The geographic selection mosaic for squirrels, crossbills and Aleppo pine

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Abstract

The interactions between many species are structured in a geographic mosaic of populations among which selection is divergent. Here we tested the hypothesis that such a geographic selection mosaic arises for common crossbills (*Loxia curvirostra*) feeding on seeds in the cones of Aleppo pine (*Pinus halepensis*) because of geographic variation in the occurrence of European red squirrels (*Sciurus vulgaris*). On the Iberian Peninsula, *Sciurus* exerted directional selection favouring larger cones with larger scales, which has caused cones there to be larger than in the Balearic Islands where *Sciurus* are absent. Moreover, cones on the Iberian Peninsula are so large that they are apparently little used by the relatively small-billed crossbills on the Peninsula; selection by *Sciurus* seems to have made the cones so difficult to feed on that crossbills rely mostly on the seeds of other conifers. Where crossbills are present but *Sciurus* are absent (Mallorca Island), cones were smaller as a result of relaxation of selection by *Sciurus*. However, cones on Mallorca had proportionally thicker scales in comparison to where both *Sciurus* and crossbills are absent (Ibiza Island), presumably as an adaptation against crossbill predation. Here crossbills specialize on Aleppo pine, have relatively large bills and have apparently coevolved in an arms race with Aleppo pine. These results suggest that *Sciurus* has influenced both the geographic selection mosaics for crossbills and conifers and the adaptive radiation of crossbills in Eurasia much like *Tamiasciurus* has done in the North America.

Introduction

Species interactions are thought to be a major force driving evolutionary change and promoting biodiversity (McPeek, 1996; Thompson, 1999a; Rausher, 2001). Moreover, the ranges of interacting species rarely coincide completely so that the interaction strengths and the interactions themselves usually vary among populations (Gómez & Zamora, 2000; Brodie *et al.*, 2002; Fedriani *et al.*, 2004). For example, coevolution between a predator and its main prey may depend on the presence of a dominant competitor (Benkman *et al.*, 2001), or even the outcome of an interaction may vary (e.g. from mutualism

to parasitism) depending on community context (Thompson & Pellmyr, 1992; Thompson & Cunningham, 2002). The result is a mosaic of populations in which the form of selection between interacting species differs, as it has been found in recent years for different systems (Benkman, 1999; Burdon & Thrall, 1999; Lively, 1999; Thompson & Cunningham, 2002; Zangerl & Berenbaum, 2003). Thompson (1994, 1999b) formalized this geographic perspective for the study of species interactions within the framework of the geographic mosaic theory of coevolution.

A well-characterized system of coevolving interactions in the context of a geographic mosaic is common crossbills (*Loxia curvirostra* complex) and Rocky Mountain lodgepole pine (*Pinus contorta* spp. *latifolia*; Benkman, 1999; Benkman *et al.*, 2001). Pine squirrels (*Tamiasciurus hudsonicus*) usually occur in lodgepole pine forests of the Rocky Mountains and are the main seed predators of lodgepole pine. *Tamiasciurus* are efficient seed predators

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because they harvest and cache large numbers of cones early in the fall before the scales start to open (Smith, 1970), whereas crossbills extract seeds from cones that accumulate and weather if not harvested by *Tamiasciurus*. Thus, *Tamiasciurus* preemptively outcompete crossbills and lodgepole pine has evolved defenses against *Tamiasciurus* where they are found. Crossbills, in turn, are uncommon, have adapted to the average cone and exert little selection on lodgepole pine. In isolated mountain ranges east and west of the Rocky Mountains where *Tamiasciurus* are absent, lodgepole pine cones have lost defenses against *Tamiasciurus*. In these ranges, crossbills are resident and common, and have been evolving reciprocally with lodgepole pine in coevolutionary arms races (Benkman *et al.*, 2003). Consequently, crossbills are subject to divergent selection depending on the presence or absence of a dominant competitor.

A central issue, however, is to determine if coevolution between crossbills and conifers is a common process, or whether the case of crossbills and lodgepole pine should be considered exceptional. Recently, Parchman & Benkman (2002) demonstrated that crossbills and black spruce (*Picea mariana*) coevolved on Newfoundland in the absence of *Tamiasciurus*. Moreover, cone structure in the presence or absence of *Tamiasciurus* showed convergent patterns in black spruce and lodgepole pine. These results suggest that a geographic mosaic of coevolution may have been a prominent process in the adaptive radiation of North American crossbills. Throughout the Eurasian range of crossbills, however, *Tamiasciurus* are absent and instead European red squirrels (*Sciurus vulgaris*) are the main tree squirrels and predispersal seed predators of conifers (Moller, 1986; Cagnin *et al.*, 2000; but see Castro *et al.*, 1999). This raises the question of whether *Sciurus* also outcompetes crossbills for conifer seeds and inhibits coevolution between crossbills and conifers.

Here, we examine whether, analogous to the situation with *Tamiasciurus* and lodgepole pine and black spruce, crossbills coevolve with Aleppo pine (*Pinus halepensis*) and whether *Sciurus* prevent coevolution between crossbills and Aleppo pine. Aleppo pine is a lowland circum-Mediterranean species, although the largest areas covered with natural forests of this pine are found in the Iberian Peninsula (Fig. 1). Aleppo pine produces abundant and regular cone crops (Ruiz de la Torre, 1979). Some mature cones open and release their seeds during late summer and fall, although a variable percentage of cones remain closed (serotinous cones) until they open in response to dry and hot weather (xeriscence) or fire (pyriscence; Nathan *et al.*, 1999). *Sciurus* are widespread in Aleppo pine forests on the Iberian Peninsula (Purroy, 2002). Crossbills also inhabit and breed in these forests, although their abundance appears low (Borrás & Senar, 2003). Aleppo pine is also present on the Balearic Islands (Fig. 1). *Sciurus* do not occur on the Balearic Islands (Purroy, 2002), but crossbills are present

on the largest island (i.e. Mallorca; Borrás & Senar, 2003). The presence of crossbills on Mallorca has been corroborated by fossil evidence, dating at least from the late Pleistocene (Alcover *et al.*, 1992; Seguí, 1997). Crossbills are resident on Mallorca, feed exclusively on seeds in the cones of Aleppo pine and are considered a distinct taxon (*L. c. balearica*; Massa, 1987; Cramp & Perrins, 1994). Crossbills have been observed sporadically on other islands of this archipelago (e.g. Ibiza and Menorca) during irruptive movements, but these islands do not support resident populations (Altaba, 2001; O. García, pers. comm.); although crossbills became established on Ibiza in ca. 2000 (J. Estarellas, pers. comm.). Insects are also important seed predators of conifer seeds (Smith & Balda, 1979), and at least two species of cone borers depredate the seeds of Aleppo pine in the western Mediterranean. Information on the impact of these seed predators on Aleppo pine on the Iberian Peninsula and the Balearic Islands is lacking. However, signs of seed predation by these insect species have been observed on the Iberian Peninsula and the three major Balearic Islands (E.T. Mezquida, pers. obs.). Consequently, variation in cone structure between the Iberian Peninsula and the Balearic Islands is unlikely the result of differences in selection exerted by insects.

We address the hypothesis that Aleppo pine cone structure differs among areas as the result of the presence of *Sciurus* on the Iberian Peninsula, the presence of crossbills on Mallorca in the absence of *Sciurus*, and the lack of both vertebrate seed predators on other islands of the Balearics. Conifer seeds are the main item in the diet of *Sciurus*, especially in late summer, fall and winter (Moller, 1983; Wauters *et al.*, 1992), and *Sciurus* cache individual or small groups of cones (i.e. scatter-hoard) that are used in spring (Wauters *et al.*, 1995; Wauters & Casale, 1996). This suggests that *Sciurus* should also be the main vertebrate seed predator in those conifer forests where they occur, as it has been found in some studies (Moller, 1986; Cagnin *et al.*, 2000). Information on *Sciurus* populations in Aleppo pine forests is scarce, although a study in a mixed Aleppo pine forest in northeast Spain found relatively high *Sciurus* abundance (Piqué, 1997). Thus, we expect *Sciurus* are the main vertebrate seed predators on the Iberian Peninsula and have had a selective impact on Aleppo pine.

One prediction we test is that if *Sciurus* are dominant seed predators, as is the case of *Tamiasciurus* in North America, cone traits that act to deter *Sciurus* should be enhanced on the Iberian Peninsula. This increase in seed defenses should result in a decrease of relative seed production per cone (i.e. a decrease in seed mass to cone mass; Smith, 1970; Benkman, 1999). Our second prediction is that in the absence of *Sciurus*, some differences in cone traits should be consistent with selection by crossbills and not simply the result of relaxation of selection by *Sciurus*. This prediction would be supported if traits that act to deter crossbills are enhanced on Mallorca and
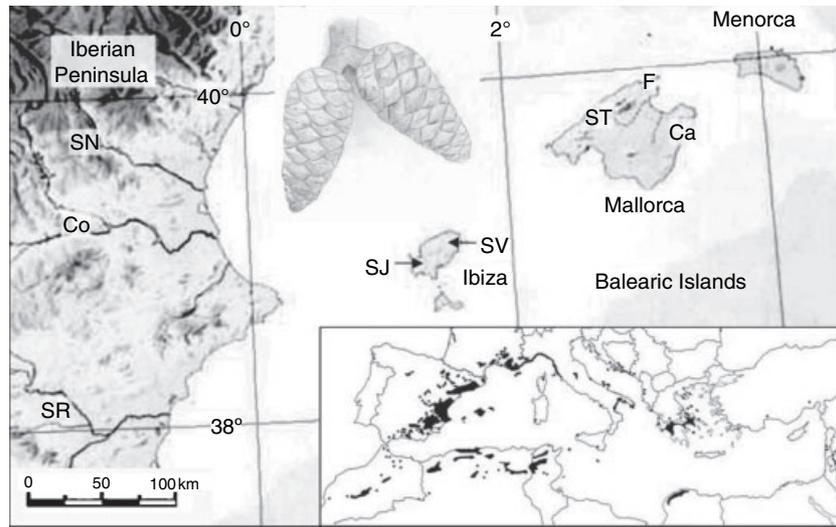
Fig. 1 The location of study sites and a typical whorl of two Aleppo pine cones. The lower inset shows the circum-Mediterranean distribution of Aleppo pine (black; modified from Fady *et al.*, 2003). The study sites on the Iberian Peninsula were in Valencia [Sierra del Negrete (SN) and Cofrentes (Co)] and Murcia provinces [Sierra del Ricote (SR)], and sites on the Balearic Islands were on the two westernmost islands: Mallorca [Canyamel (Ca), Sierra de Tramuntana (ST) and Formentor (F)] and Ibiza [San José (SJ) and Sierra de San Vicente (SV)]. European red squirrels (*Sciurus vulgaris*) and red crossbills (*Loxia curvirostra*) occur on the Iberian Peninsula, and crossbills alone occur on Mallorca.

this increase of costly investment also results in a decrease in relative seed production per cone. Our third prediction is that in the absence of both vertebrate seed predators differences in cone traits should be consistent with relaxation of selection by *Sciurus* and crossbills, and the lost of costly defenses should result in a proportional increase of seed production. Finally, we examine an alternative hypothesis that differences in cone and seed traits between the Iberian Peninsula and the Balearic Islands are the result of abiotic factors such as annual precipitation, summer drought or frequency of fires. If the presence of *Sciurus* prevents coevolution between crossbills and Aleppo pine this would indicate that the presence and absence of *Sciurus* influences the geographic selection mosaic for crossbills foraging on conifers, as does *Tamiasciurus* in North America.

Materials and methods

Geographic cone variation

We collected mature cones of Aleppo pine in early autumn 2003 before they opened to shed their seed (Ruiz de la Torre, 1979). On the Iberian Peninsula, where *S. vulgaris* and crossbills occur, we sampled three sites in Valencia (Sierra del Negrete and Cofrentes) and Murcia provinces (Sierra del Ricote) where large forests of mature Aleppo pine exist (Fig. 1). We also sampled on two of the Balearic Islands. On Mallorca, where *Sciurus* are absent and crossbills are resident, cones were sampled in three sites: Canyamel, Sierra de Tramuntana and

Formentor (Fig. 1). On Ibiza, where *Sciurus* and until recently crossbills were absent, cones were sampled in two sites: San José and Sierra de San Vicente (Fig. 1).

At each site, we sampled 25 trees each separated by a minimum distance of over 100 m. Cones were collected from the upper third of the trees using a branch cutter attached to a 9-m extendable pole. Several morphological traits were measured for three cones of each sampled tree. Following Benkman (1999) (see also Benkman *et al.*, 2003), we measured the following traits for each cone: maximum length and width of the closed cone, number of scales that fall along the vertical axis of the cone, cone mass without seeds, number of full seeds (i.e. with female gametophyte and embryo), number of empty seeds, mass of five seeds without their wings, thickness of five scales in the middle part of the cone and length of three scales in the middle part of the cone (in both cases scales were selected approximately equi-distant around the cone). We used digital callipers to make length measurements to the nearest 0.01 mm. A digital scale was used to measure mass to the nearest 0.01 mg after cones and seeds had been oven dried for 1–2 days at 60–70 °C. We also estimated the ratio of seed mass to cone mass as the total seed mass in grams (number of seeds times individual seed mass) divided by cone mass in grams. We calculated mean values for each cone and averaged the values for each tree, which was the sample unit used in the statistical analyses.

In order to compare the morphological traits of cones among areas (i.e. *Sciurus* and crossbills present, *Sciurus* absent but crossbills present, and both predators absent)

and among sites within each area, we used two-level nested ANOVAs. Cone traits were transformed (ln-transformed except number of seeds, which was square root-transformed, and seed mass/cone mass, which was not transformed) to meet the statistical requirements of this parametric test. We also used *a posteriori* Tukey tests for unequal sample sizes to examine which areas differed significantly in cone traits. We performed a principal components analysis of cone traits (based on the correlation matrix) to further characterize the variation in cone morphology among areas with different compositions of seed predators.

Targets and form of natural selection by *Sciurus*

In order to examine the relationships between seed predation by *S. vulgaris* and morphological traits of cones, we collected cones from 100 trees in an Aleppo pine forest on the Iberian Peninsula (Valle del Cabriel, Valencia province) during April 2004. We searched for signs of *Sciurus* seed predation (e.g. cone cores, scales) at the base of trees. The presence of cone cores at the base of a tree is a reliable indicator of tree selection by *Sciurus* because most cores fall under the selected tree (Moller, 1986). Harvested trees with one or more trees closer than 3 m, or with overlapping crowns, were not sampled to avoid potential mistakes. For each harvested tree sampled, we looked for a nearby tree without signs of *Sciurus* seed predation. By doing so, we avoided sampling trees in different microhabitats or outside *Sciurus* territories or home ranges. We sampled 50 harvested and 50 nonharvested trees using a branch cutter as described above. Three mature cones were collected from each tree, although it was not possible to gather three cones from four trees heavily harvested by *Sciurus*. In those cases, we also collected open cones still attached to branches to measure some traits (i.e. those not related to seeds). We counted the number of cone cores underneath harvested

trees and gathered all (or up to a maximum of 14) cone cores to subsequently measure their length with a digital calliper to the nearest 0.01 mm. For each closed cone, we measured the same morphological traits described previously. The ratio of seed mass to cone mass was calculated as the number of full seeds times individual seed mass in grams divided by cone mass in grams. Values for each cone were averaged and the mean for each tree was calculated and used as the sample unit in the analyses.

We used pairwise logistical regressions (Janzen & Stern, 1998) on cone traits from trees harvested and avoided by *Sciurus* to estimate the form of selection (both direct and indirect selection because of correlations between traits) on Aleppo pine by *Sciurus*. We assigned fitnesses of 0 and 1 to harvested and unharvested trees, respectively. Cubic splines (Schluter, 1988) were used to check for stabilizing selection on cone traits. We did not find evidence of stabilizing selection on any cone trait. We used a multiple logistic regression to determine which traits were under direct selection (i.e. the targets of selection) by *Sciurus*. To reduce multicollinearity, we included only those cone traits with correlation coefficients <0.51. We present the logistic regression coefficients (α) and estimate the selection gradient (β), as described by Janzen & Stern (1998).

Results

Geographic cone variation

All cone traits differed significantly ($P < 0.0001$) among the three areas (Table 1). Furthermore, cone traits differed among sites within each area ($F_{5,192} > 3.3$, $P < 0.01$ in all cases). Cones of Aleppo pine on the Iberian Peninsula were larger and had larger scales than those on the Balearic Islands (Table 2). Within the Balearics, cones on Mallorca were characterized by being somewhat shorter and having thicker scales and fewer

Table 1 Mean cone measurements for sites on the Iberian Peninsula, Mallorca and Ibiza.

| Measurement | Iberian Peninsula | | | Mallorca Island | | | Ibiza Island | | Among areas | |
	Sierra del Negrete	Cofrentes	Sierra del Ricote	Canyamel	Sierra de Tramuntana	Formentor	San José	Sierra de San Vicente	$F_{2,5}$	P
Cone length (mm)	79.47	76.15	71.76	65.38	58.06	58.74	65.94	62.56	72.8	<0.0001
Cone width (mm)	36.25	35.16	33.09	30.79	30.19	29.81	30.10	29.73	65.1	<0.0001
Number of scales	12.47	11.95	11.83	11.20	10.29	10.33	12.15	11.96	55.9	<0.0001
Cone mass (g)	26.25	24.62	21.34	17.66	13.67	14.94	17.71	16.50	56.0	<0.0001
Number of full seeds	74.65	73.80	72.69	66.19	57.07	38.15	85.96	83.23	47.3	<0.0001
Number of empty seeds	9.32	4.72	6.53	4.29	2.53	5.69	7.43	10.04	13.5	<0.0001
Individual seed mass (mg)	18.71	17.61	14.92	14.62	15.53	17.35	12.33	11.86	29.2	<0.0001
Seed mass/cone mass	0.060	0.056	0.054	0.057	0.065	0.049	0.064	0.067	10.5	<0.0001
Scale thickness (mm)	3.61	3.74	3.43	3.19	3.14	3.10	2.90	2.77	111.5	<0.0001
Scale length (mm)	25.83	24.91	22.88	22.35	20.78	20.84	21.06	20.35	39.5	<0.0001

The *F*- and *P*-values are the results of univariate two-level nested ANOVAs comparing the three areas. Analyses were based on the mean for each tree (25 trees were measured at each site), with three cones measured from each tree.

Table 2 Mean cone measurements for three sites on the Iberian Peninsula, three sites on Mallorca and two sites on Ibiza.

Measurement	Iberian Peninsula	Mallorca Island	Ibiza Island
Cone length (mm)	75.79a	60.73b	64.25c
Cone width (mm)	34.83a	30.26b	29.92b
Number of scales	12.08a	10.61b	12.05a
Cone mass (g)	24.07a	15.42b	17.10b
Number of full seeds	73.72a	53.80b	84.59c
Number of empty seeds	6.86a	4.17b	8.73a
Individual seed mass (mg)	17.08a	15.83a	12.09b
Seed mass/cone mass	0.057a	0.057a	0.066b
Scale thickness (mm)	3.60a	3.14b	2.83c
Scale length (mm)	24.54a	21.32b	20.70b
Number of sites per area	3	3	2

Different lowercase letters indicate differences ($P < 0.05$) among areas in cone traits, based on Tukey tests for unequal sample sizes.

heavier seeds compared with Ibiza (Table 2). The first principal component sorted cones according to cone size and the length and thickness of scales (Fig. 2), and separated cones on the Iberian Peninsula from those on the Balearic Islands. The second component was correlated with the number of scales and seeds per cone, and tended to separate cones on Mallorca from those on Ibiza (Fig. 2).
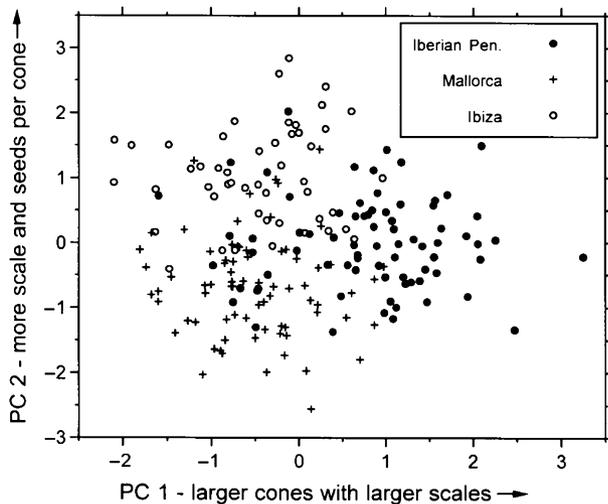

Fig. 2 Variation in cone structure among areas with *Sciurus* and crossbills (Iberian Peninsula), without *Sciurus* but with crossbills (Mallorca), and without both seed predators (Ibiza) in relation to the first two principal components (PC) of eight cone and seed traits of Aleppo pine. The first and second PC explained 63.4 and 21.9% of the variation, respectively. The cone traits with the highest loadings on the first PC were cone mass (0.97), cone width (0.95), cone length (0.95), scale length (0.92) and scale thickness (0.76). The cone traits with the highest loadings on the second PC were the number of seeds (0.84) and number of scales (0.71).

Natural selection by *Sciurus*

Mean length of cones depredated by *Sciurus* did not match mean cone length measured for each tree (Fig. 3). *Sciurus* tended to feed on larger cones when the available cones within a tree were relatively small (approximately <70 mm), whereas *Sciurus* avoided large cones when the tree produced longer (approximately >70 mm) and, thus, larger cones (Fig. 3). Based on such cone length preferences (Fig. 3), we expect that cones should evolve to lengths of 70 or more mm in areas with *Sciurus*. The average cone length on the Iberian Peninsula (75.79 mm; Table 2) was >70 mm (Fig. 3). To analyse tree selection by *Sciurus*, we conservatively included those trees in which the difference between the length of depredated cones (i.e. cone cores) and the length of cones remaining on the tree was <5 mm. Because several cone traits were highly correlated (Table 3), we included four cone traits in the multiple logistic regression: cone length, seed mass, full seed mass to cone mass and scale thickness ($r \leq 0.51$; Table 3). Multiple logistic regression indicated that cone length and perhaps scale thickness were the targets of selection (Table 4). Pairwise logistic regression showed that four cone traits were under selection (direct and indirect selection combined) by *Sciurus* (Table 4). *Sciurus* preferentially harvested shorter cones with fewer, thinner and shorter scales (Table 4), resulting in selection against trees that produced such cones.

Cone evolution in the absence of *Sciurus*

In the absence of *Sciurus*, we expect pines to evolve smaller cones as a result of relaxation of selection exerted
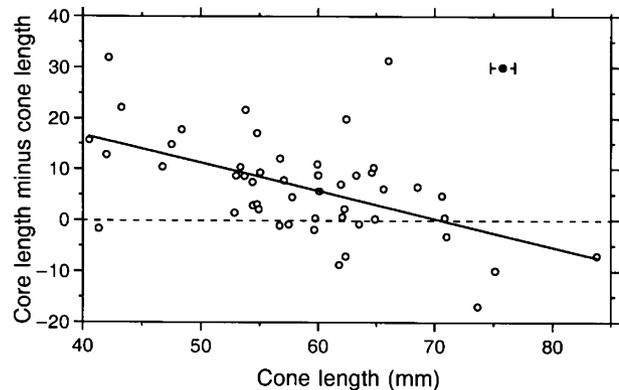

Fig. 3 Relationship between the lengths of cones measured for trees harvested by *Sciurus* and the difference between the lengths of cone cores (i.e. harvested cones) found at the base of the tree and the length of cones remaining in the tree. The filled circle indicates the mean (±SE) cone length found for three sites on the Iberian Peninsula (see Table 2). The solid line represents the best-fit linear regression ($r^2 = 0.27$, d.f. = 48, $F = 17.9$, $P < 0.0001$). The dashed line represents no difference between the lengths of predated and nonpredated cones.

Table 3 Product-moment pairwise correlations between various cone traits (transformed variables, $n = 96$ trees) in Valle del Cabriel, Valencia.
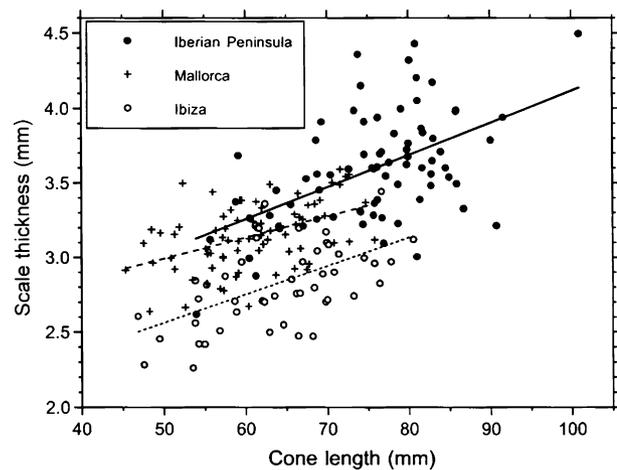
	Cone width	Number of scales	Cone mass	Number of seeds		Seed mass	Scale	
				Full	Empty		Thickness	Length
Cone length (mm)	0.809**	0.661**	0.822**	0.656**	0.123	0.318*	0.506**	0.783**
Cone width (mm)		0.482**	0.810**	0.471**	0.001	0.497**	0.598**	0.764**
Number of scales			0.464**	0.497**	0.002	0.130	0.179	0.292*
Cone mass (g)				0.671**	−0.010	0.548**	0.517**	0.780**
Number of full seeds					−0.073	0.044	0.284*	0.501**
Number of empty seeds						−0.148	−0.014	−0.004
Seed mass (mg)							0.418**	0.602**
Scale thickness (mm)								0.569**

*$P < 0.01$, **$P < 0.001$.

Table 4 Pairwise and multiple logistic regression analyses of natural selection on Aleppo pine from seed predation by *Sciurus vulgaris* in Valle del Cabriel, Valencia ($n = 67$ trees).

Trait	Pairwise logistic regression			Multiple logistic regression*			
	α	SE	P	α	SE	P	β
Cone length (mm)	0.11	0.04	0.010	0.10	0.05	0.047	0.02
Cone width (mm)	0.21	0.12	0.089	–	–	–	–
Number of scales	0.75	0.35	0.030	–	–	–	–
Cone mass (g)	0.14	0.07	0.062	–	–	–	–
Number of full seeds	0.03	0.02	0.091	–	–	–	–
Number of empty seeds	0.08	0.08	0.335	–	–	–	–
Seed mass (mg)	−0.01	0.10	0.898	−0.19	0.12	0.111	−0.03
Seed mass/ cone mass	9.01	25.92	0.728	−17.96	29.92	0.548	−2.75
Scale thickness (mm)	2.50	1.07	0.019	2.63	1.38	0.056	0.40
Scale length (mm)	0.24	0.11	0.026	–	–	–	–

*The whole multiple regression model was significant (maximum likelihood ratio test, $\chi_4^2 = 13.2$, $P = 0.011$).

Fig. 4 Scale thickness in relation to cone length for cones from the Iberian Peninsula (*Sciurus* and crossbills present), Mallorca (*Sciurus* absent and crossbills present) and Ibiza (both seed predators absent). Each symbol represents the mean of one tree. The lines represent the best-fit linear regressions for trees from the Iberian Peninsula (solid line: $r^2 = 0.39$, d.f. = 73, $F = 46.1$, $P < 0.0001$), Mallorca (dashed line: $r^2 = 0.23$, d.f. = 73, $F = 21.6$, $P < 0.0001$) and Ibiza (dotted line: $r^2 = 0.35$, d.f. = 48, $F = 26.0$, $P < 0.0001$).

by *Sciurus*. We also expect that under relaxation of selection by *Sciurus* but with selection by crossbills, cones develop proportionately thicker scales (Benkman *et al.*, 2001, 2003). However, cones in Mallorca were smaller versions of cones from the Iberian Peninsula with proportionately thinner scales (Fig. 4; ANCOVA, slopes: $F_{1,146} = 3.2$, $P = 0.07$; intercepts: $F_{1,147} = 8.8$, $P < 0.01$) and not smaller cones with thicker scales. One explanation for this result is that scale thickness is greater on the Iberian Peninsula, because selection on scale thickness by both *Sciurus* (Table 4) and crossbills was stronger than that exerted by crossbills alone. In the absence of *Sciurus* and crossbills, Aleppo pine on Ibiza has presumably reduced defenses against both seed predators, and cones had more seeds and a higher seed mass to cone mass ratio than on either the Iberian Peninsula or Mallorca

(Table 2). Furthermore, cone scales were disproportionately thinner on Ibiza than on Mallorca (Fig. 4; ANCOVA, slopes: $F_{1,121} = 2.5$, $P = 0.11$; intercepts: $F_{1,122} = 85.1$, $P < 0.0001$), implying that selection by crossbills favours thicker scales on Mallorca.

Discussion

Our results indicate that Aleppo pine has evolved defenses against its two main vertebrate seed predators. *Sciurus* appear to be the most important selective agent on Aleppo pine on the Iberian Peninsula favouring larger cones with larger scales. In the absence of *Sciurus*, Aleppo pine has evolved in response to relaxation of selection by *Sciurus* and crossbills appear to impact cone evolution,

favouring thicker scales and a lower ratio of seed mass to cone mass. Indeed, the overall level of defence in terms of the amount of cone mass per seed mass is similar regardless of whether *Sciurus* are present as long as crossbills are present. Whether crossbills are an important selective agent in the presence of *Sciurus* is unclear. Regardless, the presence of crossbills or both crossbills and *Sciurus* creates a geographic selection mosaic for Aleppo pine. In addition, the presence or absence of *Sciurus* determines whether apparent coevolution between crossbills and Aleppo pine occurs, further creating a geographic selection mosaic for crossbills. Below we discuss the evolution of defenses against predispersal seed predators in Aleppo pine and evidence for reciprocal adaptations between crossbills and Aleppo pine. We also point out the similarities and the main difference between our study and previous studies on similar systems (e.g. Benkman et al., 2001; Parchman & Benkman, 2002). As in these other studies, the evolutionary changes may have occurred fairly recently. Aleppo pine has probably been on Mallorca since the late Pleistocene based on crossbill fossils (Alcover et al., 1992; Seguí, 1997), but Aleppo pine probably was not present much earlier given the genetic similarity between Aleppo pine on Mallorca and the adjacent Iberian Peninsula (Agúndez et al., 1999).

Aleppo pine evolution in response to seed predators

Our evidence indicates that *Sciurus* preferentially harvested trees having shorter cones with thinner scales. Like other squirrels, *Sciurus* remove whole cones from the branch by biting cones off at their base and then feed on the seeds by biting off successive scales starting at the proximal end of the cone. Thus, an increase in scale thickness increases the quantity of tissue that *Sciurus* have to remove to reach the seeds (see also Benkman, 1999; Benkman et al., 2001). In some conifers, such as lodgepole pine, cones are wider at the base in areas with *Tamiasciurus* because this trait makes it more difficult for squirrels to bite off the cone from the branch (Smith, 1970; Benkman et al., 2001, 2003). However, cones of Aleppo pine are not sessile and are attached to branches by a relatively long stalk that *Sciurus* bite through to remove the cone. Consequently, an increase in cone width is not expected to deter *Sciurus* predation, in consonance with findings in another conifer (*P. mariana*) having nonsessile cones (Parchman & Benkman, 2002).

We also found that *Sciurus* showed some degree of cone selectivity within a tree, preferring larger cones when the available cones were relatively small and avoiding larger cones when cones were relatively large (Fig. 3; see also Moller, 1986). When foraging on the relatively small cones of Scots pine (*Pinus sylvestris*; mean length <40 mm), *S. vulgaris* preferred larger cones because they had more and larger seeds (Summers & Proctor, 1999). Larger cones of Aleppo pine also tend to

have more seeds, probably because of their correlation with cone length (Table 3), and thus more energy content, so *Sciurus* may select larger cones to increase the number of calories cached or ingested per cone. However, cone profitability is not only related to total energy content but to handling costs as well (e.g. Steele & Weigl, 1992). For example, previous work has shown that when *Tamiasciurus* forage on the relatively small cones of lodgepole pine (mean cone length <40 mm), *Tamiasciurus* exert direct selection on the number of seeds per cone and the seed mass to cone mass ratio (Benkman et al., 2003), which influence feeding rates (Smith, 1970), but do not exert selection on cone length or cone mass (Benkman et al., 2003; see Parchman & Benkman, 2002 for consistent results for *P. mariana*).

A preference by squirrels for smaller cones with thinner scales when foraging on relatively large-coned conifers may explain patterns of cone variation in other conifers. For example, Linhart (1978) found that cones of three conifer species in western North America were larger and had thicker scales in areas with squirrels (*Sciurus griseus* and *Tamiasciurus douglasii*) compared with areas where squirrels were absent. Cone lengths in these conifers ranged from 40 to 90 mm in one species and 70–160 mm in the other two. Similarly, Aleppo pine cones on the Iberian Peninsula average about 75 mm long, and for *Sciurus* and *Tamiasciurus*, such large cones may decrease profitability because of a disproportionate increase in handling time. Indeed, observations of *Sciurus* feeding on Aleppo pine cones suggest that *Sciurus* have more difficulties manipulating larger cones (J. Piqué, pers. comm.). Overall, this suggests that selection by squirrels tends to cause smaller coned species to evolve even smaller cones and for larger coned species to evolve larger cones.

Our quantification of the form of selection by *Sciurus* on Aleppo pine was in agreement with the geographic variation in Aleppo pine cone structure. Under relaxation of selection exerted by *Sciurus*, cones are expected to be smaller and have thinner scales, as was found for cones on Ibiza. In addition, cones had more seeds and higher seed mass to cone mass ratio, consistent with the lost of costly defenses and an increase in seed production. Cones in Mallorca were also smaller than those on the Iberian Peninsula, although scales were thicker compared with cones on Ibiza. This evidence is consistent with relaxation of selection by *Sciurus* and an increase of defenses directed at crossbills on Mallorca. Crossbills extract seeds from cones by prying apart the scales so that thicker scales increase their resistance to being spread apart. Cones in areas without squirrels have consistently evolved thicker scales to deter crossbill predation in other conifers (Benkman et al., 2001; Parchman & Benkman, 2002; T. L. Parchman and C. W. Benkman, unpubl. data), and it has been demonstrated that this cone trait was under direct selection by crossbills (Benkman et al., 2003). This investment in seed defenses against crossbills in the

absence of *Sciurus* also reduces seed production at the cone level (i.e. lower seed mass to cone mass ratio) and presumably reduces it at the tree level.

In the absence of *Sciurus* and crossbills, Aleppo pine cones had more seeds and higher seed mass to cone mass ratio, although individual seeds were lighter than those on both the Iberian Peninsula and Mallorca. We believe that the decrease in individual seed mass was a by-product of the decrease in scale size in response to relaxation of selection by seed predators, which is consistent with previous studies (Benkman *et al.*, 2001; Parchman & Benkman, 2002). Indeed, a comparison of individual seed mass among the three areas did not show differences after controlling for variation in scale thickness (ANCOVA: $F_{2,194} = 0.84$, $P = 0.43$). Alternatively, geographic variation in seed size might be the result of differences in precipitation regimes or frequency of fires favouring larger seeds in dryer areas (Leishman & Westoby, 1994) or those subjected to frequent fires (Escudero *et al.*, 2000). We used published data (Gil *et al.*, 1996) on annual and summer precipitation for each of the three areas on the Iberian Peninsula and the two islands as a surrogate of conditions experienced by seedlings during the two most crucial periods for their survival (i.e. rainy season and summer; Daskalakou & Thanos, 2004). Although these analyses are crude, the relationships between seed size and annual ($r = 0.68$, $n = 5$, $P = 0.21$) or summer precipitation ($r = 0.82$, $n = 5$, $P = 0.09$) were positive and nonsignificant in both cases. Thus, seeds did not tend to be larger in dryer areas. However, frequency of natural fires is similar among the Aleppo pine forests studied and there are no appreciable differences in fire frequencies between the mainland and the islands, or between the islands (DGCONA, 1994).

Finally, our inference that geographic variation in cone structure is the result of evolutionary responses to selection by seed predators rests on the condition that cone traits are heritable. This is reasonable given that cone and seed traits generally show moderate to high values of heritability in conifers (e.g. Stoehr & Farmer, 1986; Singh & Chaudhary, 1993; Benkman, 1999), including Aleppo pine (Matziris, 1998).

Have crossbills coevolved with Aleppo pine in the absence of *Sciurus*?

Results of the present study indicate that Aleppo pine cone structure has evolved in response to selection by crossbills on Mallorca in the absence of *Sciurus*. The relatively thick scales on Mallorca relative to Ibiza should deter crossbill seed predation and should select for a deeper bill to improve feeding performance and ultimately fitness, as in the case of the South Hills and Newfoundland crossbills in North America where squirrels are absent (Benkman *et al.*, 2001, 2003; Parchman & Benkman, 2002; Benkman, 2003). We have no information on feeding performance or the form of selection

on crossbills to evaluate whether crossbills coevolve with Aleppo pine in the absence of *Sciurus*. However, the available information indicates that Balearic crossbills (*L. c. balearica*) have shorter, more decurved and deeper bills than crossbills from the east and southeast part of the Iberian Peninsula (i.e. in areas where Aleppo pine forests predominate; Cramp & Perrins, 1994). This suggests that the bill size of the Balearic crossbill is an adaptation for foraging on Aleppo pine cones (see also Clouet, 2003), enabling them to be resident on Mallorca. Therefore, our results and previous information suggest that the Balearic crossbill and Aleppo pine have coevolved in the absence of *Sciurus*.

Despite the even larger cones with thick scales on the Iberian Peninsula, the crossbills are relatively small-billed. This indicates that crossbills on the Iberian Peninsula do not specialize on Aleppo pine and rely more on other pines like Scots pine (e.g. Castro *et al.*, 1999). Indeed, we suspect that selection by *Sciurus* favouring larger and thicker scaled cones is ultimately responsible for restricting the use of Aleppo pine by crossbills on the Iberian Peninsula and that only in the absence of *Sciurus* do crossbills coevolve with Aleppo pine. This indicates that crossbills presently have little evolutionary impact on Aleppo pine on the Iberian Peninsula, not so much because of preemptive competition from *Sciurus*, as found for *Tamiasciurus* within lodgepole pine forests in North America (Benkman, 1999; Benkman *et al.*, 2001, 2003), but because of past selection by *Sciurus* driving cones to evolve to a larger size. This difference provides the main contrast to previous studies on crossbills, squirrels and conifers in North America.

Conclusions

Cones in some conifers vary geographically in structure because of divergent selection regimes imposed by the different suite of seed predators present in different populations (Benkman *et al.*, 2001; Parchman & Benkman, 2002; Siepielski & Benkman, 2004). Crossbills in turn coevolve with conifers in those populations where squirrels are absent (coevolutionary hotspots) but coevolution does not occur (at least to the same extent) in those populations where squirrels are present (coevolutionary coldspots). We now have several examples in diverse systems of how the presence or absence of other species determines the location of coevolutionary hotspots and coldspots between interacting species (e.g. Thompson & Pellmyr, 1992; Benkman *et al.*, 2001). Our present knowledge indicates that hotspots where crossbills and conifers experience coevolution and divergent selection are located in forest islands within continents or oceanic islands in which squirrels are absent. This also calls for conservation of these and other unique areas where coevolution between crossbills and conifers are likely an ongoing

process, such as islands in the Mediterranean and Caribbean regions. Unfortunately, at least two documented examples illustrate how the introduction of *Tamiasciurus*, a dominant competitor for crossbills, determined the extinction of both the Newfoundland crossbill (*L. c. percna*) and a unique population of crossbills in the Cypress Hills (Canada) (Benkman, 1999; Parchman & Benkman, 2002).

Acknowledgments

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