

# Landscape-scale eco-evolutionary dynamics: Selection by seed predators and fire determine a major reproductive strategy

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**Abstract.** Recent work in model systems has demonstrated significant effects of rapid evolutionary change on ecological processes (eco-evolutionary dynamics). Fewer studies have addressed whether eco-evolutionary dynamics structure natural ecosystems. We investigated variation in the frequency of serotiny in lodgepole pine (*Pinus contorta*), a widespread species in which postfire seedling density and ecosystem structure are largely determined by serotiny. Serotiny, the retention of mature seeds in cones in a canopy seed bank, is thought to be an adaptation for stand-replacing fire, but less attention has been paid to the potential selective effects of seed predation on serotiny. We hypothesized that spatial variation in percentage serotiny in lodgepole pine forests results from an eco-evolutionary dynamic where the local level of serotiny depends on the relative strengths of conflicting directional selection from fire (favoring serotiny) and seed predation (favoring cones that open at maturity). We measured percentage serotiny, the abundance of American red squirrels (*Tamiasciurus hudsonicus*; the primary pre-dispersal seed predator of lodgepole pine), and several measures of forest structure in Yellowstone National Park, USA. Fire frequency strongly predicted the frequency of serotiny, a pattern that is well-supported in the literature. At sites with high fire frequency (return intervals of ~135–185 years) where fire favors increased serotiny, squirrel abundance was negatively associated with serotiny, suggesting that selection from predation can overwhelm selection from fire when squirrels are abundant. At sites with low fire frequency (return intervals of ~280–310 years), serotiny was nearly universally uncommon (<10%). Finally, forest structure strongly predicted squirrel density independently of serotiny, and serotiny provided no additional explanatory power, suggesting that the correlation is caused by selection against serotiny exerted by squirrels, rather than squirrels responding to variation in percentage serotiny.

**Key words:** American red squirrel; climate change; cross-scale interactions; eco-evolutionary dynamics; fire; lodgepole pine; *Pinus contorta*; reproductive strategy; seed predation; serotiny; *Tamiasciurus hudsonicus*; Yellowstone National Park, USA.

## INTRODUCTION

Recently, there has been growing interest in the effects of evolutionary processes on ecological dynamics (eco-evolutionary dynamics; Schoener 2011) and in the potential for intraspecific genetic variation to influence community and ecosystem structure (community and ecosystem genetics; Whitham et al. 2006, Wymore et al. 2011). Theoretical and empirical work has shown that rapid evolution can contribute to ecological change (Hairston et al. 2005), and numerous examples demonstrate that genetic variation in foundation species (i.e., species that have large impacts on ecosystem function and comprise a majority of an ecosystem's three-dimensional physical structure; Ellison et al. 2005) can cause variation in community and ecosystem structure (Whitham et al. 2006). Nonetheless, it is less clear that

these effects are relevant in natural ecosystems, and many existing studies have necessarily been conducted in simplified model ecosystems (Hersch-Green et al. 2011, Schoener 2011). While such studies are important in describing the mechanisms underlying eco-evolutionary dynamics, it is equally crucial to investigate these effects in natural ecosystems. Specifically, unanswered questions include how the strength of evolutionary effects compares to that of ecological drivers in structuring ecosystems, whether these effects occur across broad spatial extents, and how evolutionary and ecological drivers combine to produce complex landscapes.

An important question is whether we can identify traits in foundation species that have community- and ecosystem-level consequences, and isolate selective agents driving variation in these traits. Additionally, landscape complexity often arises from multiple spatially structured biotic and abiotic drivers, and biotic interactions in natural communities are often complex (Strauss et al. 2005). It is necessary to demonstrate that eco-evolutionary dynamics are important in the context of this complexity. For example, experimentally dem-

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onstrated pairwise interactions may disappear when studied in natural communities with more complex interactions; eco-evolutionary dynamics that are predicted as a result of such an experiment might have little real-world influence on landscape patterns. In contrast, eco-evolutionary effects may interact strongly with spatially structured ecological drivers to produce landscapes that result from spatial variation in natural selection. Spatially variable selection can be very important in structuring coevolutionary dynamics (Thompson 2005); it follows that similar dynamics may influence ecological structure.

Here, we report on a study investigating the potential for selection from a seed predator to drive the evolution of serotiny, an ecologically significant trait that has well-studied associations with landscape-scale community and ecosystem structure and function. Serotinous plants retain seeds in a canopy seed bank until an environmental cue (commonly stand-replacing fires; Lamont et al. 1991) causes the seed-bearing structures to open and release seeds. We focus on Rocky Mountain lodgepole pine (*Pinus contorta* subsp. *latifolia*), a foundation species that dominates vast areas of western North America (Critchfield 1980). Rocky Mountain lodgepole pine exhibits variable serotiny; individual trees produce predominantly cones of one type (i.e., either serotinous or non-serotinous; Koch 1996), but the frequency of serotiny within stands varies greatly at multiple spatial scales (Tinker et al. 1994, Benkman and Siepielski 2004).

Serotiny is an excellent candidate for demonstrating widespread and biologically significant effects of evolutionary dynamics on ecosystem structure. Serotiny in lodgepole pine is highly heritable; broad-sense heritability for serotiny is probably at least 0.5, based on recent association mapping of the trait (Parchman et al. 2012). Furthermore, because it is an important alternative reproductive strategy, serotiny should be strongly correlated with fitness and, thus, potentially subject to strong selection (Lamont et al. 1991). Furthermore, serotiny is ecologically important and can have profound effects on landscape structure. Several studies have addressed postfire dynamics in lodgepole pine forests following widespread stand-replacing fires in the Greater Yellowstone Ecosystem (GYE) in 1988. They found fine-grained (i.e., scales <1 km) variation in postfire stand density ranging over several orders of magnitude (from conversion to open habitats to very dense forest), and concluded that much of this variation could be attributed to variation in the prefire frequency of serotiny (Tinker et al. 1994, Turner et al. 1997, Schoennagel et al. 2003). Postfire stand density also affects community and ecosystem processes in recovering forests (e.g., community composition and assembly, annual net primary productivity, total leaf area; Turner et al. 2003a, b, 2004).

Most studies attempting to account for variation in the frequency of serotiny have focused on the frequency of stand-replacing fires, and high fire frequency is

hypothesized to favor increased serotiny (Givnish 1981, Schoennagel et al. 2003). Fire frequency is positively correlated with percentage serotiny in several species, and models and empirical work have suggested that increased fire frequency can select for serotiny (Perry and Lotan 1979, Muir and Lotan 1985, Lamont et al. 1991, Gauthier et al. 1996, Enright et al. 1998, Schoennagel et al. 2003, Radeloff et al. 2004). Less attention has been given to the potential of biotic interactions to influence percentage serotiny, although population models suggest that any factor that depletes the canopy seed bank (e.g., pre-dispersal seed predation) can select against serotiny (Enright et al. 1998). Serotiny increases exposure to pre-dispersal seed predation; therefore, with extensive seed predation, plants that could escape predation (i.e., by not being serotinous) would be at a selective advantage (Janzen 1969). Thus, percentage serotiny in a given stand potentially stems from a balance of selection from an abiotic factor, fire, and a biotic factor, seed predation. Isolated lodgepole pine stands that have historically lacked American red squirrels (*Tamiasciurus hudsonicus*), the primary pre-dispersal seed predator in lodgepole pine forests (Smith 1970), have a much higher percentage serotiny than stands where red squirrels are present, suggesting that opposing directional selection from fire and seed predation drives continental-scale variability in serotiny in this system (Benkman and Siepielski 2004). However, isolated ranges that lack red squirrels are uncommon compared to the wide range of lodgepole pine in North America; thus, an unanswered question is whether landscape-scale variability in percentage serotiny within continuous forests can be explained by the interaction of these two factors. If selection pressure from red squirrels drives some of the variation in serotiny, then this system would represent an important example of a widespread natural ecosystem where spatially variable selection on a heritable trait produces large-scale patterns in ecosystem structure and function.

Here, we report on the results of a field study testing the general hypothesis that variation in the relative strengths of selection from squirrels and fire drive spatial patterns in serotiny in lodgepole pine. Previous work in this system has shown that serotiny is uncommon at high elevations, probably due to low fire frequencies (Tinker et al. 1994, Schoennagel et al. 2003). We used this natural variation to evaluate the relationship between serotiny and the intensity of seed predation (estimated by squirrel density) when selection from fire was strong (at low elevations) and weak (at high elevations). We tested the following hypotheses: (1) as previously described (Tinker et al. 1994, Schoennagel et al. 2003), low fire frequencies at high elevations favor non-serotinous trees, regardless of squirrel density; (2) high fire frequencies at low elevations favor serotiny, but the strength of selection against serotiny from squirrels increases with squirrel density, resulting in a negative correlation between squirrel density and the frequency

of serotiny; and (3) squirrel density is driven by overall habitat structure regardless of serotiny (and therefore the correlation predicted in hypothesis 2 is due to natural selection imposed by the squirrels, not due to a preference for low-serotiny sites). We show that landscape-scale patterns in percentage serotiny are consistent with opposing selection from fire and seed predation, and that evolutionary dynamics appear to have large ecological ramifications in this widespread natural ecosystem.

## MATERIALS AND METHODS

### *Study area*

We performed all work in lodgepole pine forests within Yellowstone National Park, USA. We used GIS to identify areas that were dominated by lodgepole pine and had not burned within the last 60 years (because serotiny is not reliably expressed in lodgepole pine until the trees are >55 years of age; Critchfield 1980). We also stratified our study area into low- and high-elevation regions (<2456 m and  $\geq$ 2456 m, respectively), based on previous work in the region showing an elevational threshold above which serotiny is uncommon, probably due to low fire frequency at higher elevations (Tinker et al. 1994, Schoennagel et al. 2003). We randomly located 13 study plots within each of the low- and high-elevation regions. Due to a GIS error (in which a region mapped as mature lodgepole pine was dominated by other species), one of the low-elevation plots was excluded from the study, for a final sample size of 12 low- and 13 high-elevation plots. A map of the study area and sample plots is provided in Appendix A.

### *Field methods*

Sample plots averaged 32.3 ha (range 25.5–45.4 ha) and consisted of a minimum convex polygon surrounding all subsamples plus a 50-m buffer. Previous work and preliminary analyses of our data indicated that spatial autocorrelation in percentage serotiny diminishes at scales greater than 1 km (Tinker et al. 1994). We therefore enforced a minimum distance of 1 km between plots to reduce spatial autocorrelation. Within each plot, we established north–south oriented strip transects (200  $\times$  20 m), arranged in two rows with 150 m separating transect centerlines in each direction. We also established two 400-m<sup>2</sup> subplots 50 m from the end of each transect. When transects intersected barriers (e.g., standing water, topographic barriers) or patches dominated by species other than lodgepole pine, the transect was interrupted and continued on the other side of the barrier. If barriers intersected >25% of a transect's total length, that transect was dropped from the plot. We sampled 14 plots in 2010 and 11 plots in 2011. Plots sampled in 2010 had eight transects each (two rows of four transects each). Exploratory analyses of 2010 data indicated that there was little within-plot variance; thus we reduced the number of transects to six per plot in

2011. All transect and subplot subsamples were aggregated at the plot level for analysis.

Within each sample plot, we estimated squirrel density as the number of active squirrel middens per hectare. Red squirrels in lodgepole pine generally produce one large central midden per territory, although some squirrels also produce smaller satellite middens as much as 20 m from the central midden (Smith 1968, Elliott 1988). Squirrel density in lodgepole pine is likely to be stable from year to year due to low interannual variability in seed production (Rusch and Reeder 1978, Gurnell 1984, Wheatley et al. 2002). Furthermore, middens are used for multiple years, vacant middens are rapidly reoccupied, and overwinter survival of squirrels that lack a territory (and thus an associated cache and midden) is very low (Gurnell 1984, Larsen and Boutin 1994). Thus, the density of active middens is an appropriate estimate of long-term red squirrel density. Midden density was recorded along our 0.4-ha strip transects. Single observers walked transect centerlines to spot middens, and measured the right angle distance from the transect centerline to the center point of each midden with 5-cm precision. Identification of active central middens was based on size (>1 m in diameter) and the presence of recently chewed cone cores and scales on the top of the midden. Some squirrels also produce smaller satellite middens as much as 20 m from the central midden (Smith 1968, Elliott 1988). When satellite middens were identified without an accompanying central midden, we left the transect line and attempted to locate the central midden. When estimating squirrel density, we only counted active central middens that were within 20 m of the transect centerline.

To estimate percentage serotiny, we recorded the number of serotinous and non-serotinous trees within each 400-m<sup>2</sup> subplot. If a subplot contained fewer than 20 trees, we counted the closest 20 trees to the subplot center. We classified trees into four categories based on the number of mature, non-weathered (i.e., brown in color) closed cones: strongly serotinous (95–100% closed cones), weakly serotinous (50–95% closed cones), weakly non-serotinous (5–50% closed cones), and strongly non-serotinous (0–5% closed cones). In practice, most trees (99%) were strongly serotinous or strongly non-serotinous; therefore, for analysis, we considered both weakly and strongly serotinous trees as serotinous, and all other trees as non-serotinous.

To test hypotheses about the effect of forest structure on red squirrel density, we measured several aspects of forest structure within each 400-m<sup>2</sup> subplot. We measured overhead canopy cover at five points spaced 5 m apart using a spherical crown densitometer (Model A, Forestry Suppliers, Jackson, Mississippi, USA). We made a single measurement of basal area at the subplot center using a 2.0 BAF basal area prism. Finally, we recorded the species and diameter at breast height (dbh) for the 20 trees (>10 cm dbh) closest to the subplot center. We also used data from Tinker et al. (2003) to

determine stand age at the plot level. For 13 plots that fell outside the area mapped by Tinker et al. (2003), we obtained an estimate of stand age by collecting 9–32 tree cores (distributed evenly among subplots) from large lodgepole pine trees (i.e., trees that appeared to be among the oldest in the stand). All cores were mounted on wooden blocks and polished until rings were clearly visible. We determined the age of each core by analyzing a scanned image using WinDENDRO software (Regent Instruments, Quebec City, Quebec, Canada). Finally, we included GIS data for slope, aspect, and mean annual precipitation at the plot level in our analysis. Summary statistics for all variables are included in Appendix B.

#### *Statistical analysis*

For all analyses that compared multiple models, we calculated Akaike's Information Criterion adjusted for small samples sizes ( $AIC_c$ ), as well as  $\Delta AIC_c$  values and the associated model weights. These model weights can be interpreted as the probability that a given model in a set is the best model in the set (Burnham and Anderson 2001). We considered a single model in a set to be superior when  $\Delta AIC_c > 10$  for all other models in the set. In this case, we interpreted the best model and ignored all others. All analyses were performed using the R software package (R Development Core Team 2012).

To ensure that our sampling scheme was effective at minimizing spatial autocorrelation and that we had met the assumption of no spatial dependence in model residuals (Legendre 1993), we used package *spdep* in R (Bivand et al. 2012) to calculate Moran's  $I$  on the residuals of all models. We tested the null hypothesis of no spatial autocorrelation in model residuals using permutation tests with 1000 replicates. Because we found no significant spatial autocorrelation in the residuals for any of our models, these results are not presented and are not discussed further.

To describe the relationship between squirrel density and percentage serotiny in the context of other factors known to influence serotiny, we used generalized linear models (GLMs) with binomial errors and logit link functions. For all models, we used the proportion of serotinous trees as the response and included combinations of squirrel density, elevation, and stand age as predictors. We selected stand age and elevation, and excluded other possible environmental factors (e.g., soils, topography) based on previous work that has found no correlations between serotiny and any factors except stand age and elevation (Tinker et al. 1994, Schoennagel et al. 2003). For ease of interpretation and to match our experimental design that was stratified by elevation, we included elevation as a categorical variable (i.e., whether a plot was high elevation or not). We included all combinations of the three predictors in first-order additive models (i.e., no quadratic or interaction terms) in our initial model set, for a total of seven models. We also included two models incorporating

two-way interactions with squirrel density to examine whether the effects of squirrels might vary depending on the other predictors. We did not examine higher-order interactions due to the likelihood that such models would be overparameterized, given the relatively small sample size.

To analyze how habitat structure and local topography and climate affected squirrel density, we used principal components analysis (PCA) to reduce the number of variables and eliminate correlations among predictors. We included eight variables describing habitat structure and local climate and topography in the PCA, and retained for analysis all axes with eigenvalues  $>1$  (Quinn and Keough 2002). We then included the retained PCA axes in GLMs with squirrel density as the response. All models assumed Gaussian errors. We log-transformed squirrel density to better meet assumptions of normality and homoscedasticity in the residuals.

To test what factors predicted squirrel density, we first analyzed our 12 high-elevation plots (where serotiny was uncommon). We included additive effects for a maximum of three PCA axes in each model, and we compared all possible models that met these restrictions. We then constructed a model using the same predictors for low-elevation plots ( $n = 13$ ) in order to test whether the same factors predicted squirrel density regardless of elevation. Because some PCA axes may be elevation specific, we also evaluated subsets of this model by subtracting single predictors one at a time and comparing them with the original model using analysis of variance (ANOVA). To test whether percentage serotiny affected squirrel density after accounting for habitat selection based on other factors, we added percentage serotiny as a predictor to the low-elevation model and used ANOVA to test the hypothesis that the more complex model was not an improvement over the simpler model.

## RESULTS

### *Percentage serotiny*

Of our nine models predicting percentage serotiny, the model including squirrel density, elevation, and an interaction between the two had substantially more support than any other model in the set (Appendix C). Because of the strong support for this single model (model weight = 1,  $\Delta AIC_c = 33.3$  for the second strongest model), and because both squirrel density and elevation were in each of the top three models, we interpret only the strongest model here.

As expected, we found negative relationships between percentage serotiny and both elevation and squirrel density (Table 1). This model incorporated an interaction between squirrel density and elevation, resulting in a greatly reduced slope of the relationship between squirrel density and percentage serotiny at high elevations compared with low elevations (Fig. 1).

TABLE 1. Parameter estimates and standard errors (logit-transformed) for the best-supported model for percentage serotiny in lodgepole pine, *Pinus contorta*.

Parameter	Estimate	SE
Intercept	0.595	0.077
Squirrel density	-2.012	0.116
Elevation	-2.468	0.118
Squirrel density × elevation	1.135	0.172

Note: Predictors included in this model are red squirrel density (individuals/ha), elevation (categorical variable, true when elevation ≥2456 m), and an interaction between the two.

*Squirrel habitat*

We retained the first four principal components for use as predictors in regression models; these axes described 82% of the variance in the original variables (Table 2). We compared a total of 13 models using the high-elevation data. Six of these models were very similar in terms of the degree of support ( $\Delta AIC \leq 10$ ; Table 3). The best-supported model (model weight = 0.60) included PC1 and PC2. Of the six best models, PC1 was present in three, and PC2 appeared in all six. Therefore, we consider only the best-supported model, as it was both the most probable in the set and broadly representative of other highly supported models.

Our best model explained 82% of the variance in squirrel density at high elevations. We found a positive correlation with PC1, indicating that squirrels at high-elevation sites tended to be in areas with less precipitation and greater percent cover of lodgepole pine. Squirrel density was negatively correlated with PC2, indicating a preference for sites with greater overhead canopy cover, greater mean dbh, and greater variation in dbh (Fig. 2, Table 4).

A model using the same predictors (PC1 + PC2) for low-elevation plots explained 33% of the variance in squirrel density. However, PC1 largely described variation in the percent cover of whitebark pine (*P. albicaulis*), a species that is uncommon at low elevations. Comparing this model to a more parsimonious model containing only PC2, we found no improvement as a result of retaining PC1 (ANOVA  $F_{1,10} = 0.186$ ,  $P = 0.68$ ). Therefore, we used the more parsimonious model for testing the effect of percentage serotiny at low elevations (Table 4). Finally, adding percentage serotiny as a predictor to this model did not significantly improve the model (ANOVA  $F_{1,10} = 0.643$ ,  $P = 0.44$ ), implying that the variables included in the PCA are sufficient to explain variation in squirrel density independently of variation in percentage serotiny.

DISCUSSION

We observed patterns in stand-level percentage serotiny that were consistent with the hypothesis that variation in percentage serotiny in lodgepole pine is due to a balance of selection from frequent fires (favoring more serotiny) and counter selection from pre-dispersal

seed predators (favoring less serotiny) (Benkman and Siepielski 2004). These two drivers appear to interact across scales (Peters et al. 2004). Fire frequency, which is strongly associated with elevation in this system (Schoennagel et al. 2003), is likely to produce coarse-scale variation in serotiny. Serotiny is rare in high-elevation regions, which can encompass thousands of hectares, and we observed little among-plot variance in percentage serotiny in our high-elevation plots. At low elevations, fire frequencies are higher and mean percentage serotiny is consequently higher (Appendix B). However, we observed considerable among-plot variation at low elevations, suggesting that processes operating at finer scales contribute to variation in

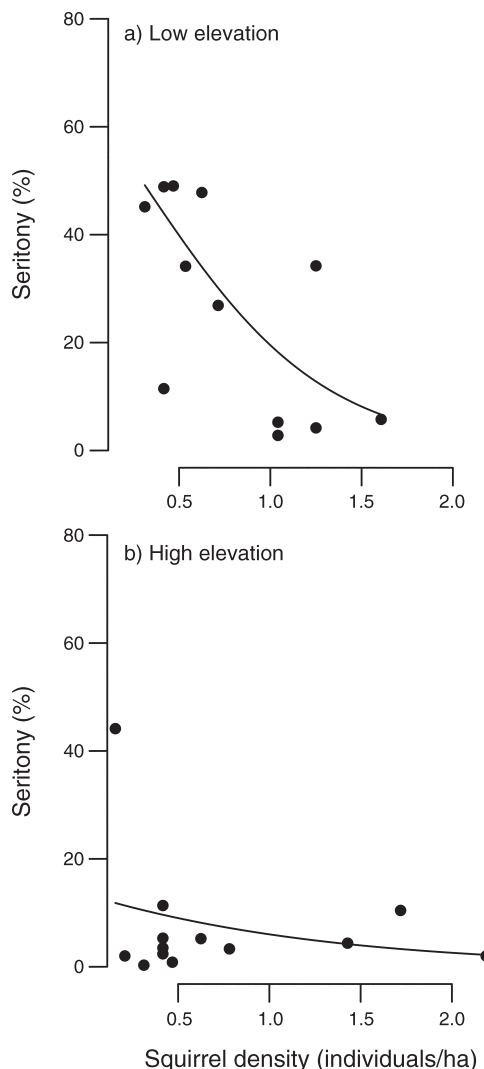


FIG. 1. Relationship between red squirrel (*Tamiasciurus hudsonicus*) density and percentage serotiny in lodgepole pine (*Pinus contorta*) at low (<2456 m) and high (≥2456 m) elevations in Yellowstone National Park, USA. Fitted lines are from a generalized linear model as described in *Materials and methods: Statistical analysis*.

TABLE 2. Factor loadings, eigenvalues, and proportion of variance explained from a principal-components analysis on microclimate, forest structure, and forest composition variables used to describe red squirrel habitat.

Variable	PC1	PC2	PC3	PC4
Northness	-0.2625	0.2851	-0.3697	0.4001
Eastness	-0.0204	-0.4837	-0.5537	-0.4015
Precipitation	-0.5157	-0.0157	-0.1014	-0.3181
Canopy cover	0.3608	-0.3790	0.2039	0.4564
dbh	-0.1323	-0.4442	-0.4139	0.5121
%CV of dbh	-0.0285	-0.5606	0.3835	-0.2231
<i>Pinus contorta</i> % cover	-0.4921	-0.0813	0.4046	-0.1853
<i>P. albicaulis</i> % cover	0.5235	0.1506	-0.1509	-0.1514
Eigenvalue	1.6665	1.2557	1.0866	1.0198
Proportion of variance explained	0.3472	0.1971	0.1476	0.1300
Cumulative proportion of variance	0.3472	0.5443	0.6918	0.8218

Note: Only axes with eigenvalues >1 are shown.

serotiny. Although the precise scale of variation in squirrel density is unknown, it is likely to be finer grained than elevation-driven differences in fire frequency, and our results suggest that this variation in percentage serotiny at scales of hundreds of hectares is a result of selection from seed predation. Here, we discuss further how serotiny varies as a function of these two factors, address potential alternative hypotheses, and discuss the implications of our findings with respect to eco-evolutionary dynamics.

*Variation in percentage serotiny in relation to fire frequency and seed predation*

In the Greater Yellowstone Ecosystem (GYE), the frequency of stand-replacing fires is much lower at high elevations than at low elevations (fire intervals of 280–310 years and 135–185 years, respectively; Schoennagel et al. 2003), and percentage serotiny at high elevations was nearly uniformly low, regardless of squirrel density (Fig. 1b; see also Tinker et al. 1994); this pattern is consistent with previous work suggesting that serotiny is not favored at low fire intervals (Enright et al. 1998, Keeley and Zedler 1998). In contrast, mean percentage serotiny was greater at lower elevations, as expected if the adaptive value of serotiny increases with increasing fire frequency (Enright et al. 1998, Schoennagel et al. 2003). However, percentage serotiny decreased strikingly with increasing squirrel density at low elevations (Fig.

TABLE 3. Model selection results, based on Akaike's Information Criterion, for the analysis of squirrel density at high elevations as a function of microclimate and forest structure variables.

Model	K	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Model weight
PC1 + PC2	3	21.74	0	0.60
PC2	2	23.87	2.14	0.21
PC1 + PC2 + PC3	4	25.27	3.53	0.10
PC1 + PC2 + PC4	4	27.21	5.48	0.04
PC2 + PC4	3	27.93	6.20	0.03
PC2 + PC3	3	28.57	6.83	0.02

Notes: Model names show the predictors for models with log(squirrel density) as the response. Only models with ΔAIC<sub>c</sub> < 10 are shown; K is number of parameters.

1a). The strong effect of squirrels on serotiny was further supported by our test showing that predation intensity (represented by squirrel density) was a better predictor of percentage serotiny than was local fire history (represented by stand age); see Appendix C. Furthermore, individual fires generally may be too large to explain the fine-grained variation in percentage serotiny that we observed at low elevations (and fine-grained variance in postfire stand density reported elsewhere; Tinker et al. 1994, Turner et al. 2004). Overall, our results indicate that although serotiny is favored by high fire frequency, selection exerted by seed predators can

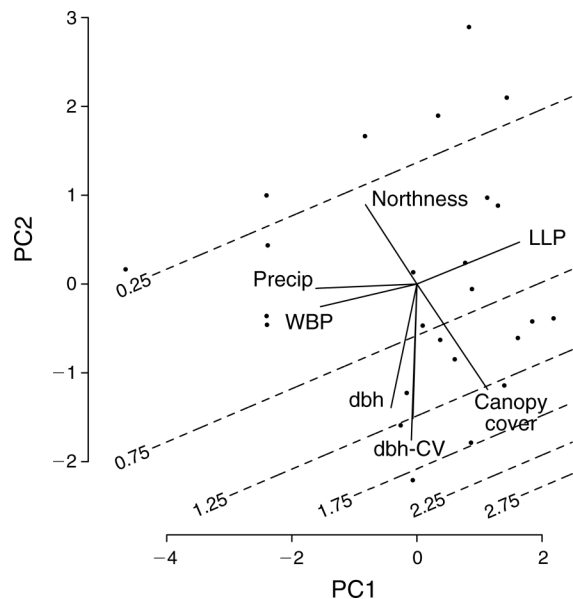


FIG. 2. Principal-components biplot showing factor loadings (solid lines) for the first two principal components (PC). The most strongly correlated variables included mean annual precipitation (precip), percent cover of whitebark pine (WBP), percent cover of lodgepole pine (LPP), northern exposure (northness), percent overhead canopy cover (canopy cover), mean tree diameter at breast height (dbh), and the coefficient of variation in dbh (dbh-CV). Dashed contour lines show squirrel density (individuals/ha), predicted from the regression model for high-elevation sites; contour values are shown at the bottom and left margins of the plot.

overwhelm the effect of high fire frequency and drive serotiny to low levels, as proposed by Benkman and Siepielski (2004).

#### *Alternative hypotheses*

In the present study, we focused on the hypothesis that spatial variation in percentage serotiny arises from a combination of selection from fire and seed predation. That higher fire frequencies exert natural selection favoring serotiny is well-supported in the literature (Gauthier et al. 1996, Enright et al. 1998, Keeley and Zedler 1998, Schoennagel et al. 2003). Therefore, we do not evaluate alternatives to differences in fire frequency as an explanation for the coarse-scale negative correlation between percentage serotiny and elevation. Because fewer studies have addressed the potential for natural selection from seed predation to affect serotiny (but see Lamont et al. 1991, Enright et al. 1998, Benkman and Siepielski 2004), we address several alternatives to this hypothesis here.

One alternative is that squirrel density is driven by food supply, and that serotinous cones are a poorer food source than non-serotinous cones, leading to the observed negative correlation between percentage serotiny and squirrel density. Within lodgepole pine forests, cones from lodgepole pines are the most important food source for red squirrels, and may largely regulate population size during years when food is scarce (Smith 1968, Gurnell 1984). Where squirrels have historically been present, serotinous cones are well-defended against predation (Smith 1970, Elliott 1974, Benkman et al. 2001), and energy intake rates may be lower for squirrels feeding on serotinous cones (Smith 1970), implying an advantage from feeding on non-serotinous cones. In a pilot study, we measured cone survival near squirrel middens and found that squirrels preferentially harvest non-serotinous cones over serotinous cones in the fall, when cones are mature but before non-serotinous cones have opened. However, the greater availability of serotinous cones (year-round as opposed to several weeks in the fall) results in annual predation rates for first-year serotinous cones that are nearly double those for non-serotinous cones. We estimated a mean survival probability of 0.44 for non-serotinous cones, compared to 0.23 for first-year serotinous cones and 0.63 and 0.78 for mature and weathered serotinous cones, respectively. Considering that serotinous cones are exposed to predation for multiple years, actual survival probabilities are even lower. For example, we estimated that the probability of a serotinous cone surviving for 10 years was 0.011 at our site (M. V. Talluto and C. W. Benkman, *unpublished manuscript*). These data suggest that we might expect purely ecological explanations (i.e., that the correlation between squirrel density and percentage serotiny is driven by food availability) to predict an increase in squirrel density with percentage serotiny instead of the observed negative correlation (Fig. 1).

TABLE 4. Parameter estimates and standard errors for the best-supported models for squirrel density at high and low elevations.

Parameter	High elevations		Low elevations	
	Estimate	SE	Estimate	SE
Intercept	-0.615	0.118	-0.345	0.124
PC1	0.169	0.064		
PC2	-0.565	0.115	-0.208	0.089

Additionally, the pattern that we observed at high-elevation sites does not support the alternative hypothesis that variation in squirrel density is driven by percentage serotiny. First, the correlation between squirrel density and percentage serotiny at high elevations was negative but weak, and driven largely by a single influential point (Fig. 1); removing this point eliminates the correlation. Second, even though serotiny was uncommon at these sites (<12% for all sites except one), squirrel density varied as much as at low elevations. If percentage serotiny were the primary driver of squirrel density, we would expect to see less variance in squirrel density at high elevations (Fig. 1).

A second possibility is that percentage serotiny drives squirrel density at low elevations, but other factors are important at high elevations where serotiny is uncommon. To evaluate this hypothesis, we used the high-elevation sites to construct a habitat model predicting squirrel density. This model was highly predictive of squirrel density (Table 4, Fig. 2). We found that squirrels were associated with denser canopies, larger trees (as measured by dbh), and greater variation in tree dbh. Furthermore, the same factors were important predictors of squirrel density at low elevations, suggesting that forest structure is an important driver of squirrel density, regardless of elevation. Finally, percentage serotiny did not predict additional variation in squirrel density at low-elevation sites. Therefore, our data do not support the hypothesis that squirrel density is driven by different factors at high and low elevations; forest structure, not serotiny, appears to be the primary driver of squirrel density, regardless of elevation.

Phenotypic plasticity could account for the patterns that we observed if trees are able to switch from making serotinous cones to non-serotinous cones when predation pressure is high. The ability to produce non-serotinous cones and escape from predation when squirrels are numerous and return to producing serotinous cones if squirrel populations decline could have considerable adaptive value. However, the high heritability of serotiny in lodgepole pine (Parchman et al. 2012), coupled with the rarity of trees that show evidence of producing both types of cones (<1% when looking at multiple cone cohorts within trees), implies that most trees produce a single type of cone and do not switch after reaching maturity.

In sum, our data are consistent with the hypothesis that squirrels select against serotiny and the associated

hypothesis that squirrel density varies in relation to drivers unrelated to serotiny. At high elevations, fire frequencies are typically low enough that serotiny is not expected to be favored (Enright et al. 1998, Keeley and Zedler 1998, Schoennagel et al. 2003). Thus, we expect little serotiny at these elevations, regardless of squirrel density (see also Tinker et al. 1994). At low elevations, high fire frequency favors the evolution of serotiny, whereas selection by squirrels favors the production of non-serotinous cones. Thus, we expect a negative correlation between squirrel density and percentage serotiny. Where squirrels are uncommon, fire is the dominant selective agent, and serotiny is common. Where squirrels are abundant, selection exerted by seed predators overwhelms selection from fire, and serotiny is rare. This is the only hypothesis that correctly predicts the patterns that we observed at both high and low elevations.

#### *Implications for eco-evolutionary dynamics*

We have shown that seed predation from a small mammal appears to drive variation in an ecologically significant trait in lodgepole pine forests, an ecosystem that dominates millions of hectares in western North America (Critchfield 1980). The importance of serotiny in these forests is difficult to overstate; the canopy seed bank produced by serotinous trees is closely associated with postfire stand density, and it probably affects community and ecosystem structure for decades or centuries following stand-replacing fires (Tinker et al. 1994, Schoennagel et al. 2003, Turner et al. 2003a, b, 2004, Kashian et al. 2005). Landscape legacy can be an important driver of contemporary ecosystem dynamics (Turner 2005). In lodgepole pine, the signature of differences in postfire stand density can be detected for at least a century following a fire (Kashian et al. 2005). Given the fire rotations in this ecosystem, it is plausible that, at a given time, the state of a large fraction of the landscape (e.g., >10%) is influenced by the legacy of postfire recovery density following stand initiation. Our study implies that selection exerted by red squirrels may influence the structure of millions of hectares of North American forests.

Lodgepole pine is a long-lived organism, and gene flow can be extensive in conifers; thus, the spatial and temporal dynamics of evolutionary change in this species are an important consideration when addressing eco-evolutionary dynamics. Perry (1978) estimated that pollen dispersal in lodgepole pine could move genes 300–500 m/year, suggesting considerable gene flow that could overwhelm the effect of selection in highly localized populations. However, lodgepole pine shows relatively short-range seed dispersal (~40 m; Perry 1978, Greene and Johnson 1989), and this species shows evidence of local adaptation in traits associated with climatic variation despite gene flow (Yeaman and Jarvis 2006). More generally, local adaptation due to strong selection despite the presence of gene flow is a well-

known phenomenon (Linhart and Grant 1996). Although gene flow via pollen dispersal probably reduces overall spatial phenotypic variance, strong selection and short-range seed dispersal are likely to be sufficient to produce spatial structure at the scale of our study plots (i.e., tens of hectares).

At low elevations in the GYE, historical fire return intervals have been estimated at 135–185 years (Schoennagel et al. 2003); these intervals probably set a lower bound on the time scale of changes in percentage serotiny in this system. Given the strong relationship between canopy seed bank and postfire seedling density, a single fire interval may be sufficient time for measurable changes in percentage serotiny. If squirrels reduce the canopy seed bank enough to decrease postfire density, then a larger proportion of seedlings in that stand will come from non-serotinous parents (either from relict trees or dispersal from outside the burn area), resulting in a greater proportion of non-serotinous trees in the mature stand. A number of studies suggest that tree squirrels feeding on conifers can remove a very high proportion of cones, suggesting that selection may be intense enough to drive local adaptation in cone traits in these systems (for a review, see Steele et al. 2005). For example, Steele and Weigl (1992) found that fox squirrels (*Sciurus niger*) feeding on *Pinus palustris* could remove as much as 85% of a stand's seeds. Given the high heritability of serotiny in lodgepole pine (Parchman et al. 2012), the magnitude of the potential effects of seed predation on the size of the canopy seed bank, and the strong relationship between the size of the canopy seed bank and postfire seedling density, it is possible that the evolution of serotiny in response to seed predation could be quite rapid.

Existing studies of eco-evolutionary dynamics have often focused on developing concepts using simplified model ecosystems, leaving unanswered the question of whether eco-evolutionary effects structure natural ecosystems (Hersch-Green et al. 2011, Schoener 2011). Our results suggest that evolutionary changes can determine contemporary ecosystem structure, that such interactions are probably ongoing, and that these interactions can vary across multiple spatial scales. These effects are likely to be most pronounced in ecosystems dominated by a single foundation species that interacts strongly with a small number of biotic and abiotic drivers. It remains to be shown how powerful eco-evolutionary effects are in natural ecosystems with more complex interactions (Strauss et al. 2005).

An important implication of eco-evolutionary dynamics in natural systems is that land management efforts must consider evolutionary processes in addition to ecological interactions (Schoener 2011). In forests in western North America, climate-driven changes in fire regimes have the potential to greatly restructure entire ecosystems at landscape and continental scales (Liu et al. 2010, Loehman et al. 2011, Westerling et al. 2011). Generally, these models do not incorporate evolution



when predicting landscape changes, and models of serotinous species have ignored the potential for interactions with seed predators to influence model predictions. Given the importance of serotiny in determining the structure of lodgepole pine forests, future models must go beyond simply predicting changes to fire regime and explore how climate change will affect the total selective regime on serotiny.

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#### SUPPLEMENTAL MATERIAL

##### Appendix A

Map of study sites within Yellowstone National Park ([Ecological Archives E094-116-A1](#)).

##### Appendix B

Summary statistics for all variables ([Ecological Archives E094-116-A2](#)).

##### Appendix C

Model selection details for models with percentage serotiny as the response variable ([Ecological Archives E094-116-A3](#)).