables are considered important if their importance value is above the absolute value of the lowest ranking predictor variable (marked with the dashed line). The dark colour indicates a variable negative effect on *Pinus nigra* relative fitness, whereas the light colour indicates a positive effect. Permutation *P*-values (1000 repetitions) $^+P < 0.10$, $^*P < 0.05$.

that cones collected at the northern forest were the largest of all those included in the study, while also having more scales which were as thick as those found in the central forest. Cones from the northern forest also exhibited the most empty seeds. Therefore, overall cone defenses against crossbill predation were found to be greater in the northern study forest, where the pine-crossbill interaction was assumed to have been stronger over evolutionary time. In turn, the multidimensional CAP analysis revealed that cones from the central and southern forests were smaller (Figure 2), suggesting that variation in relative densities of the pair of interacting species could have resulted in spatial variation in the evolved cone defenses of *P. nigra*

([Benkman and Mezquida, 2015](#)). These results essentially confirmed previous general findings about the *P. nigra*-crossbill coevolutionary system ([Benkman and Parchman, 2009](#), [2013](#); [Benkman and Mezquida, 2015](#)), but did allow me to describe a geographic mosaic of selection with implications for assisted migration.

I did not consider selection exerted by insects, because pre-dispersal seed losses to insect predation are consistently low across the distribution of *P. nigra* ([Tíscar and Linares, 2011](#)). However, the observed variability in pine traits could be influenced by the concurrent interaction of crossbills with a third partner: the red squirrel (*Sciurus vulgaris*) ([Parchman and Benkman, 2008](#)). Phenotypic selection exerted by red squirrels seems to favour pine cones with more numerous, shorter scales in *P. uncinata* that attains cones of a comparable size to *P. nigra* ([Mezquida and Benkman, 2010](#)). These two cone traits do not generally show large selection gradients in the pine-crossbill interaction ([Benkman and Mezquida, 2015](#)). Consequently, squirrel predation should not be expected to have interfered directly with the most relevant variables used here to identify the pine-crossbill selection mosaic (Figure [2](#)). Yet, an indirect effect should be considered if red squirrels outcompeted crossbills, thus limiting the amount of food available for the latter species ([Mezquida and Benkman, 2005](#); [Parchman and Benkman, 2008](#)). Red squirrels and common crossbills are indeed sympatric in the Iberian Peninsula, but red squirrels are considered unimportant selective agents on *P. nigra* as they occur at low densities in these forests ([Benkman and Parchman, 2009](#)). Thus, forests of *P. nigra* represent poor habitats for red squirrels most of the time and predation rates would remain generally low, although forests of *P. nigra* could be used intensively by transient squirrels coming from the neighbouring forests of *P. halepensis* and *P. pinaster* during mast-seeding years ([Lurz *et al*., 2000](#)).

Despite my results being consistent with the expected outcome from a spatially structured pine-crossbill interaction, there is a possibility that observed variation in *P. nigra* defensive traits was environmentally induced. Studies of plant-animal interactions do not usually consider the potential effect of environmental variability on trait variation, probably, because environmental variability is indirectly included via its effect on animal density and/or behaviour, and thus on the interaction strength and, conversely, because plant phenotypic variation affects animal behaviour, and thus plant fitness ([Herrera, 1995](#); [Mezquida and Benkman, 2014](#)). Yet, the interpretation of my results could be blurred, if environmental factors were simultaneously influencing fitness and values of phenotypic traits ([Stinchcombe *et al*. 2002](#)). Here, I found no relationship between cone and seed traits with climate. This finding was obviously limited by the number of study sites, only six, but was once again consistent with previous research on this subject ([Parchman and Benkman, 2008](#); [Mezquida and Benkman, 2010](#)). In any case, environmental variability was not expected to have a great influence on the phenotypic variability of cone traits, since the heritability of different cone traits appears to be high in *P. nigra* ([Sivacioglu and Ayan, 2010](#)). The lack of relationship between crop size and cone traits (Table A2) further suggests that resource availability and environmental variability have little influence on the size of cone traits. An additional consequence of this latter finding is that cone traits of *P. nigra* are not expected to vary between masting and non-masting years.

Results from the random-forest analysis showed that crossbills forage adaptively, and choose trees that maximize the food intake rate. Specifically, crossbills foraging on the *P. nigra* southern forests preferentially chose trees with more cones. This result was consistent with other studies ([Mezquida and Benkman, 2014](#)). In contrast, the positive relationship between mean seed mass and crossbill consumption was unexpected, since my prediction was that crossbills would preferentially feed on smaller seeds. This unexpected result could be explained by the observed positive correlation between seed mass and crop size (Table A2). It could be also the consequence of crossbills invading from adjacent *P. halepensis* forests, as those birds would be accustomed to handling the larger seeds of that pine species ([Bladé and Vallejo, 2008](#)). The number of empty seeds had an unimportant negative effect on the relative fitness of *P. nigra* (Figure [3](#)). Although I had predicted that cones should attain more numerous empty seeds in order to prevent crossbill predation, the latter results are consistent with the most recent research on the phenotypic selection exerted by crossbills on pine species ([Benkman and Mezquida, 2015](#)). An evolutionary response for this trait is less likely compared with others, because it depends on the various factors affecting pollination success ([Tíscar and Linares, 2011](#)).

As expected, cone mass and scale thickness were found both to be important and to increase the relative fitness of *P. nigra*, but these results were statistically non-significant. This lack of significance could be a function of crop size. Since cone production was low in the southern forest during the study year, crossbills just fed on the available food and, consequently, the opportunity for the selection of cone traits was also low ([Benkman and Parchman, 2013](#)). In summary, although some variables rendered non-significant results, the importance measurements produced by the random-forest analysis and the sign of the regression coefficients were congruent for most variables with existing knowledge ([Benkman and Parchman, 2009](#); [Benkman and Mezquida, 2015](#)). These findings further suggest that observed geographical variation in cone and seed traits of *P. nigra* is mainly due to spatio-temporal variability in the pine interaction with crossbills.

Implications for assisted migration

This study highlights the necessity of considering interspecific interactions when assessing the possible outcomes of assisted migration. A hypothetical scheme of assisted migration for *P. nigra* would involve translocating southern populations, which are presumably better adapted to drier, warmer conditions, towards the north of the species' range ([Benito-Garzón *et al*., 2008](#)). This movement would introduce the genotype of pines with poorly defended cones into areas where crossbills are abundant. The outcome for the resulting pine-crossbill interaction is difficult to foresee as many other interacting factors would be present ([Tíscar and Linares, 2011](#); [Tíscar et al., 2018](#)). For instance, the common crossbill distribution will be conversely influenced by pine responses to changing climate, including a reduction in the future range of this species ([Mezquida *et al*., 2018](#)). However, some evidence suggests that crossbill predation could have a significant negative impact for the translocated trees. For example, [Benkman and Parchman](#)

(2013) reported that a 4-per cent reduction in cone mass and a 7-per cent reduction in the proximal scale thickness increased the risk of crossbill predation by 10 times. Important seed losses should be expected within the translocated southern genotypes of *P. nigra*, since the per cent mean differences of cone mass and proximal scale thickness between the study southern and northern forests were 7 per cent and 11 per cent, respectively (Table 1). Yet, the actual effect of predispersal seed predation has proven small on the regeneration process of *P. nigra*, at least during mast-seeding years (Tíscar and Linares, 2011).

P. nigra tracks both the spatial and temporal environmental heterogeneity that typically occurs in Mediterranean forests (Quero *et al.*, 2011). During masting years, most trees synchronously produce a large seed crop and the dispersed seeds saturate the forest floor, making it likely that at least some of them encounter safe, wet microsites for establishment (Tíscar and Linares, 2011). In this case, it is obvious that the larger the seed crop, due to less crossbill predation, the greater the probability of recruitment. Alternatively, a few trees still produce good seed crops during non-masting years. These asynchronous crops are useful to track the temporal variability of the Mediterranean climate, since Mediterranean summers are typically hot and dry, but some rainy, cooler ones occur irregularly, generating new opportunities for seedling establishment (Matías *et al.* 2012). Summer drought is considered to be the major limiting factor for the recruitment of *P. nigra* (Tíscar and Linares, 2011). Results from this study indicate that flocks of crossbills preferably head to trees with larger crop sizes during non-masting years. The resulting foraging by crossbills could deplete local seed availability and nullify prospective regeneration during the occasional occurrence of wetter and/or cooler summers coinciding with non-masting years.

Supplementary data

Supplementary data are available at *Forestry* online.

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Conflict of interest statement

None declared.

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References

Afzal-Rafii, Z. and Dodd, R.S. 2007 Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of black pine (*Pinus nigra*) in Western Europe. *Mol. Ecol.* **16**, 723–736.

Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T. and Curtis-McLane, S. 2008 Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol. Appl* **1**, 95–111.

Alejano, R. and Martínez, E. 1996 Distribución de *Pinus nigra* Arn. subsp. *salzmannii* en las sierras Béticas. *Ecología* **10**, 231–241.

Alía, R., García, J.M., Iglesias, S., Mancha, J.A., de Miguel, J., Nicolás, J.L., *et al* 2009 *Regiones de procedencia de especies forestales en España*. Organismo Autónomo Parques Nacionales, MMAMRM.

Anderson, M.J. 2004. *CAP: a FORTRAN computer program for canonical analysis of principal coordinates*. Department of Statistics, University of Auckland, New Zealand.

Arizaga, J., Alonso, D. and Edelaar, P. 2015 Stable isotopes in a southern European crossbill population indicate restricted movement between regions with different pine species. *J. Zool.* **295**, 49–55.

Benito-Garzón, M., Sánchez de Dios, R. and Sainz Ollero, H. 2008 Effects of climate change on the distribution of Iberian tree species. *Appl. Veg. Sci* **1**, 169–178.

Benito-Garzón, M., Ha-Duong, M., Frascaria-Lacoste, N. and Fernández-Majarrés, J. 2013 Habitat restoration and climate change: dealing with climate variability, incomplete data, and management decisions with tree translocations. *Restor. Ecol.* **21**, 530–536. doi:10.1111/rec.12032.

Benkman, C.W. and Mezquida, E.T. 2015 Phenotypic selection exerted by a seed predator is replicated in space and time and among prey species. *Am. Nat.* **186**, 682–691.

Benkman, C.W. and Parchman, T.L. 2009 Coevolution between crossbills and black pine: the importance of competitors, forest area and resource stability. *J. Evol. Biol.* **22**, 942–953.

Benkman, C.W. and Parchman, T.L. 2013 When directional selection reduces geographic variation in traits mediating species interactions. *Ecol. Evol* **3**, 961–970.

Bladé, C. and Vallejo, V.R. 2008 Seed mass effects on performance of *Pinus halepensis* Mill. seedlings sown after fire. *For. Ecol. Manage.* **255**, 2362–2372.

Borrás, A., Cabrera, J. and Senar, J.C. 2008 Local divergence between Mediterranean crossbills occurring in two different species of pine. *Ardeola* **55**, 169–177.

Borrás, A. and Senar, J.C. 2003 Piquituerto común, *Loxia curvirostra*. Atlas de las aves reproductoras de Espana. DGCN-SEO/BirdLife, Madrid.

Breiman, L. 2001 Random forests. *Mach. Learn.* **45**, 5–32.

Cutler, D.R., Edwards, T.C., Beard, K.H., Cutler, A., Hess, K.T., Gibson, J., *et al* 2007 Random forests for classification in ecology. *Ecology* **88**, 2783–2792.

Davis, M.B. and Shaw, R.G. 2001 Range shifts and adaptive responses to Quaternary climate change. *Science* **292**, 673–679.

Dyderski, M.K., Paź, S., Frelich, L.E. and Jagodziński, A.M. 2018 How much does climate change threaten European forest tree species distributions? *Glob. Change Biol.* **24**, 1150–1163.

Edelaar, P., Alonso, D., Lagerveld, S., Senar, J.C. and Björklund, M. 2012 Population differentiation and restricted gene flow in Spanish crossbills: not isolation-by-distance but isolation-by-ecology. *J. Evol. Biol.* **25**, 417–430.

Herrera, C.M. 1995 Microclimate and individual variation in pollinators: flowering plants are more than their flowers1. *Ecology* **76**, 1516–1524.

Kolb, A., Ehrlen, J. and Eriksson, O. 2007 Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspect. Plant Ecol.* **9**, 79–100.

Lurz, P.W., Garson, P.J. and Wauters, L.A. 2000 Effects of temporal and spatial variations in food supply on the space and habitat use of red squirrels (*Sciurus vulgaris* L.). *J. Zool.* **251**, 167–178.

Matías, L., Zamora, R. and Castro, J. 2012 Sporadic rainy events are more critical than increasing of drought intensity for woody species recruitment in a Mediterranean community. *Oecologia* **169**, 833–844.

Mezquida, E.T. and Benkman, C.W. 2005 The geographic selection mosaic for squirrels, crossbills and Aleppo pine. *J. Evol. Biol.* **18**, 348–357.

Mezquida, E.T. and Benkman, C.W. 2010 Habitat area and structure affect the impact of seed predators and the potential for coevolutionary arms races. *Ecology* **91**, 802–814.

Mezquida, E.T. and Benkman, C.W. 2014 Causes of variation in biotic interaction strength and phenotypic selection along an altitudinal gradient. *Evolution* **68**, 1710–1721.

Mezquida, E.T., Svenning, J.C., Summers, R.W. and Benkman, C.W. 2018 Higher spring temperatures increase food scarcity and limit the current and future distributions of crossbills. *Divers. Distrib.* **24**, 473–484.

Ninyerola, M., Pons, X. and Roure, J.M. 2005 Atlas Climático Digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Bellaterra.

Parchman, T.L. and Benkman, C.W. 2008 The geographic selection mosaic for ponderosa pine and crossbills: a tale of two squirrels. *Evolution* **62**, 348–360.

Pausas, J.G., Bladé, C., Valdecantos, A., Seva, J.P., Fuentes, D., Alloza, J.A., *et al* 2004 Pines and oaks in the restoration of Mediterranean landscapes: new perspectives for an old practice. *Plant Ecol.* **171**, 209–220.

Pedlar, J.H., McKenney, D.W., Aubin, I., Beardmore, T., Beaulieu, J., Iverson, L., *et al* 2012 Placing forestry in the assisted migration debate. *Bioscience* **62**, 835–842.

Quero, J.L., Herrero, A. and Zamora, R. 2011 Linking stochasticity to determinism of woody plant recruitment in a mosaic landscape: a spatially explicit approach. *Basic Appl. Ecol.* **12**, 161–171.

Ricciardi, A. and Simberloff, D. 2009 Assisted colonization is not a viable conservation strategy. *Trends Ecol. Evol* **5**, 248–253. doi:10.1016/j.tree.2008.12.006.

Rodríguez-Sánchez, F., Hampe, A., Jordano, P. and Arroyo, J. 2010 Past tree range dynamics in the Iberian Peninsula inferred through phylogeography and palaeodistribution modelling: a review. *Rev. Palaeobot. Palynol.* **162**, 507–521.

Ruiz de la Torre, J. 2006 Dirección General para la Biodiversidad. In *Flora mayor*. Ministerio de Medio Ambiente.

Senar, J.C., Borras, A., Cabrera, T. and Cabrera, J. 1993 Testing for the relationship between coniferous crop stability and common crossbill residence. *J. Field Ornithol.* **64**, 464–469.

Sivacioglu, A. and Ayan, S. 2010 Variation in cone and seed characteristics in a clonal seed orchard of Anatolian black pine (*Pinus nigra* Arnold subsp. pallasiana (Lamb.) Holmboe). *J. Environ. Biol.* **31**, 119–123.

Stinchcombe, J.R., Rutter, M.T., Burdick, D.S., Tiffin, P., Rausher, M.D. and Mauricio, R. 2002 Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *Am. Nat.* **160**, 511–523.

Thompson, J.N. 2005 Coevolution: the geographic mosaic of coevolutionary arms races. *Curr. Biol.* **15**, 992–994. doi:10.1016/j.cub.2005.11.046.

Tíscar, P.A. and Linares, J.C. 2011 *Pinus nigra* subsp. salzmannii forests from Southeast Spain: using structure and process information to guide management. In *Pine forests: types, threats and management*. Frisiras C. T. (ed). Nova Science Publishers, Inc, pp. 1–27.

Tíscar, P.A., Lucas-Borja, M.E. and Candel-Pérez, D. 2018 Lack of local adaptation to the establishment conditions limits assisted migration to adapt drought-prone *Pinus nigra* populations to climate change. *For. Ecol. Manage.* **409**, 719–728.