

CONFLICTING SELECTION FROM AN ANTAGONIST AND A MUTUALIST ENHANCES PHENOTYPIC VARIATION IN A PLANT

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The raw material for evolution is variation. Consequently, identifying the factors that generate, maintain, and erode phenotypic and genetic variation in ecologically important traits within and among populations is important. Although persistent directional or stabilizing selection can deplete variation, spatial variation in conflicting directional selection can enhance variation. Here, we present evidence that phenotypic variation in limber pine (*Pinus flexilis*) cone structure is enhanced by conflicting selection pressures exerted by its mutualistic seed disperser (Clark's nutcracker *Nucifraga columbiana*) and an antagonistic seed predator (pine squirrel *Tamiasciurus* spp.). Phenotypic variation in cone structure was bimodal and about two times greater where both agents of selection co-occurred than where one (the seed predator) was absent. Within the region where both agents of selection co-occurred, bimodality in cone structure was pronounced where there appears to be a mosaic of habitats with some persistent habitats supporting only the seed disperser. These results indicate that conflicting selection stemming from spatial variation in community diversity can enhance phenotypic variation in ecologically important traits.

KEY WORDS: Bimodality, *Nucifraga columbiana*, phenotypic selection, phenotypic variation, *Pinus flexilis*, seed dispersal, seed predation, *Tamiasciurus*.

A fundamental problem in evolutionary biology is to determine the factors that generate, maintain, and erode phenotypic and genetic variation in ecologically important traits within and among populations (Hallgrímsson and Hall 2005; Bolnick and Lee Lau 2008). This problem is fundamental because without phenotypic variation, and a heritable basis for such variation, adaptive evolution is not possible. Introgression, mutation, and recombination are important sources of such variation, whereas persistent directional or stabilizing selection reduces both the phenotypic and genetic variation of a trait (Fisher 1930). Selection can, however,

maintain, and even enhance variation (e.g., Bolnick and Lee Lau 2008). For instance, spatial segregation in conflicting selection pressures can enhance phenotypic variation by favoring trait extremes among different populations or subpopulations.

Plants are an excellent example of organisms facing conflicting selection pressures because they interact with numerous mutualistic (e.g., mycorrhizal fungi, pollinators, seed dispersers) and antagonistic (e.g., herbivores, florivores, seed predators) species (Strauss and Irwin 2004; Morris et al. 2007; Siepielski and Benkman 2007a,b). Conflicting selection pressures stemming from mutualists and antagonists have been posited as a mechanism maintaining variation in ecologically important traits. For example, flower color polymorphisms appear to be maintained

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by conflicting selection exerted by pollinators and herbivores (Irwin et al. 2003), pathogens (Frey 2004), and spatial variation in abiotic factors (Schemske and Bierzychudek 2007). Despite the potential for conflicting ecological selection acting to enhance variation, direct evidence is limited, particularly for quantitative traits displaying continuous variation. Furthermore, a basic prediction stemming from this argument has not been investigated: more variation should be present where conflicting selective agents co-occur relative to where only one selective agent exists.

Here, we investigate the extent to which conflicting selection, exerted by a mutualistic seed disperser, Clark's nutcracker (*Nucifraga columbiana*), and an antagonistic seed predator, pine squirrels (*Tamiasciurus* spp.), enhances phenotypic variation in limber pine (*Pinus flexilis*) cone structure. Cone structure is an ecologically important trait because it is both the primary reproductive structure of the pine and the shared phenotypic interface between the pine and its seed dispersers and predators. Although we lack estimates of heritability for limber pine cone and seed traits, such traits of other conifers are highly heritable (Verheggen and Farmer 1983; Singh and Chaudhary 1993; Matziris 1998; Benkman 1999) and so should evolve in response to selection. Limber pine is largely dependent on nutcrackers for primary seed dispersal (e.g., Tomback and Linhart 1990; Lanner 1996), whereas pine squirrels are the dominant predispersal seed predator of limber pine (Benkman et al. 1984). In mountain ranges throughout the Great Basin where pine squirrels have been absent for $\geq 10,000$ years, selection by nutcrackers has driven the evolution of cone and seed traits that facilitate seed dispersal by nutcrackers (Siepielski and Benkman 2007a). In contrast, throughout most of the distribution of limber pine in the Rocky Mountains (east of the Great Basin) and Sierra Nevada (west of the Great Basin), pine squirrels are present and their pre-emptive harvesting of large numbers of cones appears to depress nutcracker abundance (Siepielski and Benkman 2007a). Furthermore, the preemptive and selective harvesting of cones by pine squirrels likely reduces phenotypic variation in cone structure, which appears to minimize the opportunity for or mask selection exerted by nutcrackers. The result is that pine squirrels drive the evolution of cone and seed traits counter to that favored by nutcrackers, and limber pine experiences a selection mosaic among regions with and without pine squirrels (Benkman 1995; Siepielski and Benkman 2007a). The antagonistic effects of squirrels are, however, potentially relaxed in local areas devoid of pine squirrels (e.g., more open pine savanna) that exist within the geographic range of limber pine occupied by pine squirrels. These open or pine squirrel free areas, however, are commonly occupied by the strong-flying nutcrackers. Consequently, nutcrackers may still exert selection on limber pine within the region with pine squirrels. Thus, if an evolutionary response to such selection oc-

curs, there should be more variation in limber pine cone structure in regions occupied by pine squirrels and nutcrackers, relative to the region without squirrels.

Materials and Methods

THE DIRECTION OF PHENOTYPIC SELECTION EXERTED BY NUTCRACKERS

To make inferences about selection and the evolutionary consequences of selection, it is important to use a measure of fitness that reflects lifetime fitness. Although using the number of seeds harvested may be appropriate for some short-lived, annual plants, such a measure for long-lived plants, such as limber pine that live hundreds of years, may inaccurately reflect lifetime fitness when selection is measured during only a small fraction of the tree's life span. Thus, we used the proportion of seeds harvested by nutcrackers as an estimate of a component of tree fitness (hereafter simply tree fitness) in the context of selection exerted by nutcrackers, because this measure should be robust to the effects of tree age (see Siepielski and Benkman 2007c for further justification). Nevertheless, estimates of selection based on the number of seeds harvested provide nearly identical estimates as those based on the proportion of seeds harvested (e.g., Siepielski and Benkman 2008). Although we only estimate selection on a single occasion here, our previous studies have shown that patterns of selection on cone structure are similar when similar cone crop sizes are produced (e.g., Siepielski and Benkman 2007c).

In August 2004, we recorded both the number of cones that had signs of nutcracker seed harvesting activity (shredded cones on trees) and the total number of cones on each tree for 69 trees in the Schell Creek Range, NV, a mountain range in the region without pine squirrels (the Great Basin; Fig. 1). The proportion of seeds harvested was calculated as the number of cones with seeds harvested divided by the total number of cones on the tree (counted with 10×40 binoculars). During August through October 2006, at South Pass, WY (Fig. 1), a study site within the region with pine squirrels (the Rocky Mountains) but where pine squirrels were absent locally, we estimated the proportion of seeds harvested by nutcrackers using two seed traps per tree placed below 75 randomly chosen trees. The South Pass population was an isolated population of limber pine where we found no evidence of squirrel activity (e.g., vocalizations or middens [cone caches]; Siepielski and Benkman 2008). At the end of October (after seeds had fallen from cones), the seeds in traps were collected and counted. For each tree, the number of full seeds (a developed female gametophyte) that fell to the ground was estimated as the number of full seeds collected in the seed traps multiplied by the inverse of the proportion of the canopy area covered by the traps. We measured the radius of the canopy (r) of each tree to estimate canopy area (πr^2). The total number of full seeds for a given tree was estimated

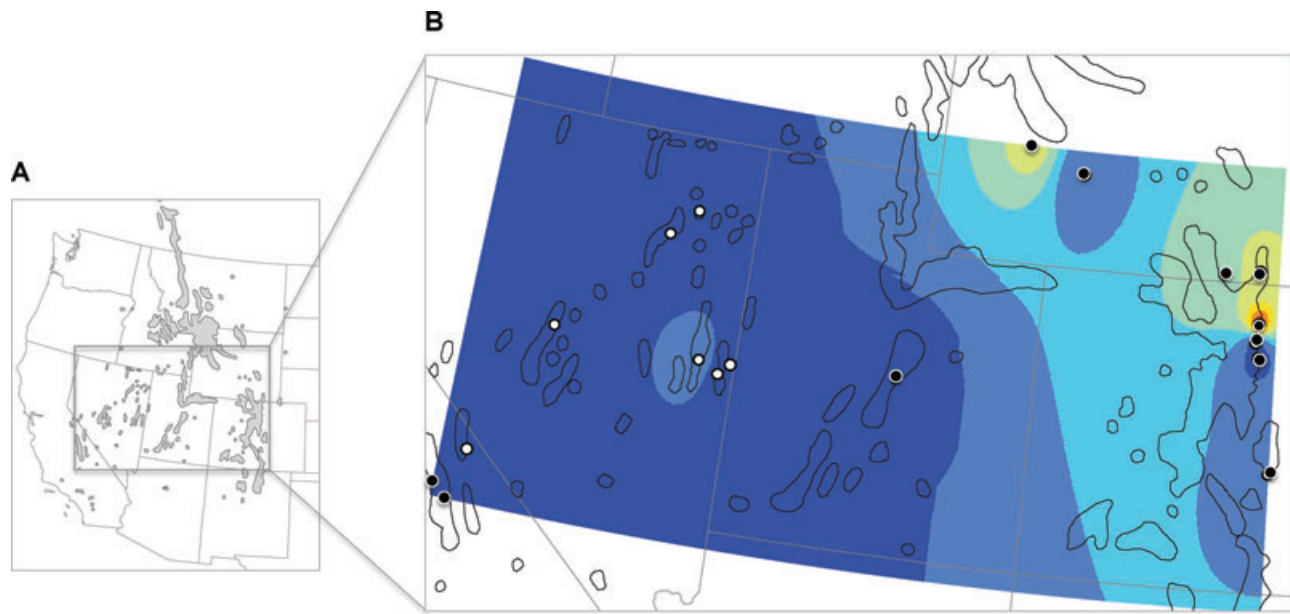


Figure 1. (A) Map of the distribution of limber pine (based on Little 1971) and (B) geographic variation in the extent of cone structure bimodality. The contour plot (B) shows the values of Δ AIC from comparisons of models of limber pine cone structure (PC1) assuming that the distributions of PC1 are composed of unimodal versus bimodal normal distributions. "Hotter" colors mean more support for a mixture of two normal distributions, i.e., greater bimodality. Study sites (white circles, squirrels absent; black circles, squirrels present) from west to east: Horseshoe Meadows, CA; Onion Valley, CA; White Mountains, CA; Toiyabe Range, NV; Schell Creek Range, NV; Ruby Mountains, NV; East Humboldt Range, NV; Mt. Moriah, NV; Snake Range, NV; Avintaquin, UT; Pinedale, WY; South Pass, WY; Jelm Mountain, WY; Red Feather Lakes, CO; Vedauwoo, WY; Rocky Mountain National Park, CO; Ward, CO; and Pikes Peak, CO.

as the number of cones on the tree multiplied by the mean number of full seeds per cone for that tree. For each tree, we counted the number of cones on the tree and haphazardly removed two unharvested cones (prior to cone opening in August). We opened the cones after oven drying them at 60–70°C for more than two days to count the number of seeds. The proportion of seeds harvested by nutcrackers was then estimated as one minus the proportion of seeds that fell to the ground; see Siepielski and Benkman (2008) for additional details. This different method for estimating tree fitness was necessary because nutcrackers were rarely observed shredding cones to remove seeds at this and other sites in the Rocky Mountains. Use of these two different methods of estimating fitness could potentially impact values of the regression coefficients, but should not affect estimates of the direction of selection.

To quantify cone structure, we haphazardly removed two unharvested cones from each tree. We did not attempt to measure within-tree variation in cone and seed traits (see below) because our analyses rely on tree means, and other studies have found that within-tree variation in cone and seed traits of pines is considerably smaller than among-tree variation (Garcia et al. 2009), and this was consistent with our observations. From each of the two cones gathered we measured a set of 10 standard cone and seed traits: cone length, closed cone width, cone mass with seeds removed, peduncle diameter; number of scales along the cone axis, proximal and distal scale thickness, total number of seeds; individ-

ual seed mass, and individual seed coat thickness (see Siepielski and Benkman [2007b,c, 2008]). 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 ≥ 2 days. We used mean trait values per tree in all analyses.

We used regression analyses (Lande and Arnold 1983) to estimate the direction of selection exerted by nutcrackers on cone and seed traits. Because we were most interested in how tree fitness was related to overall cone structure, we used principal components analysis based on the correlation matrix of the 10 cone and seed traits to extract the first principal component (PC1) to use as a variable characterizing cone structure. We focus our analysis on PC1 because much variation in cone structure can be accounted for by PC1 (Table S1) and variation in PC1 scores is consistently interpretable in terms of variation in selection exerted by nutcrackers and pine squirrels (Siepielski and Benkman 2007b,c, 2008). PC1 values 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.

THE DIRECTION OF PHENOTYPIC SELECTION EXERTED BY PINE SQUIRRELS

Pine squirrels either intensively harvest cones from trees or largely avoid them on their territories (Smith 1970; Benkman 1999;

Siepielski and Benkman 2007a). Thus, the direction of phenotypic selection exerted by pine squirrels on cone structure was estimated by using a paired design to compare cone structure between trees intensively harvested or avoided by pine squirrels. Data were gathered near Ward ($n = 32$ territories) and Sugar Loaf Mountain ($n = 18$ territories), Colorado September 2003; these two sites were ca. 14 km apart. We combined data from these two sites, because their very close proximity (e.g., gene flow from seed dispersal and pollen most likely occur between these sites) means they are likely part of the same population. Two to three cones were collected from one tree that was being intensively harvested (i.e., the squirrel was observed repeatedly removing cones from the tree) and from a nonharvested 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. Squirrels may have later harvested cones from nonharvested trees. Nevertheless, the probability of seed escape from squirrels, and thus this component of tree fitness, was greater than that for harvested trees. All of the above-mentioned cone and seed traits were measured from two cones from each tree and PC1 values calculated (see Table S1 for PC1 loadings). We used paired t -tests between the paired 50 harvested and 50 avoided trees to determine whether selection by pine squirrels would favor larger or smaller values of PC1.

FITNESS SURFACES

To visualize phenotypic selection by nutcrackers and pine squirrels, we generated fitness surfaces representing the relationship between tree fitness and cone structure using cubic splines (Schluter 1988). The dependent variables were the standardized proportion of seeds removed by nutcrackers, and whether a tree was harvested (fitness = 0) or not (fitness = 1) by pine squirrels. PC1 was the independent variable. We caution that the pine squirrel selection data do not represent a random sample from the population (i.e., it was a paired design). Thus, the fitness surface in response to selection exerted by pine squirrels is meant only to depict the direction of selection, and is not to be interpreted quantitatively.

VARIATION IN CONE STRUCTURE AMONG REGIONS WITH AND WITHOUT PINE SQUIRRELS

At each of 11 populations with pine squirrels and seven populations without pine squirrels (Fig. 1), we gathered and measured cone and seed traits (as above) from 30 to 45 randomly selected trees. We used two approaches to investigate the evolutionary consequences of conflicting selection pressures between nutcrackers

and pine squirrels on cone structure variation. First, using the population mean standard deviation (SD) of PC1 (from a PCA based on all 18 populations, which accounted for 55% of overall phenotypic variation in cone structure; Table S2) as a measure of variation, we used a general linear model (GLM) to test the prediction that more variation in cone and seed structure was present in populations with pine squirrels in comparison to populations without pine squirrels. We also considered the potential importance of abiotic factors influencing variation in cone structure by including several abiotic factors (elevation, latitude, and precipitation) in the model.

Second, if a response to selection by nutcrackers and squirrels occurs, bimodality of cone structure should only be apparent in the region with pine squirrels. Bimodality should occur if trees on squirrel territories experience strong selection for traits that differ from those favored outside of squirrel territories (e.g., by nutcrackers). Bimodality should not, however, be apparent in the region without pine squirrels where only nutcrackers exert selection. Importantly, other seed predators that could be potential selective agents, such as insects (e.g., Siepielski and Benkman 2004) and several bird species that remove seeds once cones open, are common to both regions (Hedlin et al. 1980; Sibley 2000) and so should not confound our comparisons. We examined bimodality in cone structure using two approaches. First, we visually examined frequency histograms of PC1. Second, we statistically evaluated whether the distribution of PC1 in each region (combining all populations within regions with or without pine squirrels) and within individual populations, was better represented by a single normal distribution or by a mixture of two normal distributions based on Akaike's information criterion (AIC; e.g., Brewer 2003; Hendry et al. 2006). We calculated ΔAICc as AICc for the single normal distribution minus AICc for the mixture of two normal distributions. We interpreted these ΔAICc values as described in Hendry et al. (2006; based on guidelines in Burnham and Anderson 2002). ΔAICc comparisons were made using computer software provided by M. Brewer. To show how bimodality varied geographically, we used Arcview GIS 9.2 (ESRI; Redlands, CA) to generate a contour map of ΔAICc scores from each of the populations.

Results

Selection exerted on limber pine-cone structure by nutcrackers (linear regression: limber pine fitness = $-0.12(\text{PC1}) + 1.0$, $F_{1,73} = 20.18$, $P < 0.0001$) and pine squirrels (paired $t = -12.78$, $df = 98$; $P < 0.0001$) is conflicting, favoring smaller and larger values of PC1, respectively (Fig. 2A). The difference in cone structure between squirrel harvested and nonharvested trees (e.g., the response variable in the paired t -test) was not statistically different between the two sites ($t = 0.78$, $df = 48$, $P = 0.44$), indicating

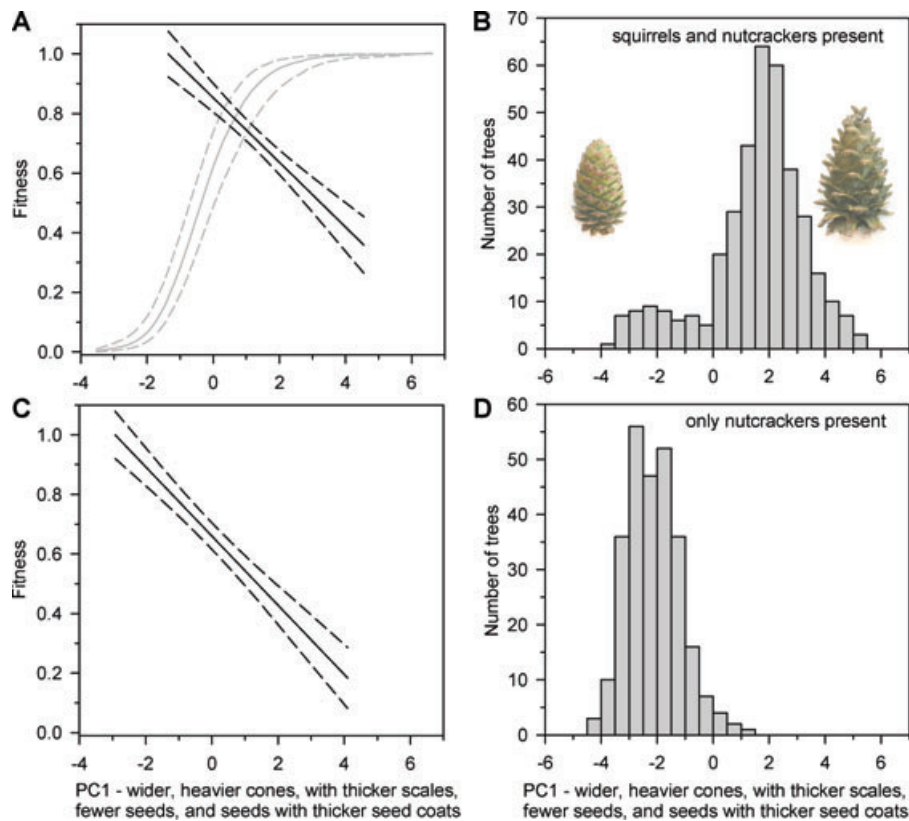


Figure 2. (A) Conflicting selection exerted by Clark's nutcrackers (solid black line) and pine squirrels (solid gray line) enhances (B) phenotypic variation in limber pine cone structure ($n = 369$ trees, 11 populations) relative to (D) areas without pine squirrels ($n = 270$ trees, 7 populations) that experience only (C) directional selection exerted by nutcrackers. The first principal component (PC1) of 10 cone and seed traits is used to characterize trait variation. Cubic splines are shown in A and C (dashed lines are 95% confidence intervals, based on 100 bootstrap replicates). Limber pine cones typifying the range of variation in cone structure are shown (B) (left cone PC1 ≈ -2.40 , right cone PC1 ≈ 2.24).

similar patterns of selection by pine squirrels between the sites. In the region without pine squirrels, limber pine experiences directional selection by nutcrackers on cone structure, favoring smaller values of PC1 (limber pine fitness = $-0.18(PCI) + 1.0$, $F_{1,67} = 24.38$, $P < 0.0001$; Fig. 2C). Both nutcrackers and squirrels prefer to forage on trees producing cones with smaller values of PC1 (i.e., smaller cones with thin cone scales and many seeds; Table S1) because they are likely rewarded with more energy per unit time foraging (Siepielski and Benkman 2007a). However, in their roles as mutualists and antagonists, respectively, overall (conflicting) selection on cone structure acts to enhance phenotypic variation in cone structure (Fig. 2B).

The consequences of this conflicting selection is evident in the SD of PC1, which was nearly two times greater in the region with pine squirrels (Fig. 2B; mean SD = 1.61 ± 0.18 [SE]) than in the region without pine squirrels (Fig. 2D; mean SD = 0.87 ± 0.05). The GLM model comparing cone structure variation between regions with and without pine squirrels, which also included abiotic factors was significant ($F_{4,13} = 6.52$, $P = 0.004$), however, only the squirrel effect was significant (one-tailed test:

$F_{1,13} = 8.65$, $P = 0.005$); all abiotic factors were nonsignificant (all $P \geq 0.06$; Table S3).

Concomitant with these findings, evidence of strong bimodality in cone structure was present in the region with pine squirrels (Fig. 2B; $\Delta AICc = 116.05$; indicative of strong support for a mixture of two normal distributions) but not in the region without pine squirrels (Fig. 2D, $\Delta AICc = -1.21$; as roughly equivalent support for a single normal distribution or a mixture of two normal distributions). Only in the regions with squirrels did individual populations show evidence of bimodality (Fig. 1B and S1, Table S4).

Discussion

Throughout most of its distribution, limber pine is essentially caught in an evolutionary tug-of-war because of conflicting selection pressures from its primary seed disperser and seed predator (Fig. 2). These conflicting selection pressures act to enhance phenotypic variation in limber pine cone structure in regions with squirrels, which is manifested at two scales: regionally

(i.e., among populations, Fig. 2) and locally (within populations, Figs. 1B and S1, and Table S4).

Two main processes could account for the greater variation in cone and seed trait structure where conflicting selection pressures operate: phenotypic plasticity and genetic divergence. Phenotypic plasticity is favored in heterogeneous environments (Pigliucci 2001), and conflicting selection is often predicted to lead to plasticity (Parsons and Robinson 2006). Furthermore, organisms with extensive dispersal, as is the case with limber pine, are often more likely to evolve plasticity because they experience heterogeneous environments (e.g., Hollander 2008). However, several lines of evidence indicate that plasticity is unlikely to explain the patterns of trait variation found here. First, for plasticity to be adaptive, limber pine must distinguish seed removal by nutcrackers and cone removal by red squirrels. We cannot envision how this would occur, especially because nutcrackers will some times remove entire cones from trees. Second, in other studies pine squirrels have been observed repeatedly harvesting cones (*Pinus contorta*) from the same trees on their territories for several years, and cones from these trees are not more well-defended than other trees in the population, as would be expected if cone and seed traits were plastic or such traits were inducible (Benkman 1999).

Although we cannot rule out phenotypic plasticity, we suspect that greater regional and local variation in limber pine cone structure in the region with squirrels is a consequence of genetic divergence in cone and seed structure in response to selection by nutcrackers and pine squirrels. We first consider the region with squirrels in which a more complex mosaic pattern of trait variation is present within populations (Fig. 1B and S1). There, most populations show unimodal distributions of large values of PC1, consistent with selection by squirrels driving the evolution of cone structure. However, several populations restricted mostly to SE Wyoming and northern Colorado display evidence of bimodality and greater phenotypic variation in cone structure (Figs. 1B and S1, Table S4). We suspect these patterns are a consequence of variation in successional dynamics of limber pine stands, accompanied by spatial variation in seed dispersers of limber pine in these stands. Limber pine is an early successional species, often colonizing recently burned or disturbed habitat (Steele 1990). At most higher elevation sites throughout the Rocky Mountains, founding stands undergo succession and are then readily colonized by pine squirrels (e.g., Fisher and Wilkinson 2005). Where pine squirrels are present, their preemptive harvesting of cones depresses nutcracker abundances so that selection by nutcrackers would be much weaker if not absent all together (Benkman et al. 1984; Benkman 1995; Siepielski and Benkman 2007a, 2008). However, along the eastern fringe of the distribution of limber pine where it begins to enter the Great Plains, the habitat appears to undergo succession less homogeneously. Although some local areas undergo succession and may be colonized by pine squirrels,

many other areas exist in open savannah like habitat or in isolated areas that pine squirrels would likely avoid or simply not colonize (e.g., Benkman et al. 1984; Fisher and Wilkinson 2005), but nutcrackers can easily access. Indeed, coexistence of competitors is often mediated by the trade-off between colonization ability and competitive ability (Levins and Culver 1971): nutcrackers are superior colonizers but poor competitors relative to pine squirrels (Benkman et al. 1984; Siepielski and Benkman 2007a).

In the local areas with pine squirrels, limber pine has apparently evolved defenses against pine squirrels. Here, nutcrackers are less-efficient seed dispersers, more seeds fall to the ground, and limber pine relies more on secondary seed dispersal by ground-foraging rodents (Siepielski and Benkman 2008). This shift to these secondary seed dispersers would be important in promoting cone divergence among subpopulations with and without pine squirrels, because rodents disperse seeds short distances (e.g., ~10 m; Siepielski and Benkman 2008) meaning that the evolutionary response to selection by pine squirrels occurs locally. In contrast, in the subpopulations without pine squirrels, selection by nutcrackers drives the evolution of cone structure, with nutcrackers presumably either dispersing many seeds locally, or dispersing seeds into new founding stands. Dispersal by nutcrackers into new stands could lead to the rapid evolution of cone and seed structure, because nutcrackers only exert selection on cone and seed traits when they are likely to provide seed dispersal benefits facilitating recruitment in pines (Siepielski and Benkman 2007c). Nutcrackers also prefer to cache seeds in open areas (Lanner 1996), and thus are unlikely to cache seeds in the later successional stands, so that seed dispersal from subpopulations without pine squirrels to subpopulations with them is limited. Although these processes would restrict gene flow from seed movement allowing for local divergence in cone structure, and this is critical (e.g., Lenormand 2002), pollen flow would likely occur among subpopulations. Presumably, either gene flow from pollen is not sufficient to retard divergence among subpopulations or cone and seed traits are maternally inherited and thus transmitted via seed. The result of this stand structure, variation in selection pressures, and shifts in seed dispersal mode is a complex mosaic of trait distributions. Such patterns argue for an important role of spatial variation in local community diversity (i.e., a metacommunity; Leibold et al. 2004; Urban et al. 2008; see also Thompson 2005) in generating conflicting selection that can enhance phenotypic variation in ecologically important traits. Indeed, many theoretical and empirical studies underscore the importance of heterogeneous environments and potential genetic \times environment interactions to the maintenance of phenotypic and genetic variation (Byers 2005). This is particularly the case when selection varies spatially and is coupled with limited gene flow (e.g., Nuismer et al. 1999; Lenormand 2002; Thompson 2005), as would occur when selection varies both regionally and locally.

At the regional level, different populations of limber pine experience selection mostly from either pine squirrels, or a combination of nutcrackers and pine squirrels. This spatial variation readily explains the regional pattern of bimodality (Fig. 2B), and is consistent with the idea that conflicting selection would facilitate the maintenance of, or enhances, phenotypic variation over broad geographic scales (e.g., Thompson 2005; Siepielski and Benkman 2007a).

We also suspect that the local absence of pine squirrels may be important for the persistence of the mutualism between nutcrackers and limber pine. Recent theoretical models suggest that mutualisms in which an antagonistic species is competitively superior to one of the mutualistic species, as is the case with pine squirrels relative to nutcrackers, may require some form of refuge from the antagonist for the mutualism to persist (e.g., Ferrière et al. 2007). Such refuges could be ecological or reflect evolutionary processes. Ecologically, the absence of pine squirrels may help to mitigate the competitive effects of pine squirrels by simply allowing nutcrackers access to a greater fraction of the seed crop. Evolutionarily, the local areas devoid of pine squirrels, and where nutcrackers drive cone evolution, would act to prevent the escalation of cone and seed traits evolving solely in response to selection by pine squirrels. Both processes would in turn allow nutcrackers to continue to be an important seed disperser for limber pine, and prevent the interaction from breaking down. Consequently, local areas without pine squirrels may represent “refugia” (e.g., Bronstein et al. 2003) from the antagonistic effects of pine squirrels, and thus be critical for the persistence of the mutualism between nutcrackers and limber pine (see also Yu et al. 2001; Ferrière et al. 2007). On the other hand, because limber pine in these areas have become less well-defended against pine squirrels, this also means that limber pine would be prone to intense seed predation if pine squirrels were able to colonize such areas. The evolution of plant reproductive traits, like most phenotypes, is thus best thought of as a balance between multiple, and perhaps often, conflicting selection pressures (e.g., Schluter et al. 1991).

Using a trait-based, geographic approach we have found evidence that conflicting selection, which should be common among communities of interacting species (e.g., Strauss and Irwin 2004), plays an important role in enhancing phenotypic variation in ecologically important traits. The maintenance of variation in traits known to experience selection has been an enduring problem in biology (e.g., Blows and Hoffmann 2005). However, when we take into account the diversity of selection pressures that organisms contend with, it is perhaps not surprising to find evidence for ample variation in traits known to be under selection. Our results are also relevant to recent studies that have investigated the link between intraspecific plant variation and variation in the communities of organisms using plants. For example, increased plant

genetic diversity often results in more diverse insect communities on host plants, presumably because the different insect species cue in on phenotypic variation produced by the different genotypes (e.g., Crutsinger et al. 2006; Johnson et al. 2006). Although not the focus of these studies, our results bring into question the directionality of community-diversity trait-diversity relationships: does intraspecific plant diversity beget community diversity of organisms using plants or does community diversity of organisms using plants drive intraspecific plant diversity? We suspect both, but resolving this conundrum will require an understanding of the evolutionary histories of plant–animal associations.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Histograms of the first principal component of 10 limber pine cone and seed traits (PC1, as in main text Fig. 1) indicate that more variation and bimodality of cone structure is present within populations in the regions with pine squirrels (Rocky Mountains and Sierra Nevada; gray bars) but not in the region without them (Great Basin, all sites in Nevada, and the White Mountains, CA; white bars).

Table S1. Principal component loadings of the 10 limber pine cone and seed traits describing cone and seed structure and the amount of variation explained by the first principal component for the populations used in the selection analyses.

Table S2. Principal component loadings of the 10 limber pine cone and seed traits describing cone and seed structure and the amount of variation explained by the first principal component from the 18 populations (11 with pine squirrels, and seven without them) used to infer geographic variation in cone structure ($n = 639$ trees).

Table S3. Results of the general linear model used to make comparisons of variation in cone structure between regions with and without pine squirrels (Full model: $F_{4,13} = 6.52$, $P = 0.0042$).

Table S4. Study sites, whether pine squirrels are present or absent, Δ AIC from comparing models of PC1 assuming that the distributions of PC1 are composed of unimodal versus bimodal normal distributions, and the qualitative categorization of bimodality.

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

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