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CONVERGENT PATTERNS IN THE SELECTION MOSAIC FOR TWO NORTH AMERICAN BIRD-DISPERSED PINES

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Abstract. The strength and outcome of interspecific interactions often vary across the landscape because of differences in community context. We investigated how the presence or absence of pine squirrels (*Tamiasciurus* spp.) influences the ecology and (co)evolution of seed-dispersal mutualisms between Clark's Nutcrackers (*Nucifraga columbiana*) and limber (*Pinus flexilis*) and whitebark (*P. albicaulis*) pines. Nutcrackers are the primary seed dispersers of these pines. Therefore both nutcrackers and pines potentially benefit from the evolution of a cone structure that enhances seed harvest by nutcrackers. Pine squirrels are the dominant predispersal seed predator of these pines throughout the Sierra Nevada–Cascades and Rocky Mountains and do not disperse seeds, so that pines benefit from deterring seed harvest by pine squirrels. To determine whether pines have evolved in response to selection by nutcrackers and pine squirrels, we conducted studies in ranges with pine squirrels and in ranges in the Great Basin and northern Montana where populations of the pines have apparently evolved without pine squirrels for 10 000 years or more. Cone evolution was convergent between both pines and among phylogeographically independent populations with and without pine squirrels, consistent with variation in selection by nutcrackers and pine squirrels. Where pine squirrels were present, they out-competed nutcrackers for seeds, nutcrackers were less abundant, and selection by pine squirrels constrained the evolution of cone and seed traits that facilitate seed dispersal by nutcrackers. In the absence of pine squirrels, nutcrackers were more than twice as abundant, selection by pine squirrels on cone structure was relaxed, and selection on cone structure by nutcrackers resulted in cones that increased the foraging efficiency of nutcrackers and improved their potential for seed dispersal.

Key words: coevolution; conflicting selection; convergent evolution; mutualism; Nucifraga columbiana; Pinus albicaulis; Pinus flexilis; *seed dispersal; seed predation;* Tamiasciurus.

Introduction

Few, if any, organisms interact solely with a single species in nature. Rather, most pairwise interactions are imbedded within larger communities of interacting taxa (i.e., a community context) that usually vary across the landscape (Strauss and Irwin 2004). This is particularly true for plants because they often interact with a diversity of species including herbivores, mycorrhizal fungi, pollinators, seed dispersers, and seed predators. As a result, different populations may experience differences in the forms or strength of selection (Thompson 2005). The factors determining the importance of a particular interaction as it occurs among communities of interacting species and in turn is translated into variation in microevolutionary processes shaping the evolution of phenotypic traits that mediate the interaction are not well understood (Strauss and Irwin 2004).

Differences in community context are particularly relevant to the coevolutionary process, because they can affect the way reciprocal interactions evolve (Thompson 1994, 2005). For example, geographic variation in the presence or absence of competitors (Benkman et al. 2001, Parchman and Benkman 2002), alternative hosts (Zangerl and Berenbaum 2003), and co-pollinators (Thompson and Cunningham 2002) can alter the potential for coevolutionary selection across the landscape. Thompson (1994, 2005) has argued that coevolution is fundamentally a population level process that often occurs in a geographic mosaic where (1) the forms of selection vary among populations yielding different evolutionary trajectories (selection mosaics), (2) coevolution occurs in some areas (coevolutionary hotspots) but not in others (coevolutionary cold spots), and (3) gene flow between populations can lead to mismatches in the traits of interacting species. Thus, the underlying (co)evolutionary and ecological dynamics of a focal interaction are best understood when they are investigated across the geographic ranges of interacting species (Thompson 2005).

One possible consequence of variation in community context is that some communities harbor species that exert conflicting selection pressures on traits important in a particular interaction, and such conflicts can mediate the coevolutionary process. For example, herbivores and predispersal seed predators, whose occurrence varies among plant populations, may select

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against floral traits facilitating pollination (Gómez 2003, Adler and Bronstein 2004, Cariveau et al. 2004) or seed dispersal (Fedriani et al. 2004). One outcome of conflicting selection pressures is that the potential for reciprocal selection and adaptation among a pair of species is limited, because a third species prevents selection from occurring or exerts selection inimical to the original pairwise interaction.

Seed dispersal mutualisms are widespread among plants (Herrera 2002) and are an excellent system to address the role of community context and conflicting selection pressures in the coevolutionary process (e.g., Jordano 1987). For example, the reproductive structures of conifers are likely under strong selection from seed dispersers and predispersal seed predators because of the fitness consequences associated with seed dispersal and seed predation (e.g., Smith 1970, Vander Wall and Balda 1977). Because the traits that facilitate seed dispersal may be some of the same traits that facilitate rather than deter seed predation, the potential for conflicting selection pressures is considerable (Benkman et al. 1984, Benkman 1995*a*). Despite the pervasiveness of seed dispersal mutualisms in nature, our knowledge of how conflicting selection pressures from organisms like seed predators alter the interaction and possibly the extent of mutualistic coevolution between plants and their seed dispersers is limited (Herrera 1985, Benkman 1995*a*).

Seed dispersal mutualisms between some large-seeded (>90 mg) pines (*Pinus* spp.) and the birds they rely on for primary seed dispersal (i.e., the initial dispersal from the tree) have considerable consequences to the many communities these pines dominate in the northern hemisphere (Lanner 1996, Tomback et al. 2001, Baumeister and Callaway 2006). At least 20 species of pines have seeds that are principally dispersed by birds, namely jays and nutcrackers (Corvidae; Tomback and Linhart 1990), and this interaction is thought to represent a coevolved mutualism (Vander Wall and Balda 1977, Tomback and Linhart 1990, Lanner 1996). With few exceptions (e.g., Benkman et al. 1984, Benkman 1995*a*, Vander Wall 1997, Tomback et al. 2005), however, prior studies on the ecology and evolution of bird-dispersed pines have largely ignored the importance of multiple interacting community members, and instead focused on cone and seed traits that facilitate seed dispersal, particularly by Clark's Nutcrackers (*Nucifraga columbiana*; see Plate 1). Such an approach, although insightful, removes the interaction from the broader community context. Most notably absent from these studies are pine squirrels (*Tamiasciurus* spp.), which are seed predators that frequently consume massive quantities of conifer seeds annually (Smith and Balda 1979) and have repeatedly been shown to be important selective agents on the cones that protect conifer seeds (Smith 1970, Benkman et al. 2001, 2003, Parchman and Benkman 2002, Siepielski and Benkman 2004). Bird-dispersed pines are no exception. The most important seed predators of bird-dispersed pines like limber pine (*P. flexilis*) and whitebark pine (*P. albicaulis*) are pine squirrels (Hutchins and Lanner 1982, Benkman et al. 1984, Benkman 1995*a*), which are found throughout most (but not all) of the ranges of these conifers. For example, pine squirrels harvest 80% of the cones of some bird-dispersed pines (Benkman et al. 1984). Moreover, Benkman (1995*a*) documented patterns of cone and seed trait differentiation in limber pine between areas with and without pine squirrels that were consistent with adaptations to deter seed predation by pine squirrels or the loss of such adaptations, respectively; however, those results were based on small sample sizes and selection was not measured. Regardless, those results suggest some bird-dispersed pines likely experience a selection mosaic, where some populations experience potentially conflicting selection from a seed predator (the pine squirrel) and its seed disperser (Clark's Nutcracker), whereas in other populations the seed predator is absent.

Here, we investigate how variation in the presence or absence of a preemptive competitor and seed predator, the pine squirrel, influences the ecology and (co)evolution of seed dispersal mutualisms between Clark's Nutcrackers and both limber and whitebark pines. First, we examine if nutcracker abundances are consistently different in the presence or absence of pine squirrels. If pine squirrels are strong preemptive competitors, we predict that nutcracker abundance should be lower in areas with pine squirrels in comparison to areas without pine squirrels. Second, we estimate the forms of phenotypic selection exerted by nutcrackers and pine squirrels on cone and seed traits of both pine species. Third, we quantify cone and seed traits in areas with and without pine squirrels to evaluate whether cone and seed traits have evolved in response to selection exerted by nutcrackers and pine squirrels. We predict selection by nutcrackers has resulted in the evolution of cone and seed traits that facilitate nutcracker seed harvest. In contrast, selection by pine squirrels should result in the evolution of cone and seed traits that deter seed predation by pine squirrels, possibly conflicting with selection by nutcrackers and thereby making seeds less accessible to nutcrackers. Because many cone traits are heritable (Cornelius 1994, Benkman 1999), an evolutionary response to selection is expected. We also examine whether the patterns of cone and seed trait evolution are convergent among areas with and without pine squirrels both within and between the two species of pines; a strong signature of adaptive evolution. Last, we predict that increased seed defenses against pine squirrels should result in lower seed harvesting rates by nutcrackers, indicating that if pine squirrels have an evolutionary impact on cone structure it constrains the mutualism between nutcrackers and pines.

METHODS

Study systems

We studied the interaction between limber and whitebark pines, Clark's Nutcrackers, and pine squirrels

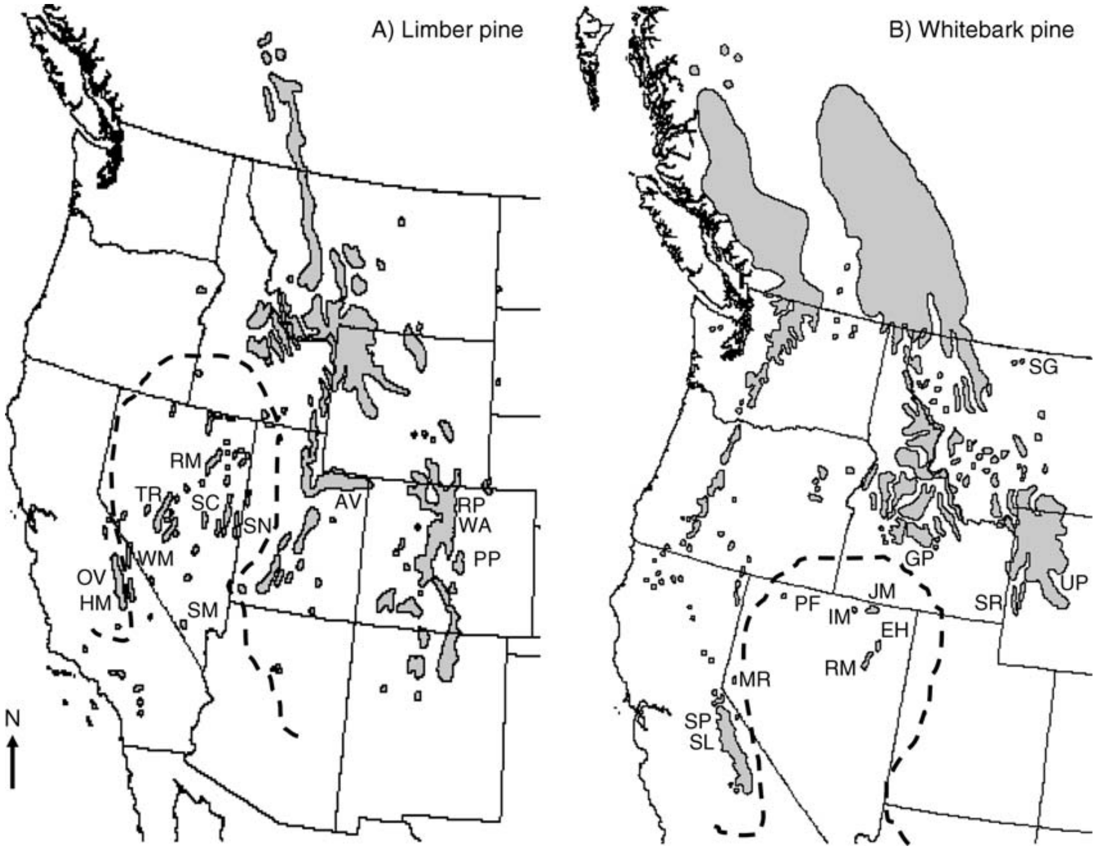

FIG. 1. The distributions of (A) limber pine (*Pinus flexilis*) and (B) whitebark pine (*P. albicaulis*), the occurrence of pine squirrels (*Tamiasciurus* spp., absent from south of the dashed line [mostly Nevada] and from the Sweet Grass Hills [SG] in northern Montana), and the location of study sites. Limber pine study sites from west to east are: Horseshoe Meadows (HM), Onion Valley (OV), White Mountains (WM), Toiyabe Range (TR), Spring Mountains (SM), Ruby Mountains (RM), Schell Creek Range (SC), Snake Range (SN), Avintaquin (AV), Rocky Mountain National Park (RP), Ward (WA), and Pikes Peak (PP). Whitebark pine study sites from west to east are: Mt. Rose (MR), Sonora Pass (SP), Saddlebag Lake (SL), Pine Forest Range (PF), Independence Mountains (IM), Jarbidge Mountains (JM), Ruby Mountains (RM), East Humboldt Range (EH), Galena Peak (GP), Sweet Grass Hills (SG), Salt River Range (SR), and Union Pass (UP). Distributions of conifers are based on Little (1971).

across three regions in western North America: the Sierra Nevada, Great Basin, and Rocky Mountains (Fig. 1). Pine squirrels may have been present in the Great Basin (Heaton 1990; see also Wells 1983, Grayson 1987) and, if so, they presumably went extinct in the last 12 000 years (Grayson 1987, Heaton 1990) perhaps related to an increasingly fragmented distribution of conifers (Arbogast et al. 2001). Pine squirrels have most likely been absent from the Sweet Grass Hills east of the Rocky Mountains in north central Montana (Fig. 1) before and following the colonization of whitebark pine about 10 000 years ago as it spread from the Rocky Mountains (Richardson et al. 2002). Phylogeographic studies of limber and whitebark pines indicate that their populations in the regions with pine squirrels, the Sierra Nevada and Rocky Mountains, are divergent from each other with no gene flow evident between them (Mitton et al. 2000, Richardson et al. 2002). Similarly, it is unlikely that gene flow occurs between the two areas without pine squirrels in the Great Basin and the Sweet Grass Hills (Fig. 1), because the nearest site in the Great Basin without pine squirrels (the Jarbidge Mountains, Nevada) is more than 800 km away (Fig. 1). The lack of gene flow between the regions with and without pine squirrels allows us to treat them as independent samples and to evaluate whether cone and seed traits are also convergent between two separate regions both with and without pine squirrels. Furthermore, limber and whitebark pines belong to separate reasonably well-supported clades (Gernandt et al. 2005) where the evolution of seed dispersal by birds (i.e., loss of seed wings [Tomback and Linhart 1990]) has been independent (A. Siepielski and C. Benkman, *unpublished data*).

Clark's Nutcrackers shred the scales of closed cones (see Plate 1) to harvest seeds, and have a unique sublingual pouch that allows them to carry upwards of 100 seeds at a time to their cache locations (see Lanner 1996). A single nutcracker harvests and caches an estimated 35 000–98 000 limber or whitebark pine seeds between early August and late October during years of large cone crops (Hutchins and Lanner 1982, Tomback 1982; see also Lanner 1996); three to five times the number of seeds required during the following nine months or so (e.g., Tomback 1982). Because nutcrackers transport seeds upwards of 22 km, place them in small caches suitable for germination, and many seeds are not retrieved, nutcrackers disperse seeds (Vander Wall and Balda 1977, Tomback 1982, Lanner 1996).

Pine squirrels (*T. douglasii* in the Sierra Nevada–Cascades and *T. hudsonicus* in the Rocky Mountains) are found throughout montane areas in North America and are common in coniferous forests (Smith 1970). Pine squirrels cache cones in middens soon after the seeds mature in late summer and early autumn, but before cone scales begin to separate (Smith 1970, Benkman et al. 1984). Seeds in cached cones provide most of the winter food for pine squirrels (Smith 1970), and are unavailable to nutcrackers. Because no animal other than pine squirrels harvests cones as intensively each year (Smith and Balda 1979, Hutchins and Lanner 1982), pine squirrels are especially effective preemptive competitors to nutcrackers. Pine squirrels are not seed dispersers, because they bury closed cones not individual seeds (Smith 1970, Benkman et al. 1984) and conditions in middens do not favor germination or seedling survival (Hutchins and Lanner 1982, Tomback 1982).

Although nutcrackers and pine squirrels are the most important consumers of seeds in limber and whitebark pine cones, small mammals and insects also consume seeds of these conifers. Because small mammalian seed predators (e.g., *Peromyscus* spp., *Tamias* spp.; see Smith and Balda 1979) mostly harvest seeds that have fallen to the ground, these mammals are not expected to have an important selective impact on seed defenses of the cone (Smith 1970, Benkman 1995*a*); although they may act as secondary seed dispersers (e.g., Tomback et al. 2005). Selection by insects can affect cone evolution (Siepielski and Benkman 2004), but the amount of seeds consumed by insects is generally much less than that consumed by nutcrackers or pine squirrels. For example, insects damaged less than 10% of the seeds in limber pine cones (Lanner and Vander Wall 1980, Benkman et al. 1984) and less than 27% of seeds in whitebark pine cones (Kegley et al. 2001). Moreover, the various cone and seed insects and small mammals in the genera that include pine seed predators (see Smith and Balda 1979) occur throughout the ranges of both conifers (Hedlin et al. 1980, Kays and Wilson 2002), so that predation by these seed predators is not expected to confound our results (i.e., comparisons between areas with and without pine squirrels).

Nutcracker abundance in relation to both the presence and absence of pine squirrels and cone abundance

We used 10-min, 50-m fixed-radius point counts to estimate nutcracker abundance in mountain ranges with and without pine squirrels. For each pine, six mountain ranges with pine squirrels and five without them were visited during both 2004 and 2005 (Fig. 1; Appendix A). Within each mountain range, we located roughly contiguous mature stands of either limber or whitebark pine and laid out a single transect bisecting the stand; the starting point was haphazardly chosen. We established five point-count locations at approximately 500-m intervals along each transect. Point counts were conducted between 06:00 and 11:00 hours in mid-August to early September when cones were closed, seeds were mature, and nutcrackers were frequently observed caching seeds, indicating nutcrackers were using local habitat and not just passing through. Both the order of visitation of sites and the order of counts were reversed the second year to minimize potential daily and seasonal effects. We assumed detection probabilities did not differ in a consistent manner between regions with and without pine squirrels because of the overall similarity of study areas and only a single observer (A. M. Siepielski) conducted the counts.

Because variation in cone crop size could also influence nutcracker abundance, we estimated cone abundance at each point count location by counting the number of cones present on the 10 nearest trees using 10×40 binoculars. Cone counts were highly repeatable, based on 50 trees recounted a week later at the study site (without pine squirrels that might remove cones) in the Schell Creek Range, Nevada (repeatability = 0.82 [Lessels and Boag 1987]). We used mean nutcracker and mean cone abundance estimates combined over all point counts within a range to provide one estimate for each parameter per year because each mountain range is the experimental unit. We used linear regressions to examine the relationships between nutcracker and cone abundances and ANCOVA to compare mean nutcracker abundance relative to cone abundance between areas with and without pine squirrels. Because year was a significant factor in the ANCOVA models ($P < 0.05$), we analyzed the data separately for each year. We checked for assumptions of normality and homogeneity of variance, and used log transformations to improve normality where necessary.

Targets and form of phenotypic selection exerted by nutcrackers

We used regression analyses to estimate the targets and form of phenotypic selection exerted by nutcrackers on cone structure (Lande and Arnold 1983). For limber pine, we recorded both the number of cones that had signs of nutcracker harvesting activity (shredded cones on trees; see Plate 1) and the total number of cones for 69 trees in the Schell Creek Range, Nevada on 9–11 August 2004. Trees were chosen haphazardly by walking through the study areas. For whitebark pine, the same data were recorded for 85 trees in the Jarbidge Mountains, Nevada on 12–14 September 2004. During sampling, cones were closed and nutcrackers had already harvested seeds from >50% of many trees (A. Siepielski, *personal observation*). Nutcrackers were usually observed storing seeds in their sublingual pouches and then flying off, indicating nutcrackers were caching seeds. We estimated the number of seeds removed as the number of full seeds (i.e., seed filled with female gametophyte) per cone multiplied by the total number of cones on the tree multiplied by the proportion of cones with seeds harvested. This value is a component of tree fitness under the assumption that the more seeds harvested the more seeds cached with the potential for germination (e.g., Tomback 1982, Lanner 1996; see also Jordano 1995), and we use it is as a surrogate for fitness in the context of selection by nutcrackers.

Because previous studies found that within-tree variation in cone and seed traits of pines is considerably smaller than among tree variation (Smith 1968, Elliott 1974), and this was consistent with our observations, we collected what appeared to be representative cones from each tree and measured two of them from each tree. If nutcrackers were selective of cones within as well as between trees, then the remaining cones we sampled would tend to be more similar to cones on avoided trees, and therefore if anything we would underestimate the strength of selection. The following cone and seed traits were measured: closed cone length, maximum width of closed cone, peduncle diameter, the thickness of six scales from the middle portion of the distal third and the proximal third of the cone (scales were selected approximately equidistant around the cone and were measured near their distal end), number of scales crossed by a transect along the central axis of the cone, the distance from the distal end of the scale to the seed, cone mass with seeds removed, number of empty and full seeds, and for five seeds from each cone we measured kernel masses, and seed coat masses and thicknesses (seed coat thickness was measured at the flattest surface of the seed coat). For limber pine, we also recorded the proportion of reflexed scales (cone scales that were curved toward the proximal end of the cone) in the proximal third of the cone (Fig. 2). The ratio of total seed mass to cone mass was calculated as the number of full seeds multiplied by the mean mass of an individual seed divided by cone mass. All length measurements were made to the nearest 0.01 mm with digital calipers. All mass measurements were made to the nearest 0.1 mg with a digital scale after cones and seeds were oven-dried at 60–70°C for $\geq$2 days. We used mean trait values per tree in all analyses.

For the selection analyses, cone and seed traits were standardized to zero mean and unit variance and individual tree fitness was converted into relative fitness by dividing individual tree fitness by mean population fitness. Multiple linear regressions were used to identify the traits under direct selection (i.e., the targets of selection) relative to the effects of other correlated traits (Lande and Arnold 1983). To avoid problems with multicollinearity that could obscure the detection of traits under direct selection we examined variance inflation factor (VIF) scores from regression models and correlation coefficients between traits (Appendices B and C); traits contributing strongly to multicollinearity (VIF scores > 10 [SAS Institute 2003]) were removed. Linear selection gradients (β) from regressions between each trait and relative tree fitness were used to identify the traits under selection (both direct and indirect) by nutcrackers; second order terms and cubic splines (Schluter 1988) were examined to determine if nonlinear selection was evident. We found departures from normality in the residuals for some whitebark pine models, thus we used the paired regression bootstrap technique of Efron and Tibshirani (1993). Tests of significance for regression coefficients were based on 1000 bootstrap replicates.

Direction of phenotypic selection exerted by pine squirrels

Pine squirrels either intensively harvest cones from trees or largely avoid trees (see Smith 1970, Benkman et al. 2003). Thus, the direction of phenotypic selection exerted by pine squirrels on cone and seed traits was estimated by using a paired design to compare differences in cone and seed traits between trees intensively harvested or largely avoided by pine squirrels. The data for limber pine were gathered near Ward ($n = 32$ territories) and Sugar Loaf Mountain ($n = 18$ territories), Colorado on 15–21 September 2003; these two sites were ~14 km apart. Data for whitebark pine were gathered from 50 squirrel territories near Union Pass in the Wind River Range, Wyoming on 20–28 September 2004. Two to three cones were collected from the base of one tree that was being intensively harvested (i.e., the squirrel was observed repeatedly removing cones from the tree and caching them) and from a non-harvested tree adjacent to each harvested tree on each squirrel territory. The adjacent (avoided) trees were of similar size, and seeds and cones were of similar ripeness as judged by visual inspection. Trees intensively harvested by pine squirrels had few cones remaining on their branches, indicating that seeds from these trees were unlikely to be harvested by nutcrackers and had little opportunity for successful dispersal. All of the abovementioned cone and seed traits were measured from two cones from each tree and differences in trait means between the paired 50 harvested and 50 avoided trees were made using paired t tests.

Geographic variation in cone and seed structure

Recently mature, closed limber and whitebark pine cones were collected from ranges with and without pine squirrels during late August and early September (Fig. 1, Appendix A). We sampled both limber and whitebark pine cones from six areas with and six areas without pine squirrels from a limited latitudinal and elevational range. We gathered cones from each of 30 trees in each area using a 9-m extension pole with a clipper attached. Trees were randomly chosen within each area by following a random compass bearing and distance, and then selecting the nearest cone-bearing tree; the initial point was haphazardly chosen.

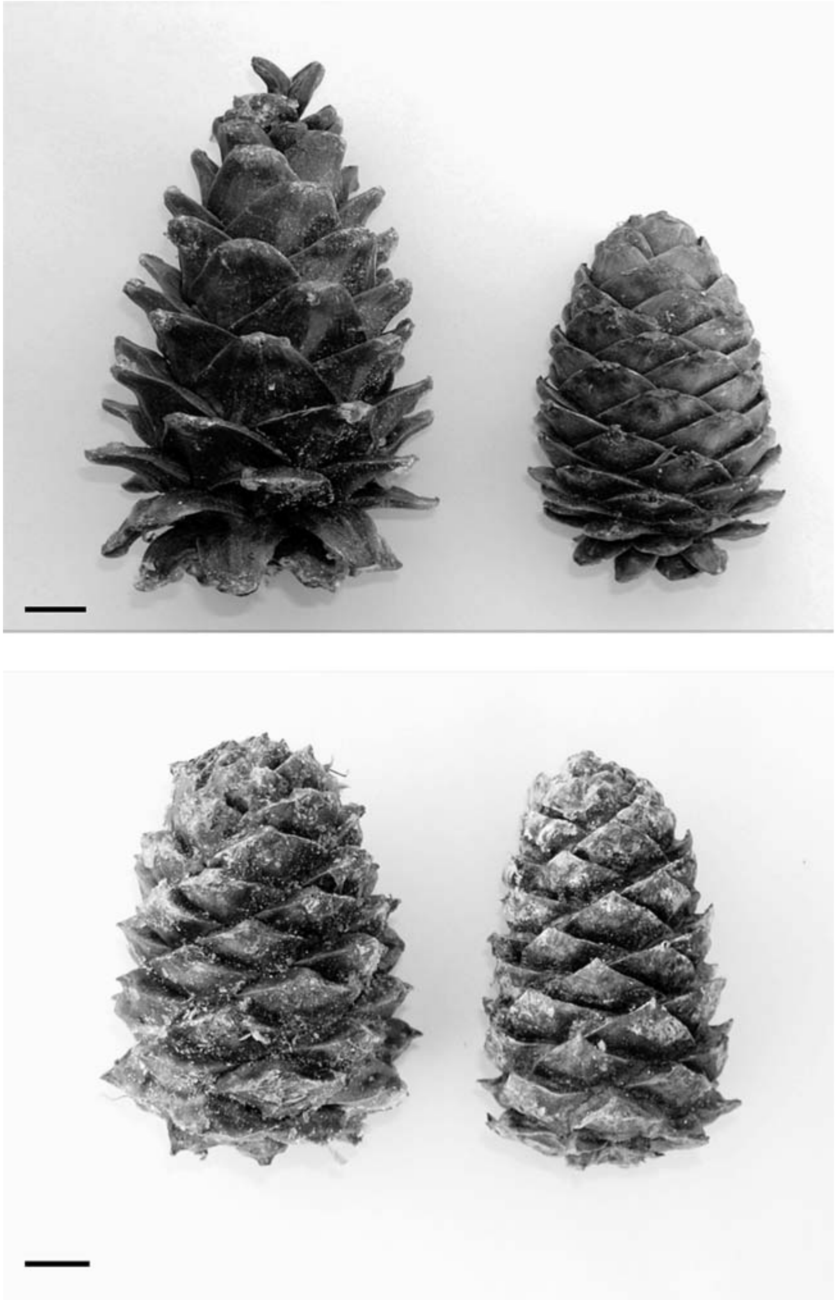

FIG. 2. Photographs of limber pine cones (top) and whitebark pine cones (bottom) from areas with (left side) or without (right side) pine squirrels. The scale bar is approximately 10 mm. These cones were chosen as representative of each region. Note the similarity between limber and whitebark pine cones from areas without pine squirrels.

We measured the same traits as above for two cones from each tree and used ANOVA and principal components analysis (PCA) on mean trait values per tree to characterize variation in cone and seed traits among study areas. First, we used two-level nested ANOVA to test for differences in cone and seed traits among and between ranges with and without pine squirrels. In addition, for both pines, we used ANOVA to compare cone and seed traits between the two regions (Sierra Nevada and Rocky Mountains) with pine squirrels, and, for whitebark pine, between the ranges in the Great Basin and the Sweet Grass Hills where pine squirrels are absent. We tested for normality and homogeneity of variance and found slight departures for some traits. However, ANOVA is generally robust to departures from normality. Plots of residuals against trait values did not reveal any consistent patterns in heterogeneity of variances. We log-transformed some variables to improve normality and variance. Second, PCA was used to reduce the dataset to two variables, which also served as a method of evaluating whether the patterns of differentiation in cone structure between areas with and without pine squirrels were similar between the two pines (e.g., Parchman and Benkman 2002). This was also used to examine if the patterns of cone and seed trait evolution for both pines were convergent between the two regions with pine squirrels and, for whitebark pine, between the two regions without pine squirrels. For this analysis, we used the correlation matrix from the following traits: cone length, cone width, cone mass, peduncle diameter, distal and proximal scale thickness, number of scales, number of full seeds, individual seed kernel mass, and seed coat thickness. We developed the principal components (PC) equation based on data for limber pine in areas with and without pine squirrels, and used this equation to derive PC scores for whitebark pine. Qualitatively, a similar pattern was found when the equations were based on whitebark pine. Spearman rank correlations between individual traits and the PC scores were used to confirm that traits loaded in a similar fashion (magnitude and sign) between the two conifers.

We generated fitness surfaces of conifer cone structure using nonparametric projection pursuit regression models (Schluter and Nychka 1994) using the data from the above selection analyses. The dependent variables were the standardized number of seeds removed by nutcrackers, and whether a tree was harvested (i.e., fitness = 0) or not (fitness = 1) by pine squirrels. We caution that the squirrel selection data do not represent a random sample from the population (i.e., it was a paired design). Thus, the fitness surfaces are meant only to visually depict the general direction of selection by pine squirrels. We used PC1 and PC2 as the independent variables so that we could draw relationships between the fitness surface and geographic differences in PC1 and PC2 between regions with and without pine squirrels. Values of λ, the smoothing parameter, were chosen by first exploring a range of values of λ, and then choosing the value that minimized the generalized cross validation (GCV) mean square error as in Schluter and Nychka (1994); the GCV score is a metric of the prediction error associated with a particular value of λ (Schluter 1988). The surface was then drawn using a smoothing spline interpolation as implemented in PROC G3GRID in SAS (SAS Institute 2003). We use these fitness surfaces only as a qualitative way of examining similarities and differences in the form of selection exerted on cone and seed structure by nutcrackers and pine squirrels and for examining whether such selection could account for differences in cone and seed traits between regions with and without pine squirrels.

Foraging rates of nutcrackers in areas with and without pine squirrels

We opportunistically recorded the length of time nutcrackers spent removing seeds from limber and whitebark pine cones using a stopwatch and 10 × 40 binoculars. During foraging bouts, we recorded the total number of seeds removed, the total length of time to the nearest second to remove those seeds, and cone ripeness (cones open or closed). We did not include time spent removing seed coats and consuming kernel, which occurred infrequently during observations. We only gathered data on adult birds because juvenile nutcrackers forage more slowly than adults (Tomback 1998). It is possible that some birds were recorded on more than one occasion as independent observations. However, we were able to distinguish among individuals over a short time period based on differences in body size, plumage, and facial characteristics (e.g., Tomback 1982), and avoided recording more than one foraging bout from the same individual so identified. We further minimized the chances of recording the same birds by moving throughout the study area during our observations.

We used a two-factor (pine squirrels: present or absent; cone: closed or open) ANOVA with an interaction term to compare differences in foraging rates (seeds per second) between areas with and without pine squirrels and to determine whether these differences were related to variation in cone ripeness, because foraging rates are expected to increase as cones ripen (e.g., Vander Wall 1988). Mean number of seeds per second per bird was used in all analyses. We checked for normality and homogeneity of variance and log transformed the data to improve normality in some cases.

Results

Nutcracker abundance in relation to cone abundance and the presence/absence of pine squirrels

Nutcrackers generally increased in abundance with increases in the number of cones per tree and were more abundant in ranges without than with pine squirrels for both limber and whitebark pines (Fig. 3).

Selection exerted by nutcrackers and pine squirrels

Nutcrackers exerted selection on cone and seed traits that was similar for both limber and whitebark pines (Table 1). For example, the target of selection (direct selection) for both pines was the number of full seeds, with selection by nutcrackers favoring cones with more full seeds (multiple regressions in Table 1). Overall, selection (direct and indirect) by nutcrackers favored trees having cones with more full seeds (Table 1; selection also favored trees having cones with more scales because there are two seeds per scale), shorter, thinner scales, and thinner seed coats (Table 1). With a decrease in cone scale size and an increase in seed number, selection by nutcrackers also favored an increase in the ratio of seed mass to cone mass (Table 1). We found no evidence of nonlinear selection.

Selection exerted by pine squirrels was also similar for both pines (Table 2). Pine squirrels preferentially harvested trees having narrower and less heavy cones with more full seeds, thinner cone scales, and thinner

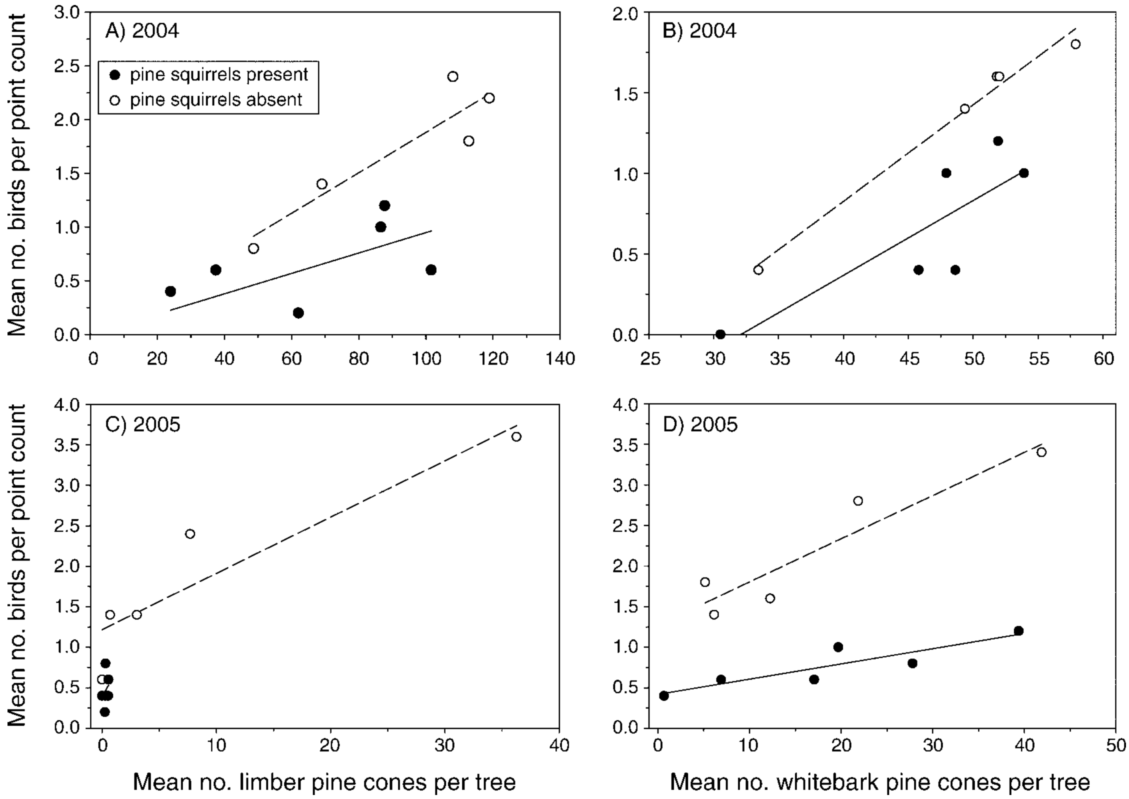

FIG. 3. The relationship between abundance of Clark's Nutcracker (*Nucifraga columbiana*) and limber or whitebark pine cone abundance between mountain ranges with and without pine squirrels in 2004 and 2005. Nutcracker abundance increased significantly with increases in cone abundance in areas without pine squirrels for both (A, C) limber pine (2004, $r^2 = 0.84$, $t_3 = 3.97$, $P = 0.028$; 2005, $r^2 = 0.84$, $t_3 = 4.01$, $P = 0.027$) and (B, D) whitebark pine (2004, $r^2 = 0.98$, $t_3 = 14.13$, $P = 0.008$; 2005, $r^2 = 0.88$, $t_3 = 4.71$, $P = 0.018$). Nutcracker abundance also increased with increases in whitebark pine cone abundance in areas with pine squirrels (2004, $r^2 = 0.68$, $t_4 = 2.95$, $P = 0.042$; 2005, $r^2 = 0.79$, $t_4 = 3.93$, $P = 0.017$), but nutcrackers did not increase in abundance with increases in limber pine cone abundance in areas with pine squirrels in either year (2004, $r^2 = 0.29$, $t_4 = 1.28$, $P = 0.268$; 2005, $r^2 = 0.067$, $t_4 = 0.54$, $P = 0.618$). In both years, nutcrackers were significantly more abundant in mountain ranges without pine squirrels than in ranges with pine squirrels whether dominated by limber pine (2004, ANCOVA, $F_{1,8} = 9.47$, $P = 0.015$; 2005, ANCOVA, $F_{1,8} = 9.96$, $P = 0.013$) or whitebark pine (2004, ANCOVA, $F_{1,8} = 17.14$, $P = 0.003$; 2005, ANCOVA, $F_{1,8} = 47.09$, $P = 0.0001$).

seed coats. For limber pine alone, pine squirrels preferentially harvested trees whose cones were shorter and had a lower proportion of reflexed cones scales. Overall, selection by pine squirrels favored a reduction in the ratio of seed mass to cone mass (Table 2), which conflicts with selection exerted by nutcrackers.

Geographic variation in cone and seed structure

Nearly all measured limber and whitebark pine cone and seed traits were significantly different between areas with and without pine squirrels and differences between these two regions accounted for on average 55.5% and 33.3% of the variation for limber and whitebark pines, respectively (Appendices D and E). Many traits were also significantly different between sites; however, on average variation between areas with and without pine squirrels was 18 and five times greater than variation among sites for limber and whitebark pines, respectively.

Limber pine cones from areas with pine squirrels were longer, wider, heavier, had fewer but thicker cone scales, and fewer seeds than areas without pine squirrels (Figs. 2 and.4; Appendix D). Whitebark pine cones from areas with pine squirrels were significantly wider and heavier but not longer, had fewer, but larger cone scales, and fewer seeds than areas without pine squirrels (Figs. 2 and 4; Appendix E). Although kernel mass did not differ between areas with and without pine squirrels, seed coat thicknesses and masses were greater and, thus, individual seeds were heavier in areas with pine squirrels than without them for both pines (Appendices D and E).

Patterns of convergence in whitebark and limber pine cone and seed structure

The first principal component (PC1) accounts for most (58.0%) of the variation in limber pine cone structure, and represents overall changes in cone size, with increasing values indicating larger, particularly wider, heavier cones with thicker cone scales, thicker peduncles, fewer seeds (and scales), and seeds with thicker seed coats (Appendix F). The second principal

TABLE 1. Selection gradients (β) from pairwise and multiple linear regression analyses of phenotypic selection exerted by Clark's Nutcrackers because of seed harvesting on limber pine in the Schell Creek Range, Nevada ($n = 69$ trees), and on whitebark pine in the Jarbidge Mountains, Nevada ($n = 85$ trees).

	Selection on limber pine		Selection on whitebark pine	
Trait	Pairwise linear regression	Multiple regression	Pairwise linear regression	Multiple regression
Cone length (mm)	−0.07 (0.14)	−0.11 (0.13)	0.26 (0.06)****	0.07 (0.03)
Cone width (mm)	−0.24 (0.14)	−0.15 (0.12)	−0.14 (0.06)*	−0.01 (0.04)
Cone width/length	−0.14 (0.14)		−0.34 (0.05)****	
Cone mass (g)	−0.15 (0.14)		0.07 (0.06)	
Number of scales	0.46 (0.13)**		0.36 (0.05)****	
Distal scale thickness (mm)	−0.34 (0.13)*		−0.30 (0.06)****	
Proximal scale thickness (mm)	−0.37 (0.13)**	0.18 (0.15)	−0.31 (0.06)****	−0.07(0.04)
Percentage reflexed proximal scales	−0.02 (0.14)		†	
Peduncle diameter (mm)	0.24 (0.14)		−0.13 (0.06)*	
Distance to seed (mm)	−0.57 (0.12)****		−0.17 (0.06)**	
Total number of seeds	0.76 (0.10)****		0.52 (0.03)****	
Number of full seeds	0.83 (90.10)****	0.91 (0.12)****	0.52 (0.03)****	0.44 (0.05)****
Number of empty seeds	−0.29 (0.14)*		−0.09 (0.06)	
Seed mass (mg)	−0.05 (0.14)		−0.19 (0.06)**	
Kernel mass (mg)	0.03 (0.14)	−0.001 (0.11)	−0.11 (0.06)	0.005 (0.03)
Seed coat mass (mg)	−0.14 (0.14)		−0.20 (0.06)**	
Seed coat thickness (mm)	−0.44 (0.13)**	−0.001 (0.14)	−0.23 (0.06)**	−0.04 (0.04)
Total seed mass/cone mass	0.65 (0.11)****		0.41 (0.05)****	
PC1	−0.47 (0.13)**		−0.44 (0.04)****	
PC2	0.29 (0.14)*		−0.47 (0.03)****	

Note: Values reported are β with SE in parentheses.
*$P \le 0.05$; ** $P \le 0.01$; **** $P \le 0.0001$.
† Trait not measured.

component (15.6% of variation) characterizes changes in cone length and mass, numbers of cone scales and seeds, with increasing values indicating increases in these traits (Appendix F). The magnitudes and signs of correlations between individual traits and PC scores were similar for both pines (Appendix F), indicating that PC1 and PC2 captured similar patterns of variation in both pines. The patterns of cone and seed trait differentiation, as well as

TABLE 2. Limber and whitebark pine cone and seed traits from adjacent trees that were either unharvested or harvested by pine squirrels.

	Selection on limber pine			Selection on whitebark pine		
Trait	Unharvested	Harvested	*t*	Unharvested	Harvested	*t*
Cone length (mm)	88.15 (1.37)	75.35 (1.29)	−7.18****	63.00 (1.35)	63.56 (1.14)	0.32
Cone width (mm)	46.11 (0.74)	39.11 (0.57)	−8.34****	48.78 (0.49)	45.69 (0.86)	−3.24**
Cone width/length	0.53 (0.007)	0.52 (0.007)	−0.47	0.79 (0.01)	0.72 (0.01)	−3.31**
Cone mass (g)	27.12 (1.15)	17.19 (0.68)	−8.11****	23.04 (0.58)	21.24 (0.64)	−2.10*
Number of scales	11.59 (0.20)	11. 21 (0.20)	−1.49	8.36 (0.21)	9.23 (0.25)	2.72**
Distal scale thickness (mm)	2.56 (0.06)	1.89 (0.05)	−9.99****	5.08 (0.09)	4.45 (0.14)	−4.12**
Proximal scale thickness (mm)	3.70 (0.09)	2.59 (0.06)	−11.89****	6.24 (0.12)	5.50 (0.14)	−3.75**
Percent reflexed proximal scales	0.72 (0.02)	0.38 (0.02)	−14.32****	†	†	†
Peduncle diameter (mm)	7.34 (0.12)	5.36 (0.10)	−14.81****	7.29 (0.13)	7.02 (0.17)	−1.30
Distance to seed (mm)	11.97 (0.22)	10.80 (0.24)	−3.61**	10.04 (0.22)	9.26 (0.18)	−2.63**
Total number of seeds	33.52 (2.51)	56.86 (2.55)	6.53****	51.81 (2.14)	66.52 (2.08)	5.57****
Number of full seeds	19.68 (2.17)	50.21 (2.50)	9.57****	45.79 (2.10)	61.42 (2.02)	6.01****
Number of empty seeds	13.85 (1.02)	6.65 (0.47)	−6.85****	6.02 (0.27)	5.10 (0.28)	−2.55*
Seed mass (mg)	88.77 (0.003)	68.77 (0.002)	−5.56****	167.69 (0.004)	171.19 (0.003)	0.99
Kernel mass (mg)	40.75 (0.001)	37.61 (0.002)	−1.47	103.38 (0.002)	106.24 (0.002)	1.09
Seed coat mass (mg)	48.01 (0.002)	31.15 (0.002)	−6.50****	64.30 (0.002)	65.64 (0.002)	0.54
Seed coat thickness (mm)	0.35 (0.007)	0.30 (0.007)	−5.10****	0.33 (0.007)	0.30 (0.01)	−2.50*
Total seed mass/cone mass	0.07 (0.008)	0.20 (0.01)	11.11****	0.23 (0.01)	0.52 (0.02)	7.21****
PC1	0.76 (0.10)	−0.76 (0.08)	−12.78****	0.72 (0.17)	−0.72 (0.30)	−6.36****
PC2	0.09 (0.14)	−0.09 (0.14)	−0.97	−0.31 (0.22)	0.31(0.20)	2.74**

Notes: Values are means with SE in parentheses. The data for limber pine were gathered near Ward and Sugarloaf Mountain, Colorado ($n = 100$ trees), and data for whitebark pine were collected at Union Pass, Wyoming ($n = 100$ trees). We sampled 2–3 cones from each of 50 unharvested and 50 pine-squirrel-harvested paired trees for each species. Statistical comparisons were made using paired *t* tests.
*$P \le 0.05$; ** $P \le 0.01$; **** $P \le 0.0001$.
† Trait not measured.

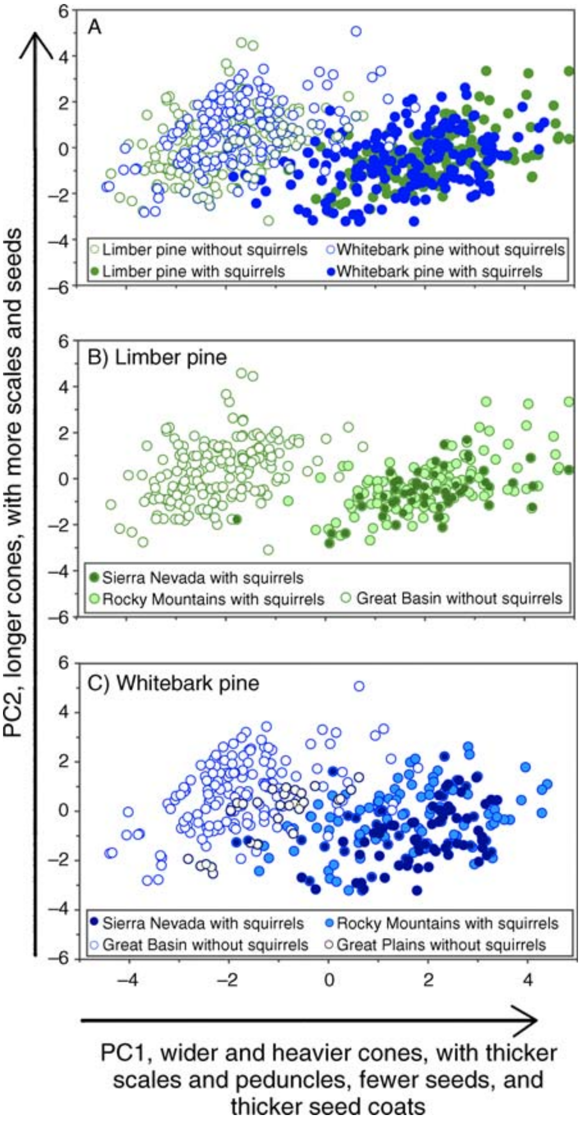

FIG. 4. (A) Convergent patterns in cone structure of limber pine (green circles) and whitebark pine (blue circles) between areas with and without pine squirrels in relation to the first two principal components of 10 cone and seed traits ($n = 360$ trees divided equally among the four categories; each circle is an individual tree; see Appendix H). (B, C) Variation in limber pine and whitebark pine cone structure in relation to the first two principal components of 10 cone and seed traits (sample sizes as in panel A), showing convergence among areas with and without pine squirrels.

the overall magnitudes of change, were similar for both pines between areas with and without pine squirrels (Figs. 2 and 4A). This indicates convergence in the same sets of traits for both pines in areas with or without pine squirrels, and is consistent with the directions of selection exerted by nutcrackers and pine squirrels (Fig. 5, Tables 1 and 2); selection by pine squirrels favors the evolution of large PC1 values whereas selection by nutcrackers in the absence of pine squirrels favors the evolution of small PC1 values.

Cone structure was also similar between the two regions with pine squirrels (Sierra Nevada and Rocky Mountains) for both limber (Fig. 4B) and whitebark pines (Fig. 4C) even though some individual cone and seed traits differed significantly between the two regions (Appendices D and E). For example, the ratio of seed mass to cone mass was lower in the Sierra Nevada compared with the Rocky Mountains for both pines. This was due in part to limber pine cones in the Sierra Nevada having fewer full seeds than cones from the Rocky Mountains, and whitebark pine having heavier cones in the Sierra Nevada than in the Rocky Mountains. However, for most comparisons the mean percent variation that was accounted for between these two regions (2.0% for limber pine and 6.3% for whitebark pine) was much less than the mean percent variation that was accounted for between areas with and without pine squirrels (55.5% and 33.3%, respectively; Appendices D and E).

Likewise, cone structure was similar between the two regions without pine squirrels for whitebark pine (Fig. 4C) even though some individual cone and seed traits differed significantly between the two regions (Appendix E). Cones from the Sweet Grass Hills were narrower, and had fewer, thicker cone scales and more empty seeds with heavier and thicker seed coats (Appendix E). However, the mean amount of variation explained between these two regions (15.1%) was less than half the mean amount of variation explained between areas with and without pine squirrels (33.3%; Appendix E). These results indicate considerable convergence, presumably as a result of selection (Fig. 5), in the same sets of cone and seed traits between the two regions with pine squirrels as well as between regions without pine squirrels (Fig. 4).

Foraging rates of nutcrackers in areas with and without pine squirrels

Nutcrackers removed limber and whitebark pine seeds from closed cones almost two times faster in areas without pine squirrels than in areas with pine squirrels (Fig. 6A; limber pine, $F_{1,106} = 160.25$, $P < 0.0001$; whitebark pine, $F_{1,130} = 264.92$, $P < 0.0001$). Similarly, nutcrackers required significantly less time to remove whitebark pine seeds from partly open cones in areas without pine squirrels than in areas with pine squirrels (Fig. 6B; $F_{1,85} = 34.90$, $P < 0.0001$). However, foraging rates on open limber pine cones did not differ between areas with and without pine squirrels (Fig. 6B; $F_{1,48} = 0.48$, $P = 0.493$); once scales spread apart variation in cone scale structure is unlikely to impact accessibility of seeds for nutcrackers (see Benkman 1987). The significant interaction terms (limber pine, $F_{1,154} = 39.77$, $P < 0.0001$; whitebark pine, $F_{1,215} = 17.29$, $P < 0.0001$) presumably reflect the greater decreases in foraging rates in areas with squirrels compared to areas without squirrels for nutcrackers foraging on closed cones (Fig. 6A; 33.4% and 38.0% decreases in seeds per second on

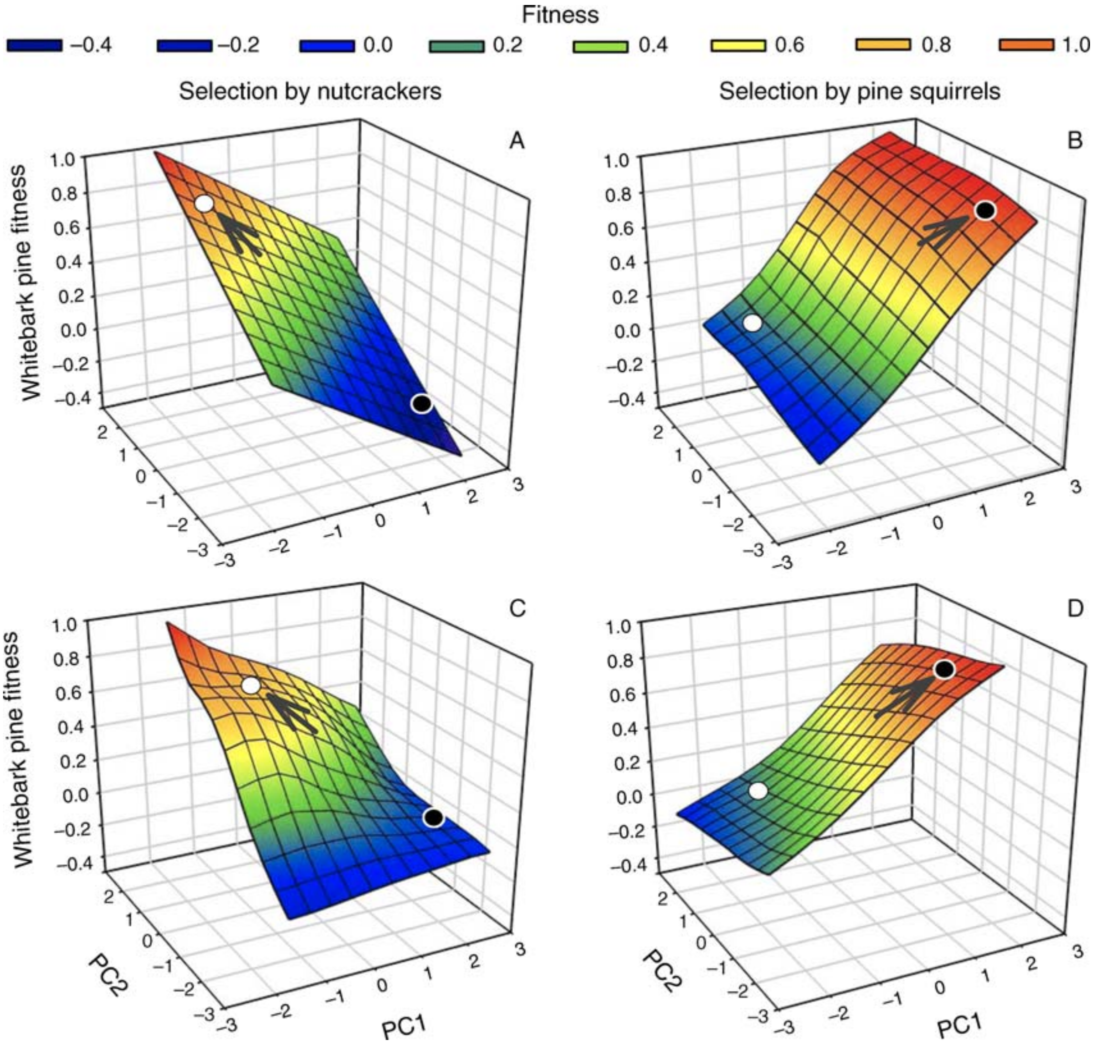

Fig. 5. Selection exerted on limber pine and whitebark pine cone structure (PC1 and PC2, as in Appendix F) by the primary seed disperser (Clark's Nutcracker; panels A and C) conflicted with selection by the dominant seed predator (pine squirrels; panels B and D). Selection or fitness surfaces were estimated with projection pursuit regressions. Solid and open circles are the centroid PC score means from areas with and without pine squirrels, respectively, from Fig. 4; arrows indicate the direction of evolution. The z-axis (fitness) is not to be interpreted quantitatively, only in a qualitative manner (e.g., increasing or decreasing fitness in relation to PC scores).

limber and whitebark pine, respectively) than on open cones (Fig. 6B; 3.6% and 21.9% decreases in seeds per second on limber and whitebark pine, respectively).

Discussion

Our results support the hypothesis that the ecological and evolutionary trajectories of limber and whitebark pines, and their potential for coevolution with nutcrackers vary across the landscape because of differences in the presence and absence of pine squirrels. Although our results indicate that selection by nutcrackers influences cone evolution, selection by nutcrackers alone cannot account for the evolution of cone and seed traits in bird-dispersed pines throughout their geographic ranges. Rather, our results indicate that selection by pine squirrels has an overwhelming influence on cone evolution where they occur. Furthermore, our results indicate that competition and selection by pine squirrels act to constrain the evolution of cone and seed traits that facilitate seed dispersal by nutcrackers. These results are in accord with the consistent finding that the underlying (co)evolutionary and ecological dynamics of a focal interaction are best understood when they are investigated across the geographic ranges of interacting species (Thompson 2005). Below we discuss our evidence for the selection mosaic, consider how pine squirrels act to constrain the potential for coevolution between nutcrackers and bird-dispersed pines, and further discuss whether nutcrackers and these pines have in fact coevolved. Because our study is based on a series of

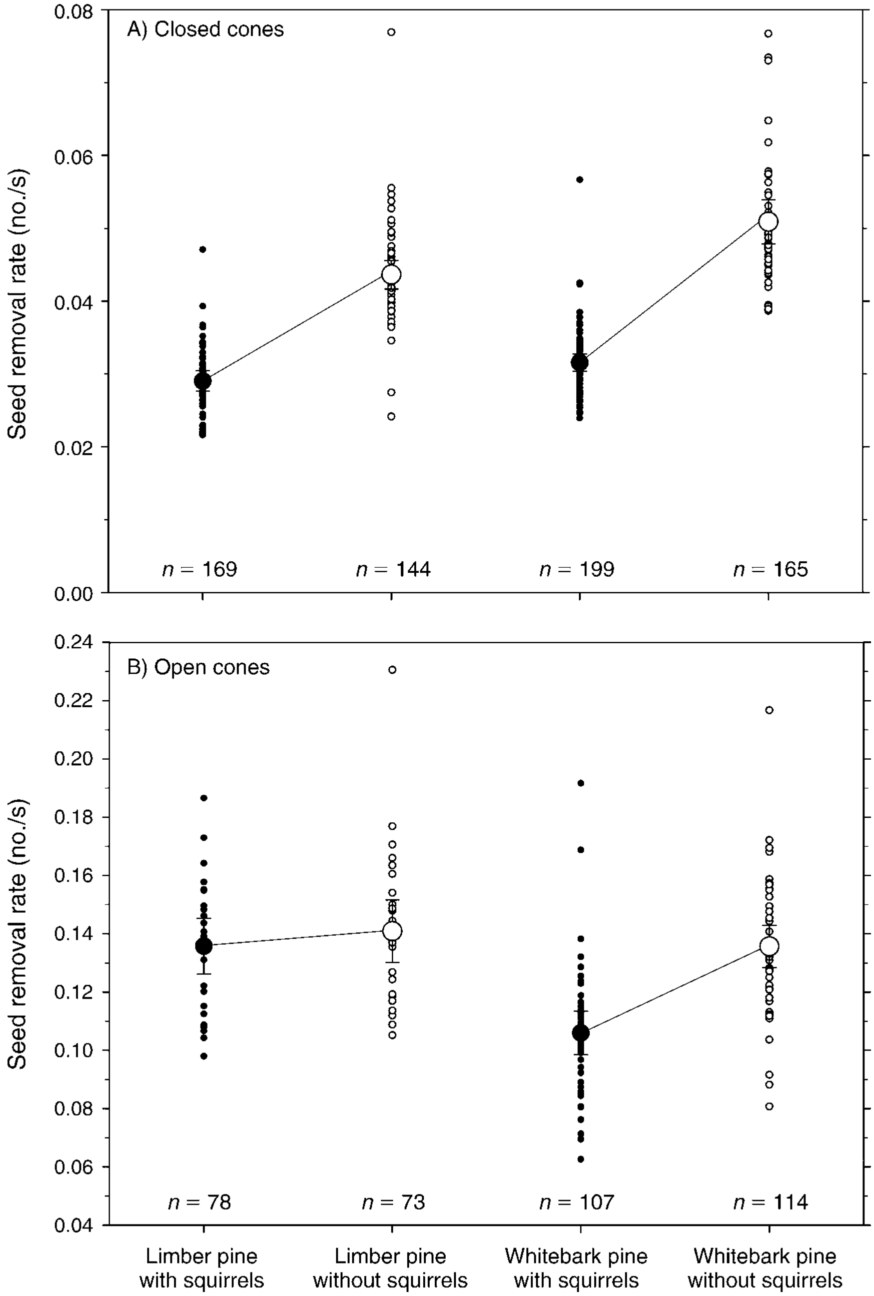

FIG. 6. Seed removal rates (mean ± SE) of Clark's Nutcrackers foraging on limber and whitebark pines in areas with (solid circles) and without (open circles) pine squirrels for both (A) closed cones and (B) open (limber pine) or partly open (whitebark pine) cones. Smaller circles are the mean seed removal rates for individual nutcrackers. Sample sizes (number of individual bird foraging rates) are given below the data points.

replicated correlations, it is not experimental. Thus, we also discuss possible alternatives that might account for the observed patterns.

Pine squirrels as preemptive competitors

The substantially lower abundances of nutcrackers in mountain ranges with pine squirrels in comparison to ranges without pine squirrels (Fig. 3), coupled with the observation that pine squirrels remove a considerable proportion of the cones before nutcrackers begin harvesting individual seeds from cones (Hutchins and Lanner 1982, Benkman et al. 1984), indicates that pine squirrels are strong preemptive competitors of nutcrackers. This further implies that competition between nutcrackers and pine squirrels is asymmetric: pine squirrels have a considerable competitive effect on

PLATE 1. Photograph of a Clark's Nutcracker (*Nucifraga columbiana*) on whitebark pine (*Pinus albicaulis*) near Carson Pass, California, USA. The shredded cones are the aftermath of nutcracker seed removal. Photo credit: Jim Dunn.

nutcrackers, whereas nutcrackers have a negligible impact on pine squirrels (e.g., Smith and Balda 1979).

Preemptive competition by pine squirrels may reduce or swamp the selective impact of nutcrackers on cone and seed traits for at least two reasons. First, preemptive competition reduces nutcracker abundance, which results in fewer seeds harvested by nutcrackers and presumably weaker selection. Such an inference is also consistent with the positive correlation between Red Crossbill (*Loxia curvirostra*; a seed predator and conifer seed specialist) abundance and the level of lodgepole pine (*Pinus contorta* spp. *latifolia*) seed defenses against crossbills (Benkman 1999, Siepielski and Benkman 2005). Second, because pine squirrels harvest a large fraction of the cones before nutcrackers harvest many seeds (Hutchins and Lanner 1982, Benkman et al. 1984), nutcrackers are left to forage on only a subset of cones and after squirrels have exerted considerable selection (e.g., Benkman 1999; see also Jordano 1987). This presumably explains why pine squirrels have such an overwhelming evolutionary impact on cone and seed traits. Similar results were found for Red Crossbills in lodgepole pine dominated forests with and without pine squirrels. Crossbills were on average six times and upwards of 20 times more abundant in the absence than presence of pine squirrels (Benkman 1999, Siepielski and Benkman 2005). Taken together, these studies suggest that the presence or absence of pine squirrels provides an ecological mechanism that gives rise to selection mosaics for several species of conifers and their seed predators and dispersers.

Selection by nutcrackers and pine squirrels

The different foraging behaviors of nutcrackers and pine squirrels are important for understanding why certain cone and seed traits experienced selection. On closed cones, nutcrackers remove seeds one at a time by using their bills to shred the woody cone scales to access underlying seeds, usually beginning near the proximal end of the cone (Vander Wall and Balda 1977; see Plate 1). Because increased cone scale thickness should slow nutcrackers, one would predict, as we found, that nutcrackers harvest seeds much faster in ranges without pine squirrels (Fig. 6), where proximal cone scales are only about half as thick (Appendices D and E), than in ranges with pine squirrels, and that nutcrackers preferentially harvest seeds from limber and whitebark pines that have cones with thinner proximal scales (Table 1). Nutcrackers also prefer to forage on cones with more full seeds (Table 1), as has been found for nutcrackers harvesting seeds from pinyon pine (*P. edulis* [Vander Wall and Balda 1977, Christensen et al. 1991]), and with thinner seed coats (Table 1). Seeds with thinner seed coats are easier to open, and thus energetically more profitable (Benkman 1995*a*). For example, nutcrackers required less time to remove the seed coat and consume the kernel of limber pine seeds in areas without pine squirrels (9.7 ± 4.6 s [mean ± SD; Vander Wall 1988]) than in areas with pine squirrels (14.4 ± 5.5 s [Benkman et al. 1984]) where seed coats are about twice as thick (Appendices E and F). In addition, the less seed coat mass per seed, the more seeds and seed kernel that can be carried by nutcrackers to a cache site for a given load, which benefits trees and nutcrackers, respectively.

Unlike nutcrackers, pine squirrels remove whole cones from trees by biting through the peduncle that attaches the cone to the branch and access seeds by biting off successive scales beginning at the proximal end of the cone. As in studies of lodgepole pine (Smith 1970, Benkman 1999), pine squirrels preferentially harvested limber and whitebark pines that had more seeds per cone (Table 2), presumably because squirrels have higher feeding rates on these cones (Smith 1970). Trees intensively harvested by pine squirrels also had thinner proximal cone scales (Table 2), presumably because less cone matter must be chewed through to access the seeds (Mezquida and Benkman 2005). On limber pine, pine squirrels avoided cones with a greater percentage of reflexed proximal scales, which should make removing cones more difficult because reflexed scales protect the peduncle connecting the cone to the branch (Table 2, Fig. 2). Samano and Tomback (2003) also found that pine squirrels avoided harvesting southwestern white pine (*P. strobiformis*) cones with reflexed proximal scales (see also Benkman et al. 1984).

Despite the differences in foraging behavior between nutcrackers and pine squirrels, they preferred many of the same cone and seed traits. This caused the conflicting selection pressures evident in the fitness surfaces, showing opposing directions of selection on overall cone structure by nutcrackers (Fig. 5A, C) and pine squirrels (Fig. 5B, D), and presumably caused the corresponding differences in cone and seed traits between regions with and without pine squirrels (Figs. 2 and 4). Selection by pine squirrels has thus led to the evolution of seed defenses that constrain the evolution of features that facilitate seed dispersal by nutcrackers, as evidenced by nutcrackers being less abundant and having slower foraging rates in areas with than without pine squirrels (Figs. 3 and 6).

Patterns of convergence in the selection mosaic

We found striking similarities in the patterns of cone and seed trait differentiation in both pine species between areas with and without pine squirrels, indicating that limber and whitebark pines have converged on similar phenotypes in areas with and without pine squirrels (Figs. 2 and 4A) presumably in response to similar selection pressures (Fig. 5, Tables 1 and 2). In the absence of pine squirrels, limber and whitebark pines have reduced their defenses aimed at deterring seed predation from pine squirrels and have also evolved adaptations in response to directional selection from nutcrackers (Fig. 5) for traits increasing seed harvest (Fig. 6). Further benefiting the trees is the concurrent reduction in their allocation of resources to defense (i.e., cone) relative to that allocated more directly into potential offspring (i.e., seeds). The greater number of seeds per cone and faster foraging rates could also help account for the greater number of nutcrackers per cone in the absence of pine squirrels (Fig. 3). Such trait evolution, along with previous studies showing that nutcrackers often cache seeds at sites suitable for germination, that stored seeds germinate (Tomback 1982, Lanner 1996), and that this is likely the most effective means of dispersal (Benkman 1995*b*) provides evidence supporting the hypothesis that the interaction between nutcrackers and these pines is mutualistic; if nutcrackers were net antagonists, then traits that deter nutcrackers should increase rather than decrease in the absence of pine squirrels. For some limber and whitebark pine cone traits the patterns are analogous to previous comparisons between areas with and without pine squirrels for lodgepole pine (Benkman et al. 2001) and black spruce (*Picea mariana* [Parchman and Benkman 2002]). For all four of these conifers there are, for example, only about half as many seeds in cones from areas with pine squirrels as in cones from areas without pine squirrels. This presumably reflects selection exerted by pine squirrels favoring trees with fewer seeds (Table 2; Benkman et al. 2001, 2003) and, in the case of bird-dispersed pines, additional selection by nutcrackers in the absence of pine squirrels favoring trees with more seeds per cone (Table 1). Such replicated patterns of convergent phenotypic evolution in response to similar forms of phenotypic selection have been documented in diverse systems (e.g., Losos 1992, Schluter and McPhail 1993, Jousselin et al. 2003), and are the signature of adaptive evolution.

In addition to the convergence between conifers, we also found striking patterns of trait convergence in the comparisons between the separate regions with and without pine squirrels (Fig. 4B, C). In the regions with pine squirrels these patterns of "convergence," however, likely reflect lack of phenotypic divergence. Although populations of both pines in the Sierra Nevada are genetically distinct from populations in the Rocky Mountains (Mitton et al. 2000, Richardson et al. 2002), indicating independent evolutionary trajectories, they were likely connected at some point in their pre-glacial histories. Such a connection may have provided a corridor and allowed pine squirrels to occur across these regions, and is consistent with the phylogeographic structure of pine squirrels (Arbogast et al. 2001). If connected, the patterns of similarity in cone and seed traits of both pines simply reflect a lack of divergence in cone structure coupled with divergence at neutral molecular markers owed to more recent isolation.

Regardless, these patterns also suggest that *T. douglassi* (Sierra Nevada–Cascades) and *T. hudsonicus* (Rocky Mountains) exert similar selection pressures on both pines.

More unequivocal evidence of convergence comes from the replicated patterns of cone and seed trait evolution in whitebark pine between the two regions without pine squirrels (Appendix E; Fig. 4C). Although pine squirrels have likely been absent from the Great Basin for over 12 000 years (Grayson 1987, Heaton 1990), the Sweet Grass Hills were only recently colonized within the past 10 000 years by whitebark pine from populations (pine squirrels present) in the Northern Rocky Mountains (e.g., Thompson and Kujit 1976, Richardson et al. 2002). As for other ranges near the Sweet Grass Hills, pine squirrels presumably never colonized these isolated ranges because during the past 10 000 years the surrounding grassland steppe habitat has been an insurmountable barrier to pine squirrels (Benkman et al. 2001). Because whitebark pine in the Sweet Grass Hills have presumably evolved for less time in the absence of pine squirrels than have whitebark pine in the Great Basin, it is perhaps not surprising that cones from the Sweet Grass Hills are somewhat intermediate in structure (Fig. 4C). Alternatively, gene flow from the Rocky Mountains is possible, although unlikely (Richardson et al. 2002), for explaining the greater similarity to Rocky Mountain cones. Nevertheless, the loss of squirrel defenses and an increase in accessibility of seeds to nutcrackers has evolved independently east and west of the Rocky Mountains, and thus reflects convergent evolution in cone structure.

Other causes of selection

Although the patterns of cone and seed trait differentiation observed between regions with and without pine squirrels are consistent with selection pressures exerted by pine squirrels and nutcrackers, we cannot ignore the possibility that trait variation may be related to selection by, for example, abiotic factors that may covary with the presence or absence of pine squirrels. There are, however, at least two reasons to suspect that variation in abiotic factors do not provide an adequate explanation for the consistent differences in cone and seed traits between areas with and without pine squirrels for both limber and whitebark pines. First, we found that cone and seed traits of whitebark pine in the Sweet Grass Hills where pine squirrels are absent, are similar to whitebark pine cones from ranges in the Great Basin (Appendix E; Fig. 4C). These results provide an independent comparison and further suggest that there is nothing particularly unique about the Great Basin, other than the absence of pine squirrels, to provide a satisfactory explanation for the consistent differences in cone and seed traits. Second, it is unlikely that the variation in most cone and seed traits is related to abiotic factors that also differ in a consistent manner between regions with and without pine squirrels (Benkman 1995*a*; see also Smith 1970).

The one known association between seed traits and abiotic conditions is seed mass. Variation in physical conditions during germination presumably have a selective influence on seed mass, as seedlings from larger seeds (i.e., larger kernels) may be favored during periods of drought or other conditions during early stages of growth (Westoby et al. 1996). We found that seed masses for both pines were greater in the Rocky Mountains and Sierra Nevada relative to seed masses in the Great Basin, however, this was because seed coats, but not kernels, were thicker and heavier (Appendices E and F). On the other hand, it is possible that thicker seed coats are favored in drier conditions to prevent desiccation of kernels. Thus, if conditions are drier in the regions with pine squirrels, then this could provide an alternative explanation for thicker seed coats in those regions. Using estimates of mean annual precipitation at our study sites, we found that, contrary to this abiotic hypothesis, mean annual precipitation tends to be higher in areas with squirrels (limber pine, 93.13 cm; whitebark pine, 118.53 cm) than without squirrels (limber pine, 81.28 cm; whitebark pine, 86.36 cm) and seed coat thickness tends to increase rather than decrease with increases in precipitation ($r = 0.32$, df = 11, $P = 0.316$; $r = 0.51$, df = 11, $P = 0.090$; limber and whitebark pine, respectively; precipitation data *available online*).[2] This indicates that variation in annual precipitation cannot account for the large differences in seed coat thickness between areas with and without pine squirrels.

The case for coevolution between nutcrackers and bird-dispersed white pines

Coevolution is the evolution of reciprocal adaptations in response to reciprocal selection (Janzen 1980). Previous studies examining the case for coevolution between nutcrackers and pines were based on observations of certain traits in pines (e.g., winglessness of seeds) and nutcrackers (e.g., the sublingual pouch) as evidence of coevolution (e.g., Vander Wall and Balda 1977, Tomback and Linhart 1990, Lanner 1996). While these traits do indicate potential reciprocal adaptations based on their current functions, whether or not limber and whitebark pines and Clark's Nutcrackers have in fact evolved in response to reciprocal selection is uncertain. We have provided the first evidence that nutcrackers do exert selection on cone and seed traits of bird-dispersed pines, and that such selection has likely led to the evolution of traits that serve as adaptations facilitating the efficient harvest of seeds. However, seed size (i.e., kernel mass), which is one of the traits that has been suggested to be an adaptation for bird dispersal (e.g., Tomback 1983, Lanner 1996), was not under selection by nutcrackers (Table 1) or by pine squirrels (Table 2).

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[2] ⟨http://www.ncgc.nrcs.usda.gov/products/datasets/climate/data⟩

This is consistent with Smith's (1970) hypothesis that seed predators affect the evolution of seed packaging (e.g., number of seeds per cone and relative cone mass) but not seed mass. Of course, selection by nutcrackers in the past may have favored the evolution of larger seeds. However, the absence of selection on both the relatively small-seeded limber pine (~90 mg) and the much larger seeded whitebark pine (~170 mg) indicates that selection by nutcrackers has not been important in the evolution of seed size among the bird-dispersed pines studied here and therefore large seed size should not be considered an "adaptation" to bird dispersal. Presumably, large-seed size is an adaptation to conditions during and after germination (Tomback and Linhart 1990), and, because large seeds are poorly dispersed by wind, adaptations of the cones and seed coats that facilitate harvesting and dispersal of seeds by birds were potentially favored (Benkman 1995*b*).

Whether or not nutcrackers are evolving in response to changes in cone or seed traits, or other features of pines, is yet to be determined. However, measurements of 121 nutcrackers reveal significant differences in bill structure between areas with and without pine squirrels. In ranges with pine squirrels, nutcrackers had longer (31.93 ± 2.45 mm [mean ± SD], $n = 78$) and deeper (11.72 ± 0.58 mm) bills than in ranges without pine squirrels (bill length, 29.31 ± 3.24 mm, $n = 43$, $F_{1,118} = 24.58$, $P < 0.0001$; bill depth, 11.38 ± 0.63 mm, $F_{1,119} = 9.15$, $P = 0.003$); two phenotypic traits with high heritabilities in songbirds (Merilä and Sheldon 2001). How differences in bill structure are related to variation in cone and seed traits has not been studied in detail (Tomback 1998), and this is one focus of our ongoing studies. However, two subspecies of Eurasian Nutcrackers (*N. caryocatactes*) have bills that are apparently adapted for foraging on pines that differ in at least seed coat thickness. Thick-billed (*N. c. caryocatactes*) and Thin-billed Nutcrackers (*N. c. macrorhynchos*) have thicker and thinner bills, and use pines with thick and thin seed coats, respectively (see Lanner 1996). This is the same pattern found between nutcrackers in ranges with and without pine squirrels, where ranges with pine squirrels have seed coats that are about twice as thick (and have much larger cone scales) as in ranges without pine squirrels (Appendices D and E). These data suggest the possibility for ongoing reciprocal selection and adaptation between nutcrackers and pines, and the presence of coevolutionary hotspots and cold- or warmspots. That is, in areas without pine squirrels nutcrackers and pines may be evolving in response to reciprocal selection (i.e., a coevolutionary hotspot), whereas elsewhere the extent of coevolution between nutcrackers and pines is limited by competition with and selection by pine squirrels.

Conclusions

Placing mutualisms such as seed dispersal into their community context is one of the major challenges toward understanding how these interactions actually function in nature (Bronstein et al. 2003). Our results indicate that the ecological and evolutionary trajectories of limber and whitebark pines, and their potential for mutualistic coevolution with nutcrackers vary across the landscape because of differences in the presence or absence of pine squirrels. Pine squirrels have an important ecological effect as competitors, but also an evolutionary one, in that adaptations that have evolved in response to seed predation from pine squirrels constrain the evolution of adaptations that facilitate seed dispersal by nutcrackers. The parallel nature of the geographic structure of coevolutionary selection between nutcrackers and bird-dispersed pines, and between crossbills and several wind-dispersed conifers in areas with and without pine squirrels (Benkman 1999, Benkman et al. 2001, 2003, Parchman and Benkman 2002) or other tree squirrels (*Sciurus* spp. [Mezquida and Benkman 2005]) indicates that their presence or absence is the ecological mechanism giving rise to selection mosaics for conifers and other species with which they interact and potentially coevolve (e.g., Siepielski and Benkman 2004). An important conclusion stemming from this and similar studies is that although selection within populations acts to reduce phenotypic or genetic variation, differences in the forms of selection between populations ultimately act to increase variation at the landscape level. This is the "duality" of selection mosaics, both creating and eliminating variation across the landscape. The widespread finding of selection mosaics (Thompson 2005) owed to variation in community context across the landscape suggests they are likely a ubiquitous feature of natural systems important in creating and maintaining biodiversity.

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APPENDIX A

Information on the type of conifer, site name, whether or not pine squirrels are present or absent, elevation and location of study sites, and the type of data collected (*Ecological Archives* M077-007-A1).

APPENDIX B

Spearman's pairwise correlations between various limber pine cone and seed traits in regions with and without pine squirrels (*Ecological Archives* M077-007-A2).

APPENDIX C

Spearman's pairwise correlations between various whitebark pine cone and seed traits in regions with and without pine squirrels (*Ecological Archives* M077-007-A3).

APPENDIX D

Mean limber pine cone measurements for sites without and with pine squirrels and ANOVAs comparing differences in cone measurements between areas without and with pine squirrels and several comparisons among sites within regions with and without pine squirrels (*Ecological Archives* M077-007-A4).

APPENDIX E

Mean whitebark pine cone measurements for sites without and with pine squirrels and ANOVAs comparing differences in cone measurements between areas without and with pine squirrels and several comparisons among sites within regions with and without pine squirrels (*Ecological Archives* M077-007-A5).

APPENDIX F

Principal component loadings and Spearman's rank correlations between principal component scores and individual cone and seed traits for limber pine and whitebark pine (*Ecological Archives* M077-007-A6).