

ECOLOGY AND CONSERVATION OF ENDANGERED TERRITORIAL SPECIES UNDER INVASION

by

Jonathan J. Derbridge

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Jonathan J. Derbridge, titled Competition Between Territorial Species and Ecologically Similar Invaders and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.



John L. Koprowski Date: 12 Dec 2017



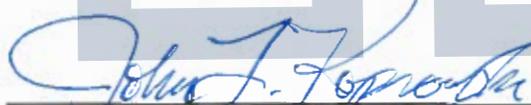
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Dissertation Director: John L. Koprowski Date: 12 Dec 2017

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ABSTRACT

Biological invasions threaten biodiversity globally, and degraded ecosystems increase the potential for invaders to compete with threatened native populations. In natural systems, niche partitioning minimizes interspecific competition, but introduced species may alter expected outcomes by competing with ecologically similar species for scarce resources. Where food production is highly variable, coexistence of native and invasive competitors may depend on dietary niche flexibility. Territorial species under invasion face additional challenges to maintain economically defensible territories. From 2011-2016, we conducted removal and behavior experiments to determine effects of non-territorial introduced Abert's squirrels (*Sciurus aberti*) on diet, space use, and territoriality of endangered Mount Graham red squirrels (MGRS; *Tamiasciurus fremonti grahamensis*) in their declining habitat in the Pinaleño Mountains, Arizona. We collected comparative data from Arizona sites of natural syntopy between Abert's and Fremont's squirrels (*T. fremonti*). Stable isotope analysis revealed similar dietary partitioning among populations. Experimental removals did not appear to affect MGRS diet. Space use by MGRS responded inconsistently to removals; territory sizes increased after the first removal, but did not change following the second removal. Territory sizes and body mass of MGRS were sensitive to conspecific population density and food production. Behavioral experiments showed MGRS were more aggressive than other Fremont's squirrels (hereafter, red squirrels). Dietary flexibility of Abert's squirrels may have facilitated coexistence with MGRS, possibly due to coevolved resource partitioning with red squirrels. However, aggressive territoriality toward Abert's squirrels may incur fitness costs for MGRS especially during poor food production years. Climate change

may reduce the advantage of ecological specialist species globally, and where introduced species are better-adapted to novel environmental conditions, native species may ultimately be replaced.

INTRODUCTION

Global-scale threats to the conservation of biodiversity include the proliferation of invasive species (Vitousek *et al.* 1997a). Humans have deliberately and unintentionally assisted non-native plants and animals to invade otherwise unavailable ecological niches, and many native species have succumbed to competition or predation against which they possessed no evolved defense (Mooney & Cleland 2001; Blackburn *et al.* 2004).

Invasive species have caused and exacerbated habitat loss for native flora and fauna (Norbury *et al.* 2013), and global climate change has set the stage for planetwide conversion of native to invaded communities (Vitousek *et al.* 1997b).

Animal populations at species' geographic ranges peripheries can be useful models for ecological studies of invasions because the relatively extreme conditions they experience facilitate answering a range of basic and applied questions about niche partitioning, competition, behavior, and population dynamics (Brown, Stevens & Kaufman 1996; Vucetich & Waite 2003; Merrick & Koprowski 2017). When ecologically similar native and introduced species interact, the typical expectation is that introduced species will be outcompeted and fail to establish (Williamson & Fitter 1996). However, anthropogenically altered native communities may provide resource opportunities for non-native species to flourish (Shea & Chesson 2002), in some cases at the expense of ecologically related natives (Tompkins, White & Boots 2003).

The space most animals typically travel in search of food is known as a home range (Burt 1943) and theoretically, an individual's energetic requirements determine its home range size, with larger animals requiring larger home ranges (McNab 1963).

Territorial species actively exclude competitors from specific portions of a home range

(Noble 1939), and territories are maintained at economically defensible sizes that enhance individual fitness (Brown 1964). As ecologically similar species become sympatric through natural processes, resource partitioning limits competition and preserves species' ecological niches (Hutchinson 1957), but in invaded communities, negative effects of competition may happen faster than resources can be partitioned (Strauss, Lau & Carroll 2006), leading to rapid decline or extirpation of native species (Davis 2003). For peripheral populations of territorial species, the addition of non-native intruders to the simultaneous effects of habitat change, population density, and food availability could make economic defensibility of territories impossible.

Territorial species defend an exclusive zone to increase probability of acquiring any combination of nest sites, mates, and food (Schoener 1968; Fretwell & Lucas 1969). To the biologist, territorial behavior is a striking representation of an evolved mechanism to preserve genes. For the individual territory owner, it is critical that the intensity of aggressive behavior matches the value of the defended resource (Brown 1964). Invasive species however, may disrupt the trade-offs for territorial behavior because niche partitioning has not occurred, and native territorial species may be unable to respond in a way that maximizes fitness (Petren & Case 1996).

Competition for food has a major role in foraging strategies of sympatric species (Pimm, Rosenzweig & Mitchell 1985), and in systems with variable food production, competing native species may adapt by altering their diets in order to avoid direct competition (Pulliam 1986). The ability for ecologically similar native species to coexist may depend on a degree of flexibility in dietary niche (Nakano, Fausch & Kitano 1999), but when invasive species have high niche overlap with threatened native species, the

consequences of niche flexibility need to be understood for effective conservation (Wiens, Anthony & Forsman 2014).

Herein, we present a study that explores the ecological responses of territorial mammalian species to invasion. We focus on a small peripheral population in anthropogenically degraded habitat under invasion by an introduced competitor that may be well adapted to changing environmental conditions. We conducted removal and behavior experiments to test hypotheses on competition for space and food, and variation in territoriality, and to examine the potential for coexistence.

PRESENT STUDY

This dissertation is composed of three manuscripts. The first manuscript, prepared for submission to the *Journal of Animal Ecology* (Appendix A), “The roles of food, population density, and introduced species in determining space use dynamics of a territorial mammal”, details a study in which we tested hypotheses on space use of an isolated territorial mammal, the Mount Graham red squirrel (*Tamiasciurus fremonti grahamensis*) in response to experimental removals of introduced Abert’s squirrels (*Sciurus aberti*). The second manuscript, prepared for the *Journal of Applied Ecology* (Appendix B), “Experimental removals reveal dietary niche partitioning facilitates coexistence between ecologically similar native and introduced species”, used the same experimental removal to examine dietary niche partitioning between Mount Graham red squirrels and Abert’s squirrels, and compared results via stable isotope analysis to populations that co-occur naturally. The third manuscript, prepared for *Biological Conservation* (Appendix C), “Unfamiliarity breeds contempt: territorial behavior varies among isolated populations in natural and invasive interactions with an ecologically similar competitor”, details a study on the behavior of Mount Graham red squirrels during simulated territorial intrusions of Abert’s squirrels. This study was also conducted in two sites where the species co-occur naturally.

LITERATURE CITED

- Blackburn, T.M., Cassey, P., Duncan, R.P., Evans, K.L. & Gaston, K.J. (2004) Avian extinction and mammalian introductions on oceanic islands. *Science*, **305**, 1955-1958.
- Brown, J.H., Stevens, G.C. & Kaufman, D.M. (1996) The geographic range: size, shape, boundaries, and internal structure. *Annual Review of Ecology and Systematics*, **27**, 597-623.
- Brown, J.L. (1964) Evolution of diversity in avian territorial systems. *The Wilson Bulletin*, **76**, 160-169.
- Burt, W.H. (1943) Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy*, **24**, 346-352.
- Fretwell, S.D. & Lucas, H.L. (1969) On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* **19**, 16-36.
- Hutchinson, G.E. (1957) Concluding remarks. *Cold Spring Harbor Symposium on Quantitative Biology*, **22**, 415-427.
- McNab, B.K. (1963) Bioenergetics and the determination of home range size. *The American Naturalist*, **97**, 133-140.
- Merrick, M.J. & Koprowski, J.L. (2017) Altered natal dispersal at the range periphery: the role of behavior, resources, and maternal condition. *Ecology and Evolution*, **7**, 58-72.
- Mooney, H.A. & Cleland, E.E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, **98**, 5446-5451.

- Nakano, S., Fausch, K.D. & Kitano, S. (1999) Flexible niche partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. *Journal of Animal Ecology*, **68**, 1079-1092.
- Noble, G.K. (1939) The role of dominance in the social life of birds. *The Auk*, 263-273.
- Norbury, G., Byrom, A., Pech, R., Smith, J., Clarke, D., Anderson, D. & Forrester, G. (2013) Invasive mammals and habitat modification interact to generate unforeseen outcomes for indigenous fauna. *Ecological Applications*, **23**, 1707-1721.
- Petren, K. & Case, T.J. (1996) An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology*, **77**, 118-132.
- Pimm, S.L., Rosenzweig, M.L. & Mitchell, W. (1985) Competition and food selection: field tests of a theory. *Ecology*, **66**, 798-807.
- Schoener, T.W. (1968) Sizes of feeding territories among birds. *Ecology*, **49**, 123-141.
- Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution*, **17**, 170-176.
- Strauss, S.Y., Lau, J.A. & Carroll, S.P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357-374.
- Tompkins, D., White, A. & Boots, M. (2003) Ecological replacement of native red squirrels by invasive greys driven by disease. *Ecology Letters*, **6**, 189-196.
- Vitousek, P.M., D'antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997a) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 1-16.

- Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997b) Human domination of Earth's ecosystems. *Science*, **277**, 494-499.
- Vucetich, J.A. & Waite, T.A. (2003) Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics*, **4**, 639-645.
- Wiens, J.D., Anthony, R.G. & Forsman, E.D. (2014) Competitive interactions and resource partitioning between northern spotted owls and barred owls in western Oregon. *Wildlife Monographs*, **185**, 1-50.
- Williamson, M. & Fitter, A. (1996) The varying success of invaders. *Ecology*, **77**, 1661-1666.

APPENDIX A: THE ROLES OF FOOD, POPULATION DENSITY, AND
INTRODUCED SPECIES IN DETERMINING SPACE USE DYNAMICS
OF A TERRITORIAL MAMMAL

Jonathan J. Derbridge and John L. Koprowski

(In the format of *Journal of Animal Ecology*)

The roles of food, population density, and introduced species in determining space use dynamics of a territorial mammal

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Abstract

1. Population density and food production play important roles in the economic defendability of exclusive space among territorial animals.
2. Resource partitioning minimizes interspecific competition in natural systems, but introduced species may alter expected outcomes by competing with ecologically similar territorial species, either restricting them to smaller territories or causing wider foraging to make up for lost resources. Such interactions could result in loss of individual fitness and declines in populations of native species.
3. Fremont's squirrels (*Tamiasciurus fremonti*) are ideal models for studying space use dynamics and invasive species effects because they comprise a wide-ranging, well-studied taxon, territoriality is key to survival, and a

subspecies, the Mount Graham (MG) red squirrel (*T. f. grahamensis*), competes with introduced Abert's squirrels (*Sciurus aberti*).

4. We combined experimental removals of introduced Abert's squirrels with analysis of home range, population density, and food production data to determine the primary drivers of space use in territorial species, and explore how space use is affected by competition with ecologically similar introduced species. We also explored the potential effects of population density, food production, and invasion on fitness through variation in body mass of MG red squirrels.
5. Territory size increased following removals, and interactions between population density and fungi production provided a novel understanding of the role of a critical food resource; at minimum levels of fungi production and high MG red squirrel population density, territory and home range sizes increased. An interaction between population density and conifer seed production predicted variation in MG red squirrel body mass; at minimum seed levels when population density was high, red squirrel body mass decreased.
6. An interplay of population density and primary food sources determined variation in space use by our model territorial species, and in years of poor food production individual fitness could be negatively affected. Populations of territorial species may experience declines in such years. Competition with ecologically similar introduced species may reduce the economic

defendability of territories, posing a conservation threat particularly in marginal or degraded habitat with variable food production.

Keywords

competition, food production, invasive species, population density, space use, territorial

Introduction

How animals use space has important implications for population dynamics (Pulliam 1988), and biodiversity conservation (Poiani *et al.* 2000). Individuals of most species occupy a home range, defined as the area normally traveled in search of food (Burt 1943) and theoretically, an animal's home range size depends on energetic requirements, which are generally correlated with body size (McNab 1963). Territorial animals actively exclude conspecifics and other competitors from a portion of their home ranges (Noble 1939), and natural selection has resulted in distinct territorial behaviors that enhance survival and reproduction under given environmental conditions such that territories remain economically defensible (Brown 1964). The ability of territorial animals to adjust space use according to proximate environmental cues is the mechanism through which economic defensibility is realized, and the general pattern is that territory and home range sizes are inversely related to population density and food production (Adams 2001). However, this response is widely variable and the ubiquitous threat of anthropogenic ecosystem change complicates management of territorial species by adding further variability to how animals are able to use space.

Home range size is predicted to be inversely related to food production (Harestad & Bunnell 1979; Relyea, Lawrence & Demarais 2000), and population density (Wolff 1985; Erlinge *et al.* 1990), and may also vary seasonally, with some species ranging

more widely in summer than winter (Myserud, Pérez-Barbería & Gordon 2001). Sex may also be a determinant of home range size, with males roaming more widely in search of mates during breeding seasons (Hanski *et al.* 2000; Dickson & Beier 2002) and females increasing home range size in order to provision young (Schradin *et al.* 2010). Further challenges to understand patterns in spatial ecology may arise from anthropogenic disturbances. Of these potential factors that affect space use, habitat fragmentation is the most well-studied and a wide range of effects includes use of less preferred habitat and increased home range size (Selonen, Hanski & Stevens 2001). Effects of introduced species on space use are relatively unstudied, but invasive populations are a global conservation threat (Vitousek *et al.* 1997) and especially where sympatric with ecologically similar species, studies that test hypotheses on competition and space use could reveal important insight for management.

Optimality models that offer theoretical predictions on territory and home range size (Hixon 1980; Schoener 1983) are the basis for decades of advances in spatial ecology (Adams 2001). The loss of food and energetic cost of excluding competitors predict that territorial animals will maintain smaller territories at the cost of reduced reproductive rates or expand territory size to make up for food lost to intruders (Brown & Orians 1970; Hixon 1980), and that the effects of competition will be related to food abundance and population density (Wolff 1985; Grant, Weir & Steingrímsson 2017). Resources are partitioned over time after ecologically similar species become sympatric through natural processes, but when such interactions result from anthropogenic invasions, negative effects of competition for resources may happen faster than resources can be partitioned, leading to rapid decline or extirpation of native species (Davis 2003).

The global proliferation of invasive populations means increasing numbers of species will become imperiled through this process (Wilcove *et al.* 1998) and documented cases provide insight on what to expect. Widespread population declines of Eurasian red squirrels (*Sciurus vulgaris*) were attributed in part to broad spatial and temporal niche overlaps with invasive eastern grey squirrels (*Sciurus carolinensis*) and coexistence between these species is considered impossible (Wauters *et al.* 2002). A similar effect of niche overlap has been implicated in the decline of northern spotted owls (*Strix occidentalis caurina*) that compete with barred owls (*Strix varia*) and occupy larger than average home ranges where the species overlap, posing a challenge to energetic constraints and ultimately fitness of spotted owls (Wiens, Anthony & Forsman 2014).

The Mount Graham (MG) red squirrel (*Tamiasciurus fremonti grahamensis*) is a useful biological model for questions on space use dynamics in disturbed landscapes and the potential for negative effects of competition with ecologically similar introduced species. This subspecies of Fremont's squirrel (*T. fremonti*) is listed as critically endangered, in part because its distribution is limited to coniferous forest fragmented by insect outbreaks and fires at the species' southern range periphery in a single mountain range, the Pinaleño Mountains, Arizona, USA (Koprowski, Alanen & Lynch 2005).

Another management concern is introduced Abert's squirrels (*Sciurus aberti*), which are syntopic with MG red squirrels throughout their range (Hutton *et al.* 2003), overlap in diets (Edelman & Koprowski 2005), and may be well adapted to recent habitat changes (Gwinn & Koprowski 2016) and forest composition predicted by climate projections (Williams *et al.* 2010). Compared to other Fremont's squirrel (hereafter, red squirrel) populations, MG red squirrels have large home ranges that fluctuate seasonally and

annually (Koprowski, King & Merrick 2008) in response to habitat alterations including fire (Blount & Koprowski 2012). The roles of population density, fluctuating food production, and competition with Abert's squirrels in MG red squirrel space use dynamics have not been explored, and analysis could increase our general understanding of how these factors interact as determinants of space use in territorial animals.

The energetic demands of territory defense against intruding neighbors may require territorial animals to maintain smaller home ranges when population density is relatively high (Hixon 1980; Schoener 1983). Abert's squirrels are non-territorial (Farentinos 1972) and observations suggest they are easily repelled (Hutton *et al.* 2003), thus an individual's competitive threat may not exceed that of an individual MG red squirrel and therefore changes in the Abert's squirrel population may have a similar per unit effect to changes in the MG red squirrel population. Alternatively, if unable to effectively repel Abert's squirrels, MG red squirrels may be forced to forage more widely to compensate for lost resources (Brown & Orians 1970; Hixon 1980), which could help explain the large home range sizes documented for this subspecies (Koprowski, King & Merrick 2008).

To determine the factors affecting variation in MG red squirrel space use, we used a before-after control-impact (BACI) study design to combine analysis of home range, population density, and food production data with an experimental removal of Abert's squirrels. For the removal component, we tested 2 hypotheses. First, the general intruder hypothesis was that MG red squirrel territory and home range sizes were limited by the population density of all potential intruders. This hypothesis assumed territorial MG red squirrels successfully defended territories against all intruders and that an inverse

relationship existed between the time and energy spent to repel intruders, and the defensible territorial space. Accordingly, we predicted that removal of Abert's squirrels should release MG red squirrels from intruder pressure and their territory and home range sizes should increase. Second, the invader hypothesis held that MG red squirrel territory and home range sizes were larger where introduced Abert's squirrels were present. This hypothesis assumed that territorial MG red squirrels were unable to repel Abert's squirrels from territories and that time and energy was spent on foraging more widely. Accordingly, we predicted that removal of invasive Abert's squirrels should also remove the need for larger territories, and that MG red squirrel territory and home range sizes should decrease. For both hypotheses we predicted territory and home range sizes to be inversely related to food production because smaller territories are more economically defensible when food is abundant (Brown 1964; Hixon 1980). Because conifer seeds provide more nutrition (Frank & Cox 2009) and comprise the largest proportion of red squirrel diet (Steele 1998), we expected conifer seed production to have a greater effect on territory and home range sizes than fungi production.

Annual production of conifer seeds and fungi is highly variable in the Pinaleno Mountains and although MG red squirrels primarily consume conifer seeds, a concurrent study estimated fungi could comprise >50% of summer and >30% of fall diet (Derbridge & Koprowski, *in review*). Competition from introduced Abert's squirrels could have detrimental effects on individual fitness of MG red squirrels by further reducing available food in low production years. Therefore, as an additional test of potential fitness consequences of invasion and variable food production, we also modeled the effects of the same predictors on MG red squirrel body mass, a reliable predictor of fitness in tree

squirrels (Wauters & Dhondt 1995). We predicted body mass would vary with food production and that there would be an inverse relationship between body mass and MG red squirrel population density. We further predicted if our invader hypothesis was supported that we would detect a removal effect on MG red squirrel body mass, specifically an increase in body mass following removal of Abert's squirrels.

Methods

Study site

Our study site was in mixed-conifer forest of Engelmann spruce (*Picea engelmannii*), Douglas-fir (*Pseudotsuga menziesii*), corkbark fir (*Abies lasiocarpa* var. *arizonica*), southwestern white pine (*Pinus strobiformis*), and ponderosa pine at 2800-3000-m elevation (Hutton *et al.* 2003) in the Pinaleno Mountains, Arizona, USA. We selected removal and control areas of ≈ 100 ha each where both squirrel species were common on either side of a road that bisected the study site (Fig. A1).

MGRS and Abert's squirrel capture and telemetry

We captured MG red squirrels from March 2008 to December 2014, and Abert's squirrels from April 2011 to October 2014 in live traps (Model No. 201 and custom model No. 202; Tomahawk Live Trap Company, Tomahawk, WI). We fitted all captured adults with VHF radio-collars (Models No. SOM 2190 and 2389; Wildlife Materials Inc., Murphysboro, IL). We recorded body mass ± 5 g of all captured animals with a Pesola spring scale (Baar, Switzerland).

We used biangulation and homing to record ≥ 25 locations of each MG red squirrel (Koprowski, King & Merrick 2008) during Jun-Nov 2008-2014, and each Abert's squirrel during Apr-Dec 2011. We used these data in Ranges 9 software

(Anatrack, Wareham, UK) to estimate individual 50% and 95% fixed kernel home ranges, inter-individual overlaps among MG red squirrels, and the mean percentage overlap of Abert's squirrel 50% and 95% home ranges on MG red squirrel 50% and 95% home ranges. We used analysis of variance (ANOVA) to compare the total number of inter-individual MG red squirrel overlaps among years 2011-2014 ($\alpha = 0.05$). For simplicity, we refer to 95% home ranges as home ranges, and 50% home ranges as territories for red squirrels and core-use areas for non-territorial Abert's squirrels (Koprowski, King & Merrick 2008; Anich, Benson & Bednarz 2009).

Food production data collection

We used conifer seed and mushroom production data collected by the University of Arizona's MGRS Monitoring Program (Koprowski, Alanen & Lynch 2005). We estimated conifer seed production from viable seeds of Engelmann spruce, Douglas-fir, and corkbark fir collected from 9 10-m x 10-m seed plots each containing 3 randomly placed 0.25-m² seed traps, and quantified as seeds/m². Seed traps were emptied and replaced following snowmelt each year, thus contents were of seeds from the previous year's crop. We estimated fungi production from genera known to be consumed by red squirrels (Smith 1968a; Smith 1968b) collected from 9 1-m x 100-m plots at 2-week intervals over 12 weeks from late July-October each year, and quantified as dry weight kg/ha.

Experimental removals

All methods were approved by the University of Arizona Institutional Animal Care and Use Committee, US Fish and Wildlife Service, and Arizona Game and Fish Department. We used live-trapping and hunting to remove Abert's squirrels from the experimental

removal area during March-September 2012 and 2014. We live-trapped Abert's squirrels and euthanized them with an overdose of inhalant isoflurane (Leary *et al.* 2013). Other Abert's squirrels were removed through coordination with local hunters with whom we verified all kill locations, species identification, sex, and age of animals.

We estimated removal success by counting incidents of Abert's squirrel feeding sign (i.e., remains of conifer cones, stripped twigs, clipped branches marked and scattered in patterns not seen in MG red squirrel sign) on 16 50-m line transects each in the removal and control areas 9 times at 7-day intervals, May-July, 2012, and 8 times at 14-day intervals, May-Aug 2014. We removed feeding sign from transects at each check to avoid double-counting. We also used remote cameras (Bushnell Trophy Cam model 119436; Bushnell Outdoor Products, Overland Park, KS) to establish 6 50-m transects in each area, June-August 2014. Two cameras faced inwards at opposite ends of the transect, and a third camera faced perpendicular to the line at 25 m. We quantified all single Abert's squirrel photographs for each hour of transect operation. We used Welch's two-sample *t*-test ($\alpha = 0.05$) to compare counts of Abert's squirrel feeding sign and photographs between the removal and control areas.

Statistical analysis

We used linear mixed effects models to estimate the effects of removal, area (control or removal), conifer seed production, fungi production, and MG red squirrel population density on territory and home range sizes of radio-collared MG red squirrels 2008-2014. We used the same linear mixed effects model approach to estimate the effect of removal, area, population density, and food production on body mass of individual MG red

squirrels. We accounted for variation arising from repeated measures of individual MG red squirrel home range size and body mass by specifying individuals as random effects.

Distributions of home range and density data were right-skewed and food production data distributions were bimodal, therefore we log transformed these response and predictor variables before estimating our models. To investigate whether the effect of MG red squirrel population density on territory size, home range size, or body mass depended on the level of food production, we included interactions between conifer seed production and population density, and fungi production and population density.

We developed a candidate set of 8 models that all included removal, area, population density, and at least one of the food production covariates. To explore the role of removal, we ran each model with and without the removal term, thus we estimated 16 models for each response. We used Akaike's Information Criterion (AIC) to select models that best fit the data, ranked models according to Δ AIC, and used the highest-ranked models for inference. Finally, we used estimates from the highest-ranked models to predict territory, home range and body mass across a range of population densities at minimum, mean, and maximum values of our conifer seed and fungi production data. We ran models in R 3.4.2 (R Development Core Team 2017) using the R package *nlme*.

Results

We radio-collared 70 MG red squirrels and estimated 137 territory and home range sizes ($\bar{x} = 2.0$ /individual) during 2008-2014. We radio-collared 17 Abert's squirrels and estimated 17 territory and home range sizes 2011-2012. Mean MG red squirrel territory and home range size from 2008-2014 was 0.76 ha (SD = 0.77) and 2.48 ha (SD = 2.78), respectively (Fig. A2), and generally decreased during this period (Fig. A3). Mean

Abert's squirrel core-use area and home range size, 2011 was 4.30 ha (SD = 1.84) and 12.99 ha (SD = 4.83), respectively. Mount Graham red squirrel population density in the study area doubled during 2008-2014 (\bar{x} = 0.13/ha, range = 0.12; Fig. A3).

We removed 18 and 34 Abert's squirrels in 2012 and 2014, respectively (Fig. A1). Counts of Abert's squirrel feeding sign did not differ between removal (\bar{x} = 0.50, SD = 1.10) and control (\bar{x} = 1.00, SD = 0.82) area transects in 2012, but did differ between removal (\bar{x} = 1.00, SD = 1.32) and control (\bar{x} = 2.56, SD = 1.55) area transects in 2014 ($t_{22,7} = 3.57$, $P = 0.002$). From camera data, counts of Abert's squirrel photographs differed during both transect periods; Abert's squirrels were more common on removal (\bar{x} = 7.17, SD = 4.71) compared to control (\bar{x} = 2.33) (SD = 1.97) transects ($t_{10} = -2.32$, $P = 0.043$) in the winter following the 2012 removal, and less common on the removal (\bar{x} = 4.17, SD = 2.32) compared to control (\bar{x} = 12.17, SD = 7.47) transects ($t_6 = 2.51$, $P = 0.046$) during the 2014 removal.

In 2011, prior to the first removal, mean percentage overlap of Abert's squirrel core-use areas and home ranges on MG red squirrel territories and home ranges was 27.05% (SD = 33.61%; \bar{x} = 0.94 Abert's squirrels, SD = 0.85), and 44.74% (SD = 26.16%; \bar{x} = 3.00 Abert's squirrels, SD = 1.71), respectively. Mean percentage overlap among MG red squirrel territories and home ranges in 2011 was 21.80% (SD = 20.69%; \bar{x} = 1.0 red squirrels, SD = 0.73) and 28.82% (SD = 18.38%; \bar{x} = 2.75 red squirrels, SD = 1.00), respectively. The number of inter-individual MG red squirrel overlaps did not vary on territories ($F_{3,75} = 1.77$, $P = 0.160$) or home ranges ($F_{3,75} = 1.77$, $P = 0.081$) among years, 2011-2014. Conifer seed production (\bar{x} = 140.2, range = 476.3 seeds/m²)

and fungi production ($\bar{x} = 6.6$, range = 10.9 dry weight kg/ha) were highly variable among years, 2008-2014 (Fig. A4).

Linear mixed effects models

The same 6 models were ranked highest in explanatory value from the candidate sets of territory and home range models (Table A1), no other models received sufficient support (Burnham, Anderson & Huyvaert 2011). The highest-ranked model fit for territory and home range variation included the same covariates, with an interaction between MG red squirrel population density and fungi production on territory ($t_{63} = -5.73$, $P < 0.001$) and home range ($t_{63} = -5.51$, $P < 0.001$) size (Table A2 a & b). For removal years (i.e., 2012 and 2014), the models estimated mean increases in MG red squirrel territory and home range sizes of 58% ($t_{63} = 2.37$, $P < 0.021$) and 66% ($t_{63} = 2.33$, $P = 0.023$), respectively. Across all years (i.e., 2008-2014), area was a useful predictor; mean territory size was 38% ($t_{68} = -2.97$, $P = 0.004$) smaller, and mean home range size was 40% ($t_{68} = -2.68$, $P = 0.009$) smaller in the removal area (Table A2 a & b). Almost identical patterns resulted from our predictions based on interaction terms, territory and home range size increased with increasing population density at minimum levels of fungi production, and decreased as population density increased at maximum levels of fungi production regardless of removal year or area being specified (Fig. A5).

From our test of predictors of MG red squirrel body mass, the full model was the highest-ranked (Table A3) and included an interaction between population density and conifer seed production ($t_{61} = 2.81$, $P = 0.007$); removals did not affect body mass (Table A4). Body mass decreased with population density at minimum levels of seed

production, but did not change as a function of population density at maximum levels of seed production (Fig. A6).

Discussion

Territory and home range sizes of our model species were associated with population density and fungi production, and with the removal of an introduced competitor.

Population density and seed production also interacted as predictors of body mass. In general MG red squirrels followed the pattern of inversely related population density and territory size that is commonly reported in rodents (Wolff 1985; Erlinge *et al.* 1990).

Complete removal of Abert's squirrels from our specified study area was not possible due to habitat connectivity and the introduced species' distribution throughout all forested areas of the Pinaleño Mountains. Our overall results from efforts to monitor removal success provided evidence that we did achieve a measurable population reduction. Although presence of Abert's squirrel feeding sign was statistically different only during the second removal in 2014, feeding sign was more common on control area transects during both removals. Camera data indicated Abert's squirrels were more common on removal transects after the first removal in 2012, but we initiated these transects 10 weeks after concluding removals that demonstrated that Abert's squirrels rapidly recolonize the areas. During the second removal in 2014, we initiated camera transects during the peak of removal activity, and camera data revealed a clear difference in presence of Abert's squirrels in removal and control areas. The rapid rate of recolonization could limit inference on the effect of removal on MGRS space use, but more importantly it demonstrates that in some cases endangered species conservation planning may need to include management of a permanently invaded community.

We based our removal hypotheses on theoretical predictions that territorial animals must choose between expelling all intruders at the cost of limiting space use, or expanding space use to compensate for resources lost to successful intruders (Hixon 1980). Our general intruder hypothesis was supported by a threefold expansion of territory and home range sizes following the first removal, in 2012. Another study that used an experimental removal reported an inverse relationship between population density and territory size of red squirrels (*T. hudsonicus*); when the red squirrel population was temporarily reduced, territory sizes increased (Boutin & Schweiger 1988). Increased MG red squirrel territory and home range sizes observed in 2012 suggest Abert's squirrels forced MG red squirrels to maintain smaller home ranges. However, removal area territories and home ranges were small in 2011, and the subsequent dramatic expansion was in part a return to the mean.

Our invader hypothesis assumed MG red squirrels were unable to repel Abert's squirrels and were therefore forced to forage more widely. The overall decline in territory and home range sizes during the 4 years of the BACI study supports this hypothesis, however space use was unchanged following the second removal in 2014. The invader hypothesis also predicted an increase in MG red squirrel body mass related to Abert's squirrel removals but body mass was not affected by removals. The evidence suggests MG red squirrels were not forced to forage more widely by Abert's squirrels. In general, intra- and interspecific competition cause animals to reduce home range size (Wolff 1985; Erlinge *et al.* 1990), and the inconsistent response to removals may mean Abert's squirrels have little effect on MG red squirrel space use. Our models yielded

alternative and more compelling explanations for variation in MG red squirrel space use and body mass.

The influence of population density on space use was strongly dependent on fungi production, and counter to our expectation, conifer seed production had no effect on territory and home range sizes. Red squirrels consume fungi throughout the year (Currah *et al.* 2000) and in years of low conifer seed production fungi may comprise 90% of diet (Vernes, Blois & Bärlocher 2004); recent estimates indicate $\approx 50\%$ MG red squirrel diet could be fungi (Derbridge & Koprowski, *in review*). The variability in abundance of conifer seeds and fungi means red squirrels must adapt between primarily conifer seed or fungi diets in years when the alternative source is in low supply. The digestible protein content of fungi is relatively low, but red squirrels consume and store mushrooms and truffles even when conifer seeds are abundant (Vernes, Blois & Bärlocher 2004), and may provide unknown nutritional benefits (Frank 2009).

Multiple years of low fungi and conifer seed production during 2008-14 likely intensified competition for each sporocarp when MG red squirrel population density was high, driving territorial animals to expand their boundaries to satisfy energetic requirements. When high fungi production was predicted, increasing population density resulted in smaller home range sizes, reflecting the reduced incentive for expansive foraging. Abert's squirrels consume the same diet items but removals did not alter the interaction of fungi availability and MG red squirrel density on space use. Fungi availability also has strong influence on population density and distribution of northern flying squirrels (*Glaucomys sabrinus*) and Townsend's chipmunks (*Tamias townsendii*),

which are both more abundant in old growth forest where fungi production is high (North, Trappe & Franklin 1997).

Broad spatial overlap between these native and introduced squirrels should be viewed as evidence for competition between ecologically similar species. Before removals, higher inter- than intraspecific overlap by Abert's squirrels on red squirrels in the control and removal areas demonstrated the ubiquitous presence of introduced Abert's squirrels in areas inhabited by the endangered MG red squirrel. Wherever such overlaps occur, territorial species pay an energetic cost, either through expelling invaders (Powers & McKee 1994) or through foraging more widely to compensate for lost resources (Wiens, Anthony & Forsman 2014). We confirmed MG squirrels defend larger territories than red squirrels in other populations (Koprowski, King & Merrick 2008), and the additional energetic cost of repelling invasive Abert's squirrels could have fitness consequences, especially in years of poor food production. Expanding territory size to counter invasion may be a risky strategy; the decline of northern spotted owls under invasion from barred owls is likely related to the cost of maintaining enlarged territories that are not economically defensible (Wiens, Anthony & Forsman 2014).

We addressed fitness effects of variable food production in models that focused on body mass. The predicted effect of MG red squirrel population density and levels of conifer seed production on body mass implies consequences of competition from Abert's squirrels could be more severe in years of high population density and low seed production because body mass is related to over-winter survival in red squirrels (Larivee *et al.* 2010).

Striking differences in interactions with population density and food production suggest the distribution of food sources along different spatial dimensions is important to understand territory size and shape (Zhang *et al.* 2014). Conifer seed production had no effect on territory or home range size, possibly because sufficient seeds were available within a territory in most years. We could not account for variation of vertical space use in relation to seed production. This is a commonly acknowledged bias in home range analysis of scansorial, volant, fossorial, and aquatic species because their areas normally traveled in search of food (*sensu* Burt 1943) vary vertically (Don 1983; Tracey *et al.* 2014). Conversely, patchy distributions of fungi in low production years require animals to expand foraging areas along the ground. Where animals are forced to expand home ranges in a patchy mosaic of dead and healthy trees in fire-scarred landscape (e.g., the Pinaleno Mountains), fitness costs may be incurred if absence from territories reduces their economic defendability (Brown 1964). Our concurrent work on diets confirms fungi are critical resources to MG red squirrels (Derbridge & Koprowski, *in review*) and helps explain the strong effect of fungi production on territory and home range size.

We have provided new insight on the complex ecological interactions that influence space use dynamics of territorial animals. The typical relationship between population density and territory and home range size is a generally reliable guide for predicting space use (Adams 2001), however threatened populations often occupy marginal or degraded habitat at the periphery of a species' geographic range where general guidelines may be of limited use (Vucetich & Waite 2003). Conservation of peripheral populations may require knowledge of present and future habitat composition,

primary diet items and their production cycles, and the potential for competition with introduced species (Davis 2003).

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References

- Adams, E.S. (2001) Approaches to the study of territory size and shape. *Annual Review of Ecology and Systematics*, **32**, 277-303.
- Anich, N.M., Benson, T.J. & Bednarz, J.C. (2009) Estimating territory and home-range sizes: Do singing locations alone provide an accurate estimate of space use? *The Auk*, **126**, 626-634.
- Blount, S.J. & Koprowski, J.L. (2012) Response of the Mount Graham red squirrel (*Tamiasciurus hudsonicus grahamensis*) to postfire conditions. *The Southwestern Naturalist*, **57**, 8-15.

- Boutin, S. & Schweiger, S. (1988) Manipulation of intruder pressure in red squirrels (*Tamiasciurus hudsonicus*): effects on territory size and acquisition. *Canadian Journal of Zoology*, **66**, 2270-2274.
- Brown, J.L. (1964) Evolution of diversity in avian territorial systems. *The Wilson Bulletin*, **76**, 160-169.
- Brown, J.L. & Orians, G.H. (1970) Spacing patterns in mobile animals. *Annual Review of Ecology and Systematics*, **1**, 239-262.
- Burnham, K.P., Anderson, D.R. & Huyvaert, K.P. (2011) AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, **65**, 23-35.
- Burt, W.H. (1943) Territoriality and home range concepts as applied to mammals. *Journal of Mammalogy*, **24**, 346-352.
- Currah, R., Smreciu, E., Lehesvirta, T., Niemi, M. & Larsen, K. (2000) Fungi in the winter diets of northern flying squirrels and red squirrels in the boreal mixedwood forest of northeastern Alberta. *Canadian Journal of Botany*, **78**, 1514-1520.
- Davis, M.A. (2003) Biotic globalization: does competition from introduced species threaten biodiversity? *AIBS Bulletin*, **53**, 481-489.
- Derbridge, J.J. & Koprowski, J.L. (, *in review*) Experimental removals reveal dietary niche partitioning facilitates coexistence between ecologically similar native and introduced species *Journal of Applied Ecology*, *in review*.
- Dickson, B.G. & Beier, P. (2002) Home-range and habitat selection by adult cougars in southern California. *The Journal of Wildlife Management*, **66**, 1235-1245.

- Don, B. (1983) Home range characteristics and correlates in tree squirrels. *Mammal Review*, **13**, 123-132.
- Edelman, A.J. & Koprowski, J.L. (2005) Diet and tree use of Abert's squirrels (*Sciurus aberti*) in a mixed-conifer forest. *The Southwestern Naturalist*, **50**, 461-465.
- Erlinge, S., Hoogenboom, I., Agrell, J., Nelson, J. & Sandell, M. (1990) Density-related home-range size and overlap in adult field voles (*Microtus agrestis*) in southern Sweden. *Journal of Mammalogy*, **71**, 597-603.
- Farentinos, R.C. (1972) Social dominance and mating activity in the tassel-eared squirrel (*Sciurus aberti ferreus*). *Animal Behaviour*, **20**, 316-326.
- Frank, C.L. (2009) The nutritional ecology of fungal sporocarp hoarding by Mt. Graham red squirrels. *The last refuge of Mt. Graham red squirrel: ecology of endangerment* (eds H.R. Sanderson & J.L. Koprowski), pp. 284-296. University of Arizona Press, Tucson, AZ.
- Frank, C.L. & Cox, S.R. (2009) The adaptive significance of seed hoarding by the Mt. Graham red squirrel. *The last refuge of the Mt. Graham red squirrel: ecology of endangerment* (eds H.R. Sanderson & J.L. Koprowski), pp. 266-283. The University of Arizona Press, Tucson, AZ.
- Grant, J.W.A., Weir, L.K. & Steingrímsson, S.Ó. (2017) Territory size decreases minimally with increasing food abundance in stream salmonids: Implications for population regulation. *Journal of Animal Ecology*.
- Gwinn, R.N. & Koprowski, J.L. (2016) Differential response to fire by an introduced and an endemic species complicates endangered species conservation. *Hystrix, the Italian Journal of Mammalogy*, <http://dx.doi.org/10.4404/hystrix-27.2-11447>

- Hanski, I.K., Stevens, P.C., Ihalempiä, P. & Selonen, V. (2000) Home-range size, movements, and nest-site use in the Siberian flying squirrel, *Pteromys volans*. *Journal of Mammalogy*, **81**, 798-809.
- Harestad, A.S. & Bunnell, F. (1979) Home range and body weight-a reevaluation. *Ecology*, **60**, 389-402.
- Hixon, M.A. (1980) Food production and competitor density as the determinants of feeding territory size. *The American Naturalist*, **115**, 510-530.
- Hutton, K.A., Koprowski, J.L., Greer, V.L., Alanen, M.I., Schaufert, C.A., Young, P.J. & Jones, C.A. (2003) Use of mixed-conifer and spruce-fir forests by an introduced population of Abert's squirrels (*Sciurus aberti*). *The Southwestern Naturalist*, **48**, 257-260.
- Koprowski, J.L., Alanen, M.I. & Lynch, A.M. (2005) Nowhere to run and nowhere to hide: Response of endemic Mt. Graham red squirrels to catastrophic forest damage. *Biological Conservation*, **126**, 491-498.
- Koprowski, J.L., King, S.R.B. & Merrick, M.J. (2008) Expanded home ranges in a peripheral population: space use by endangered Mt. Graham red squirrels. *Endangered Species Research*, **4**, 227-232.
- Larivee, M.L., Boutin, S., Speakman, J.R., McAdam, A.G. & Humphries, M.M. (2010) Associations between over-winter survival and resting metabolic rate in juvenile North American red squirrels. *Functional Ecology*, **24**, 597-607.
- Leary, S.L., Underwood, W., Anthony, R., Gwaltney-Brant, S., Poison, A. & Meyer, R. (2013) AVMA guidelines for the euthanasia of animals: 2013 edition. American Veterinary Medical Association Schaumburg, IL.

- McNab, B.K. (1963) Bioenergetics and the determination of home range size. *The American Naturalist*, **97**, 133-140.
- Mysterud, A., Pérez-Barbería, F.J. & Gordon, I.J. (2001) The effect of season, sex and feeding style on home range area versus body mass scaling in temperate ruminants. *Oecologia*, **127**, 30-39.
- Noble, G.K. (1939) The role of dominance in the social life of birds. *The Auk*, 263-273.
- North, M., Trappe, J. & Franklin, J. (1997) Standing crop and animal consumption of fungal sporocarps in Pacific Northwest forests. *Ecology*, **78**, 1543-1554.
- Poiani, K.A., Richter, B.D., Anderson, M.G. & Richter, H.E. (2000) Biodiversity conservation at multiple scales: functional sites, landscapes, and networks. *BioScience*, **50**, 133-146.
- Powers, D.R. & McKee, T. (1994) The effect of food availability on time and energy expenditures of territorial and non-territorial hummingbirds. *Condor*, 1064-1075.
- Pulliam, H.R. (1988) Sources, sinks, and population regulation. *The American Naturalist*, **132**, 652-661.
- Relyea, R.A., Lawrence, R.K. & Demarais, S. (2000) Home range of desert mule deer: testing the body-size and habitat-productivity hypotheses. *The Journal of Wildlife Management*, **64**, 146-153.
- Schoener, T.W. (1983) Simple models of optimal feeding-territory size: A reconciliation. *The American Naturalist*, **121**, 608-629.
- Schradin, C., Schmohl, G., Rödel, H.G., Schoepf, I., Treffler, S.M., Brenner, J., Bleeker, M., Schubert, M., König, B. & Pillay, N. (2010) Female home range size is

- regulated by resource distribution and intraspecific competition: a long-term field study. *Animal Behaviour*, **79**, 195-203.
- Selonen, V., Hanski, I.K. & Stevens, P.C. (2001) Space use of the Siberian flying squirrel *Ptevomys volans* in fragmented forest landscapes. *Ecography*, **24**, 588-600.
- Smith, C.C. (1968a) The adaptive nature of social organization in the genus of three squirrels *Tamiasciurus*. *Ecological Monographs*, **38**, 31-64.
- Smith, M.C. (1968b) Red squirrel responses to spruce cone failure in interior Alaska. *The Journal of Wildlife Management*, **32**, 305-317.
- Steele, M.A. (1998) *Tamiasciurus hudsonicus*. *Mammalian Species*, **586**, 1-9.
- Tracey, J.A., Sheppard, J., Zhu, J., Wei, F., Swaisgood, R.R. & Fisher, R.N. (2014) Movement-based estimation and visualization of space use in 3D for wildlife ecology and conservation. *PLoS One*, **9**, e101205.
- Vernes, K., Blois, S. & Bärlocher, F. (2004) Seasonal and yearly changes in consumption of hypogeous fungi by northern flying squirrels and red squirrels in old-growth forest, New Brunswick. *Canadian Journal of Zoology*, **82**, 110-117.
- Vitousek, P.M., D'antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 1-16.
- Vucetich, J.A. & Waite, T.A. (2003) Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics*, **4**, 639-645.
- Wauters, L. & Dhondt, A.A. (1995) Lifetime reproductive success and its correlates in female Eurasian red squirrels. *Oikos*, 402-410.

- Wauters, L., Gurnell, J., Martinoli, A. & Tosi, G. (2002) Interspecific competition between native Eurasian red squirrels and alien grey squirrels: does resource partitioning occur? *Behavioral Ecology and Sociobiology*, **52**, 332-341.
- Wiens, J.D., Anthony, R.G. & Forsman, E.D. (2014) Competitive interactions and resource partitioning between northern spotted owls and barred owls in western Oregon. *Wildlife Monographs*, **185**, 1-50.
- Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. (1998) Quantifying threats to imperiled species in the United States. *BioScience*, **48**, 607-615.
- Williams, A.P., Allen, C.D., Millar, C.I., Swetnam, T.W., Michaelsen, J., Still, C.J. & Leavitt, S.W. (2010) Forest responses to increasing aridity and warmth in the southwestern United States. *Proceedings of the National Academy of Sciences*, **107**, 21289-21294.
- Wolff, J.O. (1985) The effects of density, food, and interspecific interference on home range size in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Canadian Journal of Zoology*, **63**, 2657-2662.
- Zhang, Z., Sheppard, J.K., Swaisgood, R.R., Wang, G., Nie, Y., Wei, W., Zhao, N. & Wei, F. (2014) Ecological scale and seasonal heterogeneity in the spatial behaviors of giant pandas. *Integrative Zoology*, **9**, 46-60.

Figure Legends

Figure A1. The study site was approximately 200 ha bisected by a road in mixed-conifer forest at 2800-3000-m elevation in the Pinaleño Mountains, Arizona, USA. Removal and control areas (≈ 100 ha each) were designated east and west of the road, respectively. The map shows 50% fixed kernel density estimates (territories/core-use areas) based on telemetry locations of radio-collared Mount Graham red squirrels Jun-Nov 2011 and Abert's squirrels Apr-Dec 2011, and removal locations of Abert's squirrels. Removal locations in the control area were either of animals tracked there from the removal area during hunting, or captured there but known to be predominantly resident in the removal area.

Figure A2. Mean and standard deviation of territory (a) and home range (b) size (ha) estimated from telemetry locations of radio-collared Mount Graham red squirrels during 2011-2014 in a 200-ha study site in the Pinaleño Mountains, Arizona, USA.

Figure A3. Mean population density and territory size of Mount Graham red squirrels during 2008-2014 in a 200-ha study site in the Pinaleño Mountains, Arizona, USA.

Figure A4. Conifer seed and fungi production, 2008-2014, in a 200-ha study site at 2800-3000 m elevation in the Pinaleño Mountains, Arizona, USA. Conifer seed production calculated as viable seeds/ha in the study site in a given year. Fungi production was calculated as dry weight/ha (kg) in the study site in a given year. The variables $\log(\text{seed})$ and $\log(\text{fungi})$ were used in models to explain variation in territory size, home range size, and body mass of Mount Graham red squirrels.

Figure A5. Territory size of Mount Graham red squirrels predicted by population density at minimum, mean, and maximum levels of fungi production recorded in a 200-ha study site in the Pinaleño Mountains, Arizona, USA, 2008-2014. Predictions are from the highest ranking model in a candidate set of linear mixed models estimating predictors of space use in Mount Graham red squirrels. Predictions were estimated for removal and control area territory and home range sizes during removal years and non-removal years. Interactions plotted almost identically in each case. The plot for territory sizes in the removal area during a removal year is presented.

Figure A6. Predicted body mass (g) of Mount Graham red squirrels as a function of population density at minimum, mean, and maximum levels of conifer seed production recorded in the study site, 2008-2014. Predictions are from the highest ranking model in a candidate set of linear mixed effects models estimating predictors of body mass in Mount Graham red squirrels. Interactions plotted almost identically in each case. The plot for body mass in the removal area during a removal year is presented.

Table A1. Models estimated to explain variation in 137 territory and home range sizes of Mount Graham red squirrels. We used linear mixed effects models with individual squirrels as random effects, and removal (r), area (a), population density (d), conifer seed production (s), and fungi production (f) as fixed effects. We developed a set of 8 candidate models that all included removal, area, population density and at least one of the food production covariates, and estimated all models with and without the removal term. We used the model with the lowest AIC score for inference. Models with ΔAIC score ≤ 7 are presented.

a)

Model	Territory			Home range		
	AIC	ΔAIC	K	AIC	ΔAIC	K
$r+a+d+f+(d*f)$	321.88	0	8	340.41	0	8
$a+d+f+(d*f)$	323.79	1.9	7	342.43	2	7
$a+d+s+f+(d*s)+(d*f)$	326.12	4.2	9	343.91	3.5	9
$r+a+d+s+f+(d*f)$	327.26	5.4	9	344.95	4.5	9
$r+a+d+s+f+(d*s)+(d*f)$	327.89	6	10	345.58	5.2	10
$a+d+s+f+(d*f)$	328.46	6.6	8	345.79	5.4	8

Table A2. Model estimates from the highest ranking models used to explain variation in territory (a) and home range (b) size (ha) of Mount Graham red squirrels. Removal and area were indicator variables coding for squirrels in the removal area during removal year, and squirrels either in or not in the removal area, respectively. Density was the total number of individual adult MG red squirrels in the study area alive during September in a given year. Fungi was the calculated dry weight/ha (kg) in the study area in a given year.

a)

	Estimate	SE	df	<i>t</i> -value	<i>P</i> -value
(Intercept)	-9.315	2.246	68	-4.146	0.000
Removal	0.456	0.192	63	2.371	0.021
Area	-0.515	0.173	68	-2.973	0.004
Log(density)	3.121	0.785	63	3.976	0.000
Log(fungi)	6.045	1.032	63	5.856	0.000
Log(density)*Log(fungi)	-2.054	0.359	63	-5.730	0.000

b)

	Estimate	SE	df	<i>t</i> -value	<i>P</i> -value
(Intercept)	-9.063	2.525	68	-3.589	0.001
Removal	0.507	0.217	63	2.334	0.023
Area	-0.470	0.175	68	-2.681	0.009
Log(density)	3.415	0.882	63	3.872	0.000
Log(fungi)	6.573	1.164	63	5.646	0.000
Log(density)*Log(fungi)	-2.228	0.404	63	-5.513	0.000

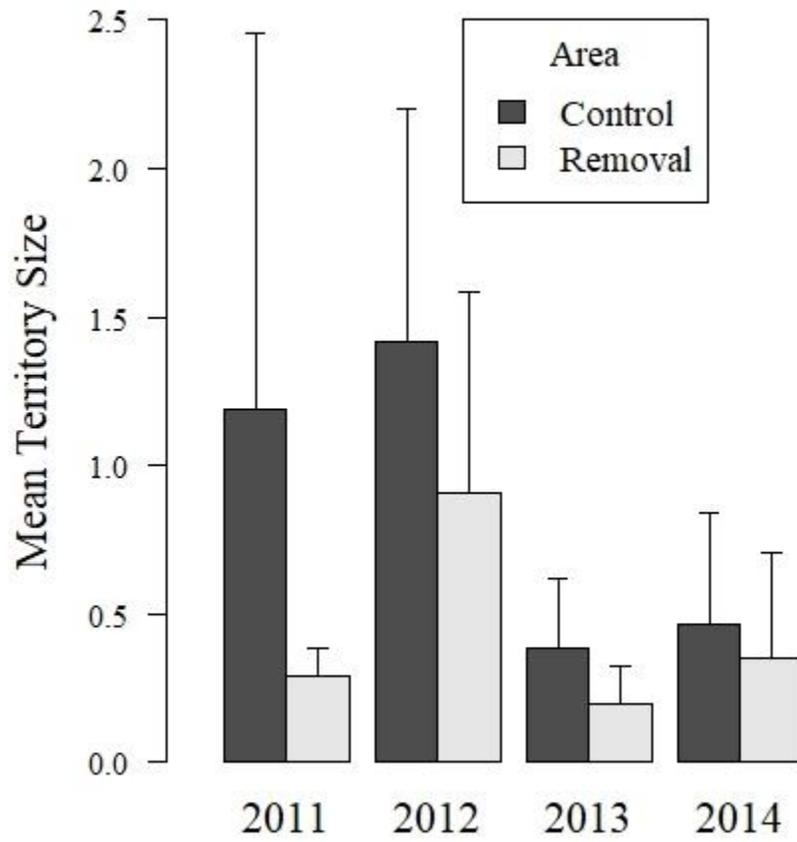
Table A3. Models estimated to explain variation in multiple body mass (g) measurements of 70 adult Mount Graham red squirrels 2008-2014. We used linear mixed effects model with individual squirrels as random effects, and removal (r), area (a), population density (d), conifer seed production (s), and fungi production (f) as fixed effects. We developed a set of 8 candidate models that all included removal, area, population density and at least one of the food production covariates, and estimated all models with and without the removal term. We used the model with the lowest AIC score for inference. Models with Δ AIC score ≤ 11 are presented.

Model	AIC	Δ AIC	K
$r+a+d+s+f+(d*s)+(d*f)$	1099.81	0	10
$r+(d*s)+(d*f)$	1102.13	3.2	9
$a+(d*s)+(d*f)$	1107.44	7.60	9

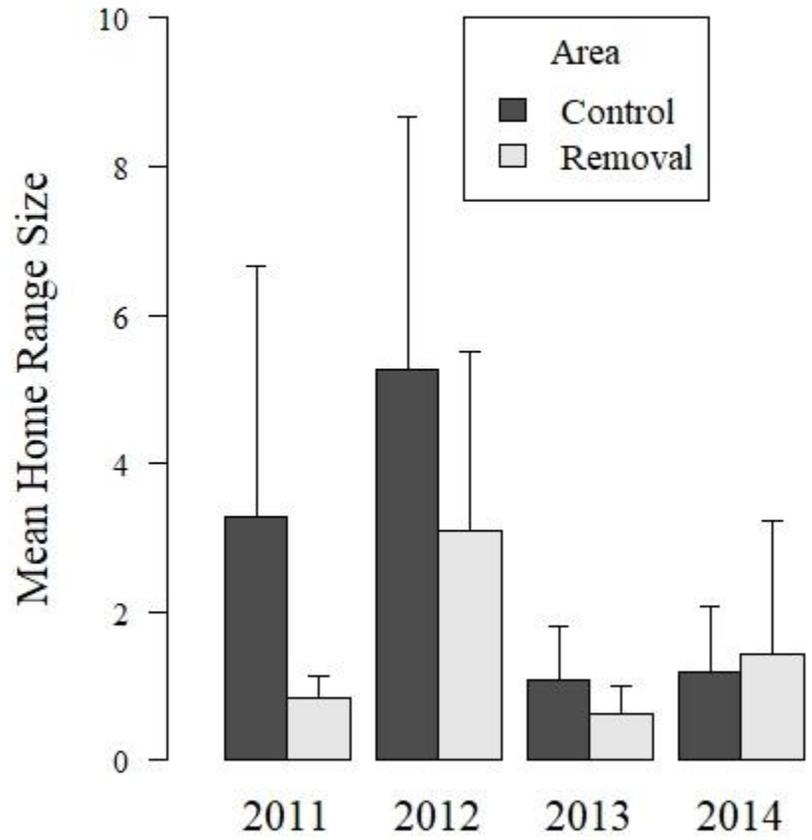
Table A4. Model estimates from the highest ranking models used to explain variation in body mass (g) of Mount Graham red squirrels. Removal and Area were indicator variables coding for squirrels in the removal area during removal year, and squirrels either in or not in the removal area, respectively. Density was the total number of individual adult MG red squirrels in the study area alive in September of a given year. Seed was the calculated viable seeds/ha in the study area in a given year. Fungi was the calculated dry weight/ha (kg) in the study area in a given year.

	Estimate	SE	df	<i>t</i> -value	<i>P</i> -value
(Intercept)	565.807	123.752	68	4.572	0.000
Removal	-1.506	5.111	61	-0.295	0.769
Area	1.450	3.142	68	0.461	0.646
Log(density)	-119.793	44.010	61	-2.722	0.008
Log(seed)	-55.660	19.880	61	-2.800	0.007
Log(fungi)	-43.837	28.045	61	-1.563	0.123
Log(density)*Log(seed)	19.104	6.795	61	2.811	0.007
Log(density)*Log(fungi)	18.076	9.980	61	1.811	0.075

Figure A2.



a)



b)

Figure A3.

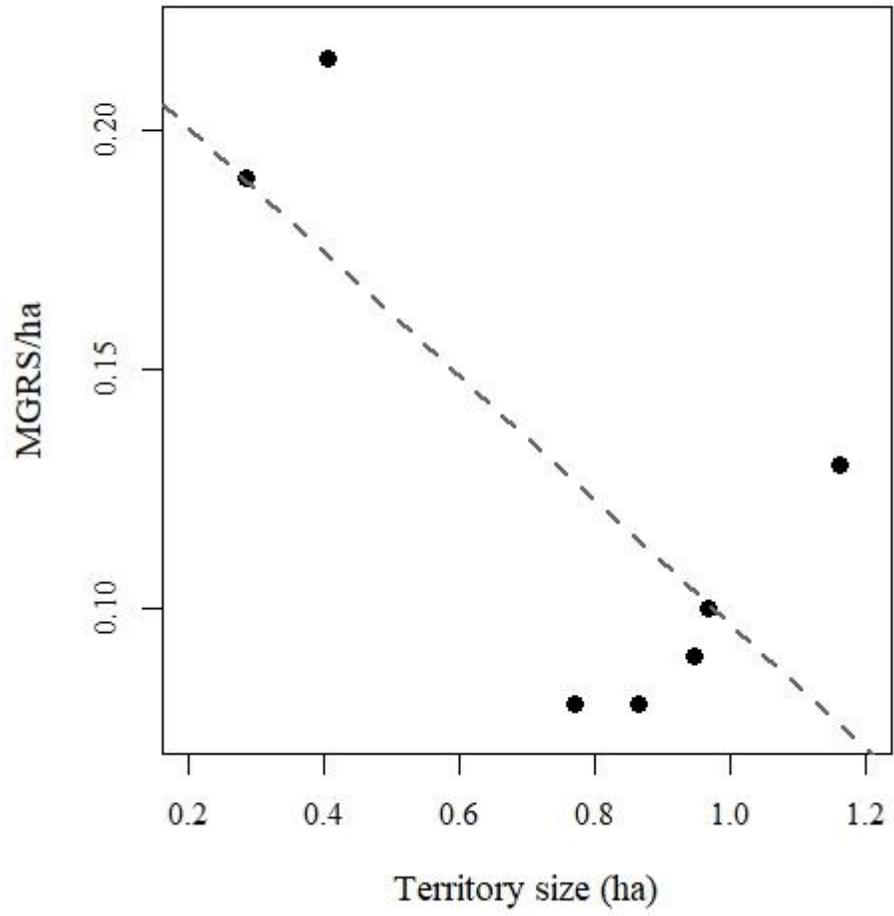


Figure A4.

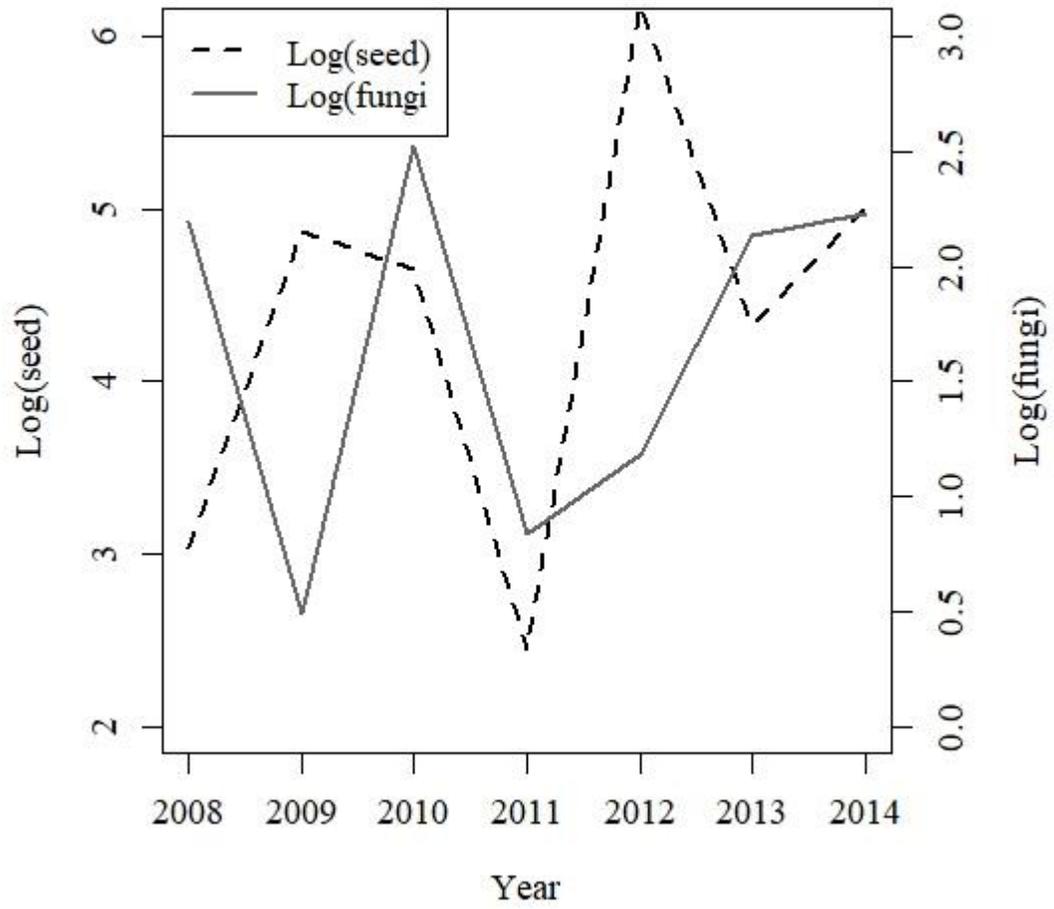


Figure A5.

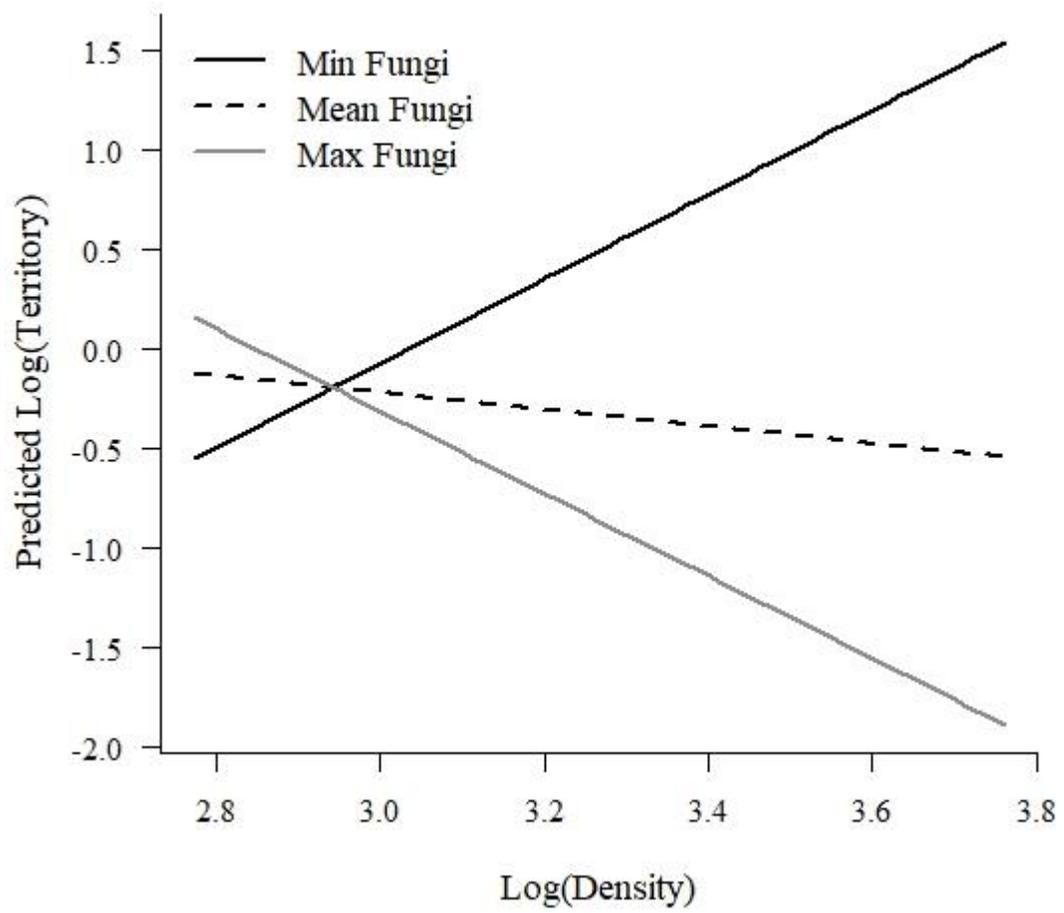
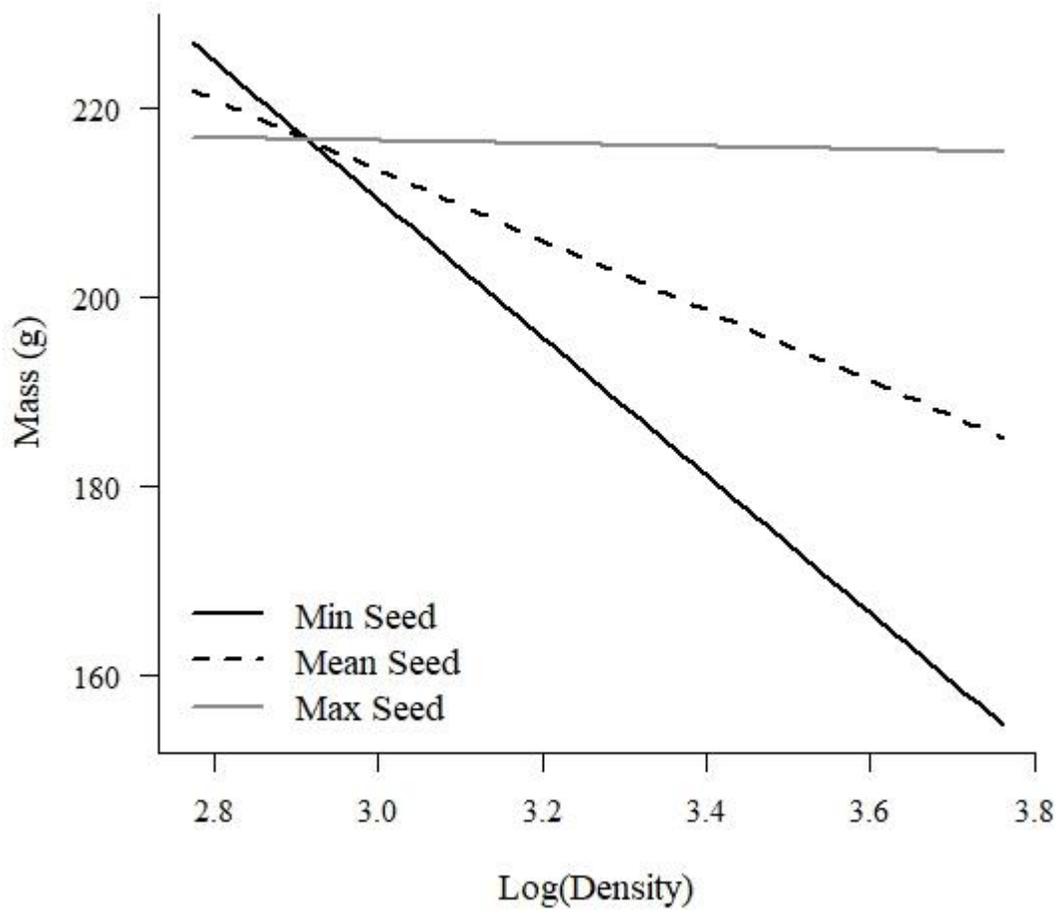


Figure A6.



APPENDIX B: EXPERIMENTAL REMOVALS REVEAL DIETARY
NICHE PARTITIONING FACILITATES COEXISTENCE BETWEEN
ECOLOGICALLY SIMILAR NATIVE AND INTRODUCED SPECIES

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Experimental removals reveal dietary niche partitioning facilitates coexistence between ecologically similar native and introduced species

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Summary

1. Niche overlap between native species and ecologically similar invaders can lead to competitive exclusion of threatened native species, but if two such species also co-occur naturally elsewhere, the anthropogenic interaction may mirror coevolved niche partitioning that reduces competition and promotes coexistence.
2. A single, insular population of Fremont's squirrel (*Tamiasciurus fremonti*) the Mount Graham red squirrel (MGRS; *T. f. grahamensis*) in the Pinaleno Mountains, Arizona, USA, is critically endangered and resource competition with introduced Abert's squirrels (*Sciurus aberti*) may threaten its long-term persistence. The species are naturally syntopic in other mountain sites, and both consume diets comprised primarily of conifer seeds and fungi.
3. We conducted experimental removals of introduced Abert's squirrels and used stable isotope analysis of diets before and after removals, and of diets in naturally

syntopic populations to test the hypothesis that dietary niche partitioning can facilitate coexistence between native and introduced species.

4. Mount Graham red squirrels and introduced Abert's squirrels partitioned the dietary niche similarly to naturally syntopic populations. Removals had no apparent effect. Diet of MGRS was more closely linked to availability of resources than to presence of Abert's squirrels.
5. Flexibility in dietary niche of introduced Abert's squirrels may have allowed them to exploit a resource opportunity in syntopy with MGRS, which occur in lower density than other red squirrel populations. Highly variable food production of MGRS habitat may mean effects of competition are more likely in poor years, and territorial defense against non-native Abert's squirrels likely imposes fitness costs on individual MGRS.
6. *Synthesis and applications.* Competitive exclusion of the endangered MGRS appears unlikely to occur through competition for food with introduced Abert's squirrels. However, similarity in diets means negative effects of competition could occur in future. Red squirrels are winter specialists, but as in other southern peripheral populations facing climate change challenges, specializing in winter survival will ultimately cease to be an advantage and where introduced populations of ecologically similar species are better adapted to changing conditions, they may replace native species.

Keywords

diet, conservation, invasive, niche, stable isotopes, squirrel, syntopy, wildlife

Introduction

Invasive species are permanent components of most ecosystems (Vitousek *et al.* 1997) and understanding the effects of competition between invaders and ecologically similar natives is important to conserve biodiversity (Davis 2003). Unlike invasive predators, which have directly caused extinctions of native species (Sax & Gaines 2008), competitive exclusion by invaders is challenging to quantify because it may not be observable and population declines may have multiple causes (Sax *et al.* 2007). Native wildlife population declines due to invasive species have been documented (Herbold & Moyle 1986; Petren & Case 1996), but competition could also lead to niche partitioning and coexistence (Shea & Chesson 2002; Davis 2003). The ability to predict the outcome of biological invasions would be valuable to conservation of threatened populations.

Co-occurring ecologically similar species occupy niches that limit competition and allow syntopic populations to persist (Brown, Kotler & Mitchell 1994; Chesson 2000), but species introductions impose novel ecological interactions that can lack the stabilizing niche differences necessary for coexistence (Chesson 2000). Invaders may be superior competitors for common resources or evolve rapidly to take advantage of novel resource opportunities (Shea & Chesson 2002) and competitively exclude native species (Mooney & Cleland 2001; Sakai *et al.* 2001). For example, the absence of niche partitioning (Wauters *et al.* 2002) and more efficient resource use (Kenward & Holm 1993) by invasive grey squirrels (*Sciurus carolinensis*) contributed to the precipitous decline of Eurasian red squirrels (*Sciurus vulgaris*) in parts of Europe (Gurnell & Pepper 1993; Bertolino *et al.* 2000).

In systems with variable food production, competing native species may adapt by altering their diets and feeding strategies in order to avoid direct competition (Pulliam

1986). In two closely related species of charr (*Salvelinus* sp.) that occur in zones of sympatry, niche partitioning varied over time, and according to species and size of individuals (Nakano, Fausch & Kitano 1999). Niche partitioning may also be flexible in novel interactions where an introduced species occupies habitat similar to its native range, which could promote coexistence with native competitors.

We studied a model system involving interactions between Abert's squirrels (*Sciurus aberti*) and Fremont's squirrels (*Tamiasciurus fremonti*) in Arizona, USA, where the species co-occur both naturally and in anthropogenic syntopy. One Fremont's squirrel (hereafter, red squirrel) subspecies, the Mount Graham red squirrel (MGRS; *T. f. grahamensis*) occurs in a single, critically endangered population in the Pinaleno Mountains, an isolated mountain range surrounded by desert grassland. Abert's squirrels introduced in the 1940s (Davis & Brown 1988) have established throughout all forested areas of the Pinaleno Mountains (Hutton *et al.* 2003). Elsewhere in Arizona, red squirrels and Abert's squirrels are naturally syntopic in zones of overlap between preferred forest types (Ferner 1974; Hall 1981); red squirrels inhabit primarily mixed-conifer and spruce-fir forest (Smith 1981), and Abert's squirrels are typically associated with ponderosa pine (*Pinus ponderosa*) forest (Keith 1965). Red squirrels are small (200-250 g) larder-hoarders, that display vigorous movements and vocalizations in defending territories (Smith 1968a; Koprowski 2005) whereas the larger (500-750 g) Abert's squirrels are secretive, occasional scatter-hoarders (Keith 1965; Allred 2010).

A potentially important effect of interaction is through competition for food. Diets of Abert's and red squirrels consist primarily of conifer seeds and fungi (Nash & Seaman 1977; Steele 1998). Both species also consume pollen from staminate cones of

conifers during late spring and early summer (Keith 1965; Smith 1968a). During periods of scarcity, Abert's squirrels also depend on phloem stripped from conifer twigs (Patton 1974; Allred 2010), and red squirrels rely on food cached in their larder-hoards or middens (Steele 1998). Such divergent strategies, in addition to differential use of available food in periods of relative abundance may facilitate coexistence in natural syntopy (Chesson 1994). As Abert's squirrel are 2-3 times larger than red squirrels, their ability to depress availability of diet items could have negative effects on MGRS especially during years of low food production. It is unknown, to what extent dietary resource partitioning occurs in the invaded community of the Pinaleno Mountains. However, the source population for Abert's squirrel translocations was naturally syntopic with red squirrels (Davis & Brown 1988), and niche separation may be maintained by coevolved differences in habitat selection (Rosenzweig 1981).

To examine the potential for coexistence between ecologically similar native and introduced species, we combined an experimental removal of introduced Abert's squirrels with a stable-isotopic study of dietary niche partitioning among naturally syntopic red squirrels and Abert's squirrels. We tested the hypothesis that dietary niche partitioning facilitated coexistence. We predicted: 1) low overlap in proportions of common items in MGRS and Abert's squirrel diets; 2) Abert's squirrel removals would not cause MGRS diet shifts; 3) evidence of dietary niche partitioning, specifically in total proportion of fungi consumed; 4) overlaps in diet proportions would be related to variability in food production; and 5) dietary niche interactions between MGRS and Abert's squirrels would be similar to those in sites of natural syntopy.

Materials and Methods

Study site

We collected data from study sites in coniferous forest of 3 mountain ranges in Arizona, USA, where red squirrels and Abert's squirrels were common. We conducted experimental removals on Abert's squirrels at our primary site, Mount Graham (MG), in the Pinaleno Mountains, Graham County. This site was ≈ 200 ha of mixed-conifer forest composed of Engelmann spruce (*Picea engelmannii*), Douglas-fir (*Pseudotsuga menziesii*), corkbark fir (*Abies lasiocarpa* var. *arizonica*), southwestern white pine (*Pinus strobiformis*), and ponderosa pine at 2800-3000-m elevation (Hutton *et al.* 2003). Two sites of natural syntopy between our model species were in the San Francisco Peaks (SF), Coconino County, and the White Mountains (WM), Greenlee County. The SF site was ≈ 100 ha in mixed-conifer forest dominated by Douglas-fir and ponderosa pine at 2500-m elevation. The WM site was divided into 2 ≈ 50 -ha areas 2800-m and 2400-m elevation, dominated by Douglas-fir and corkbark fir, and ponderosa pine and Gambel oak (*Quercus gambelii*), respectively. Red squirrel population densities at the upper and lower elevation WM sites were ≈ 0.3 /ha and ≈ 0.1 /ha, respectively (Derbridge & Koprowski, unpublished data). We also collected samples from introduced non-syntopic (INS) Abert's squirrels at a ponderosa pine forest area, ≈ 3 km southeast from the MG site at 2750-m elevation, where MGRS were not present.

Experimental removals

All methods were approved by the University of Arizona Institutional Animal Care and Use Committee, US Fish and Wildlife Service, and Arizona Game and Fish Department. We used a combination of live-trapping and hunting to remove Abert's squirrels from a 100-ha treatment area within the MG site during 2 removal periods, March-September

2012 and 2014 (Fig. B1). We captured Abert's squirrels in Tomahawk wire mesh live-traps (custom model No. 202; Tomahawk Live Trap Company, Tomahawk, WI), and euthanized them with inhalation of isoflurane. We coordinated with local hunters to remove other Abert's squirrels.

We estimated efficacy of removal by surveys of Abert's squirrel feeding sign (i.e., remains of conifer cones, stripped twigs, clipped branches marked and scattered in patterns not seen in MG red squirrel sign) on 16 50-m line transects each in the removal and control areas. We marked transects along randomly selected azimuths taken at 16 points selected at random in each area, and surveyed transects 9 times at 7-day intervals, May-July, 2012, and 8 times at 14-day intervals, May-Aug 2014. We also used remote cameras (Bushnell Trophy Cam model 119436; Bushnell Outdoor Products, Overland Park, KS) to establish 6 50-m transects in each area, June-August 2014. Two cameras faced inwards at opposite ends of the transect, and a third camera faced perpendicular to the line at 25 m. We quantified all single Abert's squirrel photographs for each hour of transect operation. We used Welch's two-sample *t*-test ($\alpha = 0.05$) to compare counts of Abert's squirrel feeding sign and photographs between the removal and control areas.

Diet sample collection and preparation

We collected known diet items of our model species from the MG site during 2011-2014, and from the SF and WM sites in 2014 and 2015, including seeds from ovulate cones of conifers (Froehlich 1990; Dodd, States & Rosenstock 2003; Edelman & Koprowski 2005), epigeous fungi (i.e., mushrooms) and hypogeous fungi (i.e., truffles and false truffles [hereafter truffles]) (Keith 1965; Smith 1968a). We also collected conifer twigs

from branches clipped by Abert's squirrels, and pollen cones from conifers at which we found squirrel feeding sign.

Both squirrel species molt in spring and fall; tails are molted only in fall (Layne 1954; Keith 1965). Hair contains isotopic records of diet for the period of growth. Thus, dorsal hairs collected June–September, or October–May contain isotopic diet records of about 1 month during summer and fall, respectively; tail hairs contain a fall diet record. We used Tomahawk wire mesh live traps (models #201 and custom #202; Tomahawk Live Trap Company, Tomahawk, WI) to capture squirrels, and collected summer- and fall-growth dorsum and tail hair samples from MGRS and Abert's squirrels throughout the study, and from red squirrels and Abert's squirrels at the SF and WM sites, 2014-2015. We also collected hair samples from Abert's squirrels removed by live-trapping or hunting during experimental removals 2012-2014, from hunted INS Abert's squirrels in 2015, and from hunted specimens at the WM and SF sites in 2014 and 2016, respectively.

We used an ultrasonic cleaner (Model FS20H, Fisher Scientific, Pittsburgh, PA) to remove coarse debris from hair samples in glass vials of deionized water. After air-drying for 24 h, we rinsed samples under a ventilation hood in a 2:1 chloroform/methanol solution to remove fine debris and oils. We removed and air-dried kernels from seed husks of conifer cones. We air-dried fungi samples and used a scalpel to cut out clean sporocarp interiors. We used a scalpel to strip outer bark from twigs, and separately removed the exposed phloem layer. We ground diet items and hairs to powder in a Wig-L-Bug[®] DS-80 amalgamator (Crescent Dental Co., Chicago, IL). We agitated staminate cones inside a clear plastic sample bag to separate pollen from cones. We weighed 1-2

mg of all samples in a DigiWeigh DWP-2004 laboratory scale (DigiWeigh Scales, Carlisle, PA).

We sent samples to the Environmental Isotope Laboratory (Department of Geosciences, University of Arizona) for analysis of C and N stable isotopes on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL) coupled to a Costech elemental analyzer. We express isotope values in delta notation (δ) as:

$$\delta Z = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where Z is ^{13}C or ^{15}N , and R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Standardization was based on acetanilide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}\text{C}$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}\text{N}$. Based on repeated internal standards, precision was better than ± 0.10 for $\delta^{13}\text{C}$ and ± 0.2 for $\delta^{15}\text{N}$.

Stable isotope diet analysis

We calculated mean and standard deviation $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for all diet items and squirrel species, and used t -tests to determine if diet items and squirrel species were isotopically distinct ($\alpha=0.05$). Because Abert's squirrels rarely consume spruce seeds (Edelman & Koprowski 2005; Allred 2010), we calculated separate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ that omitted spruce seeds from the Abert's squirrel diet analysis. We used a Bayesian stable isotope mixing model with rodent-specific diet-hair trophic discrimination factors of 3.4‰ for C and 2.4‰ for N (Kurle *et al.* 2014) to estimate the proportional contribution of each diet item to the fall and summer diets (Moore and Semmens 2008) of red squirrels and Abert's squirrels. We used Markov chain Monte Carlo (MCMC) methods to estimate the parameters of the mixing model, which produces simulations of plausible diet proportions and residual error consistent with the data. We ran 3 parallel MCMC chains with a burn-

in of 10,000 iterations. We generated posterior samples using 40,000 iterations of the model and a thinning rate of 10. We estimated these mixing models in R 3.4.2 (R Development Core Team 2017) and JAGS using the R package *rjags*.

Effect of food production on diet

We used 21 years (1994-2014) of conifer seed and mushroom production data collected by the University of Arizona's MGRS Monitoring Program (Koprowski, Alanen & Lynch 2005) to examine the relationship between annual variation in availability of common diet items and composition of MGRS and introduced Abert's squirrel diets. Conifer seeds were collected from 9 10-m x 10-m seed plots each containing 3 randomly placed 0.25-m² seed traps. Viable seeds of Engelmann spruce, Douglas-fir, and corkbark fir were quantified as seeds/m². All mushrooms of genera known to be consumed by red squirrels (Smith 1968a; Smith 1968b) were collected from 9 1-m x 100-m plots at 2-week intervals over 12 weeks from late July-October each year, and quantified as dry weight kg/ha.

We used analysis of variance (ANOVA) to test for among-year differences in conifer seed and mushroom production during our study, 2011-2014. Because $\delta^{13}\text{C}$ of seeds and mushrooms were isotopically distinct (Fig. B2), we used $\delta^{13}\text{C}$ as an index of the effect of variability in conifer seed and mushroom production on $\delta^{13}\text{C}$ of MGRS and introduced Abert's squirrels. We used $\delta^{13}\text{C}$ of individual squirrels as the response variable in linear regression models with predictors of seed and mushroom availability in each year 2011-2014. We scaled seed and mushroom data by taking the mean and standard deviation of 21 years of availability data, subtracting this mean from each year of study and dividing by the 21-year standard deviation. We assumed that because

mushrooms and truffles formed a distinct $\delta^{13}\text{C}$ group (Fig. B2) and truffle production is somewhat proportional to mushroom production (Vogt, Edmonds & Grier 1981), our model would provide insight on fungi availability in general. We included the covariates season (summer/fall), group (before-/after-removal), area (treatment/control), and sex. We considered the possibility that removals could affect variation in $\delta^{13}\text{C}$ by including a group*area interaction in models. We used a backwards elimination process to remove non-significant variables one at a time to choose a final model by Akaike's Information Criterion (AIC) when only significant variables or the main variables of biological interest (i.e., conifer seeds and mushrooms) remained. We conducted this analysis in R 3.4.2 (R Development Core Team 2017).

Results

Experimental removals

We removed 18 and 34 Abert's squirrels from the treatment area in 2012 and 2014, respectively (Fig. B1). Mean presence of Abert's squirrel feeding sign was reduced in both years after removal, although not significantly between treatment ($\bar{x} = 0.50$, SD = 1.10) and control area transects ($\bar{x} = 1.00$, SD = 0.82) in 2012 ($t_{30} = 1.17$, $P = 0.251$); however transects did differ in 2014 ($t_{22.7} = 3.57$, $P = 0.002$) with less feeding sign on treatment ($\bar{x} = 1.00$, SD = 1.32) compared to control ($\bar{x} = 2.56$, SD = 1.55) area transects.

From camera data, Abert's squirrels were more common on treatment ($\bar{x} = 7.17$, SD = 4.71) compared to control ($\bar{x} = 2.33$, SD = 1.97) transects ($t_{10} = -2.32$, $P = 0.043$) during the winter following the first removal, and less common on treatment ($\bar{x} = 4.17$, SD = 2.32) compared to control ($\bar{x} = 12.17$, SD = 7.47) transects during the second removal ($t_6 = 2.51$, $P = 0.046$).

Stable isotope data

We collected 110 and 71 hair samples from MGRS and Abert's squirrels, respectively, at the MG site, and 7 from INS Abert's squirrels, and 47 from red and Abert's squirrels at the SF and WM sites (Table B1). From 71 samples, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of diet items were different for ≥ 1 isotope in most comparisons, but epigeous fungi were not isotopically distinct from phloem or pollen, therefore we created 2 grouped diet sources from individual isotope values of epigeous fungi and phloem (i.e., epphlo), and epigeous fungi and pollen (i.e., epipol) to be used in fall and summer diet estimates, respectively (Fig. B2). Although this grouping process limits inference on single diet items, it is recommended over discarding known diet items from analysis (Phillips *et al.* 2014). We commonly observed Abert's squirrels consuming pollen, and stomachs of 8 Abert's squirrels removed in May-July during the study were filled with a homogenous thick pollen-yellow paste (Derbridge, personal observation).

Stable isotope diet analysis

Mount Graham red squirrel diets consisted of $\geq 60\%$ conifer seeds and $\leq 40\%$ fungi, pollen, and phloem in fall and summer, while Abert's squirrel diet was $\leq 52\%$ conifer seeds and $\geq 48\%$ fungi, pollen, and phloem at the MG site. Consumption of conifer seeds by introduced Abert's squirrels and MGRS did not overlap in fall or summer, and truffle consumption did not overlap in summer. The INS Abert's squirrels consumed a lower proportion of conifer seeds and higher proportion of truffles than MGRS and Abert's squirrels at the MG site (Table B2).

The proportional contribution of all diet items to MGRS fall diet was the same in treatment and control areas before and after the first removal; all 95% credible intervals

overlapped (Table B3a; Fig. B3). Mean conifer seed consumption decreased in both areas after the second removal, and mean fungi consumption increased (Table B3b; Fig. B3). Abert's squirrels consumed more fungi and phloem than MGRS throughout the study (Fig. B3). Mount Graham red squirrel summer diet did not differ before and after the second removal; all 95 % credible intervals overlapped (Table B3c; Fig. B4).

Red squirrels at the SF site consumed primarily conifer seeds in fall ($\bar{x} = 58\%$) and SF Abert's squirrels consumed primarily truffles ($\bar{x} = 68\%$). At the WM site, fungi comprised $\geq 70\%$ of red squirrel fall diet, and conifer seeds comprised $\geq 55\%$ of WM Abert's squirrel diet. Summer diet estimates of red squirrels at the SF and WM sites followed the same pattern as fall diets (Table B2).

Carbon index models

From 2011-2014, conifer seed production ($\bar{x} = 181.2/\text{m}^2$; $\text{SD} = 212.1$; $F_{3,32} = 21.9$; $P < 0.001$) and mushroom production ($\bar{x} = 5.8 \text{ kg/ha}$; $\text{SD} = 5.4$; $F_{3,32} = 7.29$; $P < 0.001$) differed among years. Final models for MGRS and introduced Abert's squirrels retained additional covariates, group and season, and group, sex and season, respectively (Table B4 a & b). Mushroom production influenced $\delta^{13}\text{C}$ of MGRS; conifer seed production influenced $\delta^{13}\text{C}$ of Abert's squirrels. Season affected both species; estimates indicated MGRS $\delta^{13}\text{C}$ was relatively enriched in summer, and Abert's squirrel $\delta^{13}\text{C}$ was relatively depleted (Table B5 a & b).

Discussion

We found broad support for the hypothesis that dietary niche partitioning can facilitate coexistence between ecologically similar native and introduced species. Experimental removals of Abert's squirrels had no effect on MGRS diet, and stable isotope analysis

revealed consistent differences in proportions of primary food items in diets. As predicted, consumption of fungi differed, diets were related to availability, and dietary niche interactions resembled those of naturally co-occurring populations.

Our results from monitoring of removal success provided evidence that we achieved measurable population reduction. It was not possible to remove all Abert's squirrels from the treatment area and we expected recolonization to occur due to habitat connectivity and the introduced species' ubiquity throughout forested areas of the Pinaleno Mountains. Camera data indicated Abert's squirrels were common on treatment area transects after the first removal in 2012 likely because we initiated these transects 10 weeks after concluding removals when Abert's squirrels were already recolonizing the area. During the second removal in 2014, we initiated camera transects during the peak of removal activity, and camera data revealed a clear difference in presence of Abert's squirrels in treatment and control areas. Although presence of Abert's squirrel feeding sign was statistically different only during the second removal in 2014, feeding sign was more common on control area transects during both removals. Fall diet estimates were based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of hair grown primarily during October and early November, likely before Abert's squirrels could have recolonized sufficiently to compromise the assumption of removal.

Overall, our results demonstrate the potential for flexibility in resource partitioning according to variation in food production (Nakano, Fausch & Kitano 1999). Introduction to the Pinaleno Mountains in the 1940s likely represented a niche opportunity (Shea & Chesson 2002) for Abert's squirrels because high resource availability, especially at lower elevations where MGRS were rare or absent, allowed a

positive rate of increase. Even in areas of syntopy with MGRS, a combination of resource availability and niche flexibility may have facilitated coexistence. Direct costs of invasion for MGRS do not appear to arise from reduced foraging opportunities, but dietary niche separation may be an ecological foundation for future competitive exclusion by the invader. To understand the relevance for invasive species management, it will be important to understand how Abert's squirrels were able to adjust dietary niche and if further conservation implications should be expected for MGRS in competition with the introduced species.

Abert's squirrels likely achieved dietary niche separation from MGRS in several ways. First, the introduced species consumed more truffles in general but more than double the amount consumed by MGRS in summer. Second, Abert's squirrels consumed more mushrooms throughout the year (States & Wettstein 1998; Dodd, States & Rosenstock 2003), but also more phloem and pollen in fall and summer, respectively. Because mushrooms were isotopically identical to phloem and pollen, we were unable to quantify their individual contributions to niche partitioning. However, phloem is a rare food item for MGRS (Froehlich 1990) and common for Abert's squirrels (Keith 1965), thus any proportion of phloem that provided nutrition and contributed to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Abert's squirrels likely represented partitioning.

Abert's squirrels spend much of the earliest period of summer consuming pollen (Keith 1965) and it may have been the most abundant food source for Abert's squirrels at the MG site before fungi production followed summer rains in early July. Climate change has caused milder winters and earlier springs in many alpine environments, and reduced snow cover along with altered phenology of diet sources favors species able to

expand their latitudinal and elevational geographic ranges (Williams, Henry & Sinclair 2015). When introduction of exotic species to novel environments coincides with phenological changes that benefit the invader, ecologically similar native species may be displaced (Gidoïn, Roques & Boivin 2015). Abert's squirrels have likely benefitted from increasingly milder winters during 75 years in the Pinaleño Mountains, and continuing changes to southwestern US forests (Williams *et al.* 2010) will likely provide further resource opportunities.

A third explanation for Abert's squirrel and MGRS dietary separation may be that they were able to partition the conifer seed component of dietary niche. Spruce cones, with thin scales and tiny seeds, and white pine cones, with coarse, resin-drenched scales and large seeds, present foraging tradeoffs that may allow MGRS and Abert's squirrels to be superior competitors for these respective resources (Connell 1980). Niche partitioning according to seed type or size plays an important role in community structure for many taxa including other mammals (Brown & Lieberman 1973) and species of ants (Davidson 1977), but if this trade-off has been important for resource partitioning between MGRS and Abert's squirrels, the decline of spruce in the Pinaleño Mountains due to insect damage and fire (Koprowski, Alanen & Lynch 2005) may not favor MGRS. Die-offs of spruce (*Picea* sp.) and expansion of invasive species populations are already widespread in North American forests (Logan, Régnière & Powell 2003).

Although the absence of a removal effect on MGRS diet alone could not clearly demonstrate niche partitioning (Rosenzweig 1981), our comparisons across sites were consistent with the experimental results. Red squirrels and Abert's squirrels in syntopy appear to consume different proportions of conifer seeds and fungi. This is further

supported by the relative contributions of seeds to WM and SF Abert's squirrel diets. Red squirrel density was low ($\approx 0.1/\text{ha}$) where WM Abert's squirrels were sampled, but higher ($\approx 0.3/\text{ha}$; Derbridge & Koprowski, unpublished data) where SF Abert's squirrels were sampled, and the relatively low component of seeds in SF Abert's squirrel diet suggests the dietary niche of Abert's squirrels may be flexible perhaps in response to red squirrels. Exclusion of competitors by territorial species has broad implications for organization of communities. For example, in competition hierarchies of boreal ants, aggressive territorial species coexist with submissive species, but the latter species is restricted in abundance and to less-preferred food (Savolainen & Vepsäläinen 1988).

The hypothesis that dietary niche partitioning facilitated coexistence was further supported by our carbon index models. The greatest difference in conifer seed consumption between MGRS and Abert's squirrels occurred in 2011, when seed production was lowest during the study. Mount Graham red squirrels likely relied on seeds stored in middens during the previous year to avoid starvation following this crop failure (Smith 1968b), but the strong influence of mushrooms represented by a negative estimate (i.e., depleted $\delta^{13}\text{C}$) in our availability model confirms this diet item's importance in a highly variable environment. When the largest cone crop during the study occurred the following year, conifer seeds contributed the largest proportions to diets of both species. The relative enrichment in $\delta^{13}\text{C}$ of Abert's squirrels demonstrated the introduced species' ability to exploit the resource opportunity of abundant seed production. The contrasting years and diet estimates suggest resource partitioning reduced competition for conifer seeds when in limited supply, and that seed production maintained the introduced Abert's squirrel population in good years. Similar patterns of

coexistence based on dietary niche separation were observed among gerbils (*Gerbillus* sp.) and jerboas (*Jaculus jaculus*), and the ability of jerboas to roam widely to take advantage of patchily available resources (Brown, Kotler & Mitchell 1994) may mirror the strategy employed by Abert's squirrels that move among red squirrel territories in search of food. Dietary niche partitioning between Mount Graham red squirrels and Abert's squirrels resembled partitioning in naturally syntopic populations of these species, which suggests competition for food does not preclude coexistence. This consistent separation may be a consequence of natural selection for a system of habitat selection that tends towards minimizing competition (Rosenzweig 1981) between red squirrels and Abert's squirrels, as has been demonstrated through removal experiments on other rodents (Abramsky & Sellah 1982). The Abert's squirrels introduced in the 1940s were translocated from the San Francisco Peaks, one of our sites of natural syntopy, and they may have been adapted for coexistence with red squirrels. Coevolution is expected to limit niche overlap and dampen effects of competition (Smith, Mooney & Agrawal 2008).

Other components of ecological niche overlap may differ among populations and provide evidence MGRS are negatively affected by introduced Abert's squirrels. Models of optimal territoriality predict aggressive territory owners must choose between expelling all intruders or reducing territory size and reproductive output (Hixon 1980). In a separate study, broad home range overlap between MGRS and Abert's squirrels (Derbridge & Koprowski *in review*) suggested interspecific encounters were common and territorial MGRS incurred invasion costs through energy expended in repelling Abert's squirrels. Niche theory predicts per-capita growth rates to be limited by gains

from resource consumption and losses from maintenance requirements, and native species could be competitively excluded if they have higher maintenance requirements than ecologically similar invaders (Shea & Chesson 2002). In the Pinaleno Mountains, resources support invasive Abert's squirrels whose presence likely increases maintenance requirements for MGRS because they are forced to defend territories against the non-native intruders.

Conservation of forest obligate species is challenged by global transformations of habitat related to climate change (Allen *et al.* 2010). In the southwestern US, where declining winter precipitation is predicted (Williams *et al.* 2010), lower frequency of harsh winters will benefit Abert squirrels, which are prone to high mortality when foraging is inhibited during extended periods of deep snow (Stephenson & Brown 1980). By contrast, red squirrels may be negatively affected as mild winters reduce competitive advantage for winter specialist species adapted to sub-nivean existence (Merritt, Lima & Bozinovic 2001). Earlier springs may also favor invasive species adapted to mild winters if changing phenology of plants provides food and habitat earlier. For instance, an invasive seed-specialized wasp species replaced a closely related native species where host plant seeds were available for ovipositioning earlier in the year (Gidoin, Roques & Boivin 2015). Abert's squirrels in the Pinaleno Mountains, would likely benefit from earlier availability of pollen from staminate conifer cones.

Our study exemplified a unique situation in which the same two species occurred in natural and anthropogenic syntopy. Future studies on other components of niche overlap could help determine the potential for stable coexistence between our model species and provide insight for other invaded communities. Many of the challenges faced

by MGRS are common to small populations at southern range peripheries. Such species may be less resistant to invasion effects as they tend to possess lower genetic diversity (Vucetich & Waite 2003) and occur in lower densities than populations at range centers (Lomolino & Channell 1995). Mount Graham red squirrels have extremely low genetic variation (Fitak, Koprowski & Culver 2013) which could inhibit their potential to adapt to a changing environment. They also have much larger home ranges than other red squirrel populations (Koprowski, King & Merrick 2008), and low population density may increase resource availability for Abert's squirrels whose introduced population in the Pinaleño Mountains is central to the species' native range. Resource partitioning between ecologically similar native and introduced species may not keep pace with rapid forest ecosystem change, and the risk of competitive exclusion may be greater for vulnerable native populations.

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Mammalogists guidelines for the use of wild mammals in research (Sikes, Gannon, & The Animal Care and Use Committee of the American Society of Mammalogists 2011).

References

- Abramsky, Z. & Sellah, C. (1982) Competition and the role of habitat selection in *Gerbillus allenbyi* and *Meriones tristrami*: a removal experiment. *Ecology*, **63**, 1242-1247.
- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S.W., Semerci, A. & Cobb, N. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, **259**, 660-684.
- Allred, S. (2010) *The Natural History of Tassel-eared Squirrels*. University of New Mexico Press, Albuquerque, NM.
- Bertolino, S., Currado, I., Mazzoglio, P.J. & Amori, G. (2000) Native and alien squirrels in Italy. *Hystrix*, **11**, 65-74.
- Brown, J.H. & Lieberman, G.A. (1973) Resource utilization and coexistence of seed-eating desert rodents in sand dune habitats. *Ecology*, **54**, 788-797.
- Brown, J.S., Kotler, B.P. & Mitchell, W.A. (1994) Foraging theory, patch use, and the structure of a Negev Desert granivore community. *Ecology*, **75**, 2286-2300.
- Chesson, P. (1994) Multispecies competition in variable environments. *Theoretical Population Biology*, **45**, 227-276.

- Chesson, P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, **31**, 343-366.
- Connell, J.H. (1980) Diversity and the coevolution of competitors, or the ghost of competition past. *Oikos*, **35**, 131-138.
- Davidson, D.W. (1977) Species diversity and community organization in desert seed-eating ants. *Ecology*, **58**, 711-724.
- Davis, M.A. (2003) Biotic globalization: does competition from introduced species threaten biodiversity? *AIBS Bulletin*, **53**, 481-489.
- Davis, R. & Brown, D.E. (1988) Documentation of the transplanting of Abert's squirrels. *The Southwestern Naturalist*, **33**, 490-492.
- Derbridge, J.J. & Koprowski, J.L. The roles of food, population density, and introduced species in determining space use dynamics of a territorial mammal. *Journal of Animal Ecology*, **In review**.
- Dodd, N.L., States, J.S. & Rosenstock, S.S. (2003) Tassel-eared squirrel population, habitat condition, and dietary relationships in north-central Arizona. *The Journal of Wildlife Management*, **67**, 622-633.
- Edelman, A.J. & Koprowski, J.L. (2005) Diet and tree use of Abert's squirrels (*Sciurus aberti*) in a mixed-conifer forest. *The Southwestern Naturalist*, **32**, 490-492.
- Ferner, J.W. (1974) Habitat relationships of *Tamiasciurus hudsonicus* and *Sciurus aberti* in the Rocky Mountains. *The Southwestern Naturalist*, **18**, 470-473.
- Fitak, R.R., Koprowski, J.L. & Culver, M. (2013) Severe reduction in genetic variation in a montane isolate: the endangered Mount Graham red squirrel (*Tamiasciurus hudsonicus grahamensis*). *Conservation Genetics*, **14**, 1233-1241.

- Froehlich, G.F. (1990) Habitat use and life history of the Mount Graham red squirrel. MS thesis, University of Arizona.
- Gidoïn, C., Roques, L. & Boivin, T. (2015) Linking niche theory to ecological impacts of successful invaders: insights from resource fluctuation-specialist herbivore interactions. *Journal of Animal Ecology*, **84**, 396-406.
- Gurnell, J. & Pepper, H. (1993) A critical look at conserving the British red squirrel *Sciurus vulgaris*. *Mammal Review*, **23**, 127-137.
- Hall, J.G. (1981) A field study of the Kaibab squirrel in Grand Canyon National Park. *Wildlife Monographs*, **75**, 3-54.
- Herbold, B. & Moyle, P.B. (1986) Introduced species and vacant niches. *The American Naturalist*, **128**, 751-760.
- Hixon, M.A. (1980) Food production and competitor density as the determinants of feeding territory size. *The American Naturalist*, **115**, 510-530.
- Hutton, K.A., Koprowski, J.L., Greer, V.L., Alanen, M.I., Schauffert, C.A., Young, P.J. & Jones, C.A. (2003) Use of mixed-conifer and spruce-fir forests by an introduced population of Abert's squirrels (*Sciurus aberti*). *The Southwestern Naturalist*, **48**, 257-260.
- Keith, J.O. (1965) The Abert squirrel and its dependence on ponderosa pine. *Ecology*, **46**, 150-163.
- Kenward, R. & Holm, J. (1993) On the replacement of the red squirrel in Britain. A phytotoxic explanation. *Proceedings of the Royal Society of London B: Biological Sciences*, **251**, 187-194.

- Koprowski, J.L. (2005) Annual cycles in body mass and reproduction of endangered Mt. Graham red squirrels. *Journal of Mammalogy*, **86**, 309-313.
- Koprowski, J.L., Alanen, M.I. & Lynch, A.M. (2005) Nowhere to run and nowhere to hide: Response of endemic Mt. Graham red squirrels to catastrophic forest damage. *Biological Conservation*, **126**, 491-498.
- Koprowski, J.L., King, S.R.B. & Merrick, M.J. (2008) Expanded home ranges in a peripheral population: space use by endangered Mt. Graham red squirrels. *Endangered Species Research*, **4**, 227-232.
- Kurle, C.M., Koch, P.L., Tershy, B.R. & Croll, D.A. (2014) The effects of sex, tissue type, and dietary components on stable isotope discrimination factors ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) in mammalian omnivores. *Isotopes in Environmental and Health Studies*, **50**, 307-321.
- Layne, J.N. (1954) The biology of the red squirrel, *Tamiasciurus hudsonicus loquax* (Bangs), in central New York. *Ecological Monographs*, **24**, 227-268.
- Logan, J.A., Régnière, J. & Powell, J.A. (2003) Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and the Environment*, **1**, 130-137.
- Lomolino, M.V. & Channell, R. (1995) Splendid isolation: patterns of geographic range collapse in endangered mammals. *Journal of Mammalogy*, **76**, 335-347.
- Merritt, J.F., Lima, M. & Bozinovic, F. (2001) Seasonal regulation in fluctuating small mammal populations: feedback structure and climate. *Oikos*, **94**, 505-514.
- Mooney, H.A. & Cleland, E.E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, **98**, 5446-5451.

- Nakano, S., Fausch, K.D. & Kitano, S. (1999) Flexible niche partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. *Journal of Animal Ecology*, **68**, 1079-1092.
- Nash, D.J. & Seaman, R.N. (1977) *Sciurus aberti*. *Mammalian Species*, **80**, 1-5.
- Patton, D.R. (1974) Estimating food consumption from twigs clipped by the Abert squirrel. Rocky Mountain Forest and Range Experiment Station, USDA Forest Service
- Petren, K. & Case, T.J. (1996) An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology*, **77**, 118-132.
- Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X. & Ward, E.J. (2014) Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, **92**, 823-835.
- Pulliam, H.R. (1986) Niche expansion and contraction in a variable environment. *American Zoologist*, **26**, 71-79.
- Rosenzweig, M.L. (1981) A theory of habitat selection. *Ecology*, **62**, 327-335.
- Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305-332.
- Savolainen, R. & Vepsäläinen, K. (1988) A competition hierarchy among boreal ants: impact on resource partitioning and community structure. *Oikos*, **51**, 135-155.

- Sax, D.F. & Gaines, S.D. (2008) Species invasions and extinction: the future of native biodiversity on islands. *Proceedings of the National Academy of Sciences*, **105**, 11490-11497.
- Sax, D.F., Stachowicz, J.J., Brown, J.H., Bruno, J.F., Dawson, M.N., Gaines, S.D., Grosberg, R.K., Hastings, A., Holt, R.D. & Mayfield, M.M. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology & Evolution*, **22**, 465-471.
- Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution*, **17**, 170-176.
- Smith, C.C. (1968a) The adaptive nature of social organization in the genus of three squirrels *Tamiasciurus*. *Ecological Monographs*, **38**, 31-64.
- Smith, C.C. (1981) The indivisible niche of *Tamiasciurus* - an example of non-partitioning of resources. *Ecological Monographs*, **51**, 343-363.
- Smith, M.C. (1968b) Red squirrel responses to spruce cone failure in interior Alaska. *The Journal of Wildlife Management*, **32**, 305-317.
- Smith, R., Mooney, K. & Agrawal, A. (2008) Coexistence of three specialist aphids on common milkweed, *Asclepias syriaca*. *Ecology*, **89**, 2187-2196.
- States, J. & Wettstein, P. (1998) Food habits and evolutionary relationships of the tasseled squirrel (*Sciurus aberti*). *Ecology and Evolutionary Biology of Tree Squirrels* (eds M.A. Steele, J.F. Merritt & D.A. Zegers), pp. 185-194. Virginia Museum of Natural History Special Publication 6, Martinsville, VA.
- Steele, M.A. (1998) *Tamiasciurus hudsonicus*. *Mammalian Species*, **586**, 1-9.

- Stephenson, R.L. & Brown, D.E. (1980) Snow cover as a factor influencing mortality of Abert's squirrels. *The Journal of Wildlife Management*, **44**, 951-955.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494-499.
- Vogt, K.A., Edmonds, R.L. & Grier, C.C. (1981) Biomass and nutrient concentrations of sporocarps produced by mycorrhizal and decomposer fungi in *Abies amabilis* stands. *Oecologia*, **50**, 170-175.
- Vucetich, J.A. & Waite, T.A. (2003) Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics*, **4**, 639-645.
- Wauters, L., Gurnell, J., Martinoli, A. & Tosi, G. (2002) Interspecific competition between native Eurasian red squirrels and alien grey squirrels: does resource partitioning occur? *Behavioral Ecology and Sociobiology*, **52**, 332-341.
- Williams, A.P., Allen, C.D., Millar, C.I., Swetnam, T.W., Michaelsen, J., Still, C.J. & Leavitt, S.W. (2010) Forest responses to increasing aridity and warmth in the southwestern United States. *Proceedings of the National Academy of Sciences*, **107**, 21289-21294.
- Williams, C.M., Henry, H.A.L. & Sinclair, B.J. (2015) Cold truths: how winter drives responses of terrestrial organisms to climate change. *Biological Reviews*, **90**, 214-235.

Figure Legends

Figure B1. The Mount Graham study area in the Pinaleño Mountains, Arizona, USA, showing overlap of 50% home range (core-use) areas between Mount Graham red squirrel (dark grey) and introduced Abert's squirrels (light grey) in 2011, the year before experimental removals of Abert's squirrels began. The black line indicates a road that bisected the study area; the Abert's squirrel treatment area was west of the road. Black crosses (2012) and white circles (2014) indicate Abert's squirrel removal locations.

Figure B2. Individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of Mount Graham red squirrels and Abert's squirrels, and mean and SD of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of their diet items. Stable isotope values were calculated from squirrel hairs and samples of diet items collected 2011-2014 in the Pinaleño Mountains, Arizona, USA.

Figure B3. Mean and SD (black bars) proportional contribution of seeds, truffles, and mushrooms and phloem (i.e., epphlo) to the fall diets of Mount Graham red squirrels in treatment (squares) and control (triangles) areas before and after introduced Abert's squirrel removals in 2012 and 2014. We used a Bayesian stable isotope mixing model to estimate diet from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in hairs collected from squirrels during captures and removals in the Pinaleño Mountains, Arizona, USA.

Figure B4. Mean and SD (black bars) proportional contribution of seeds, truffles, and mushrooms and pollen (i.e., epipol) to the summer diets of Mount Graham red squirrels in treatment (squares) and control (triangles) areas before and after Abert's squirrel removals in 2014. We used a Bayesian stable isotope mixing model to estimate diet from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in hairs collected from squirrels during captures and removals in the Pinaleño Mountains, Arizona, USA.

Table B1. Mean and standard deviation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from red squirrels, Abert's squirrels, and their diet sources at Mount Graham (MG) in the Pinaleno Mountains, San Francisco Peaks (SF) and White Mountains (WM) study areas, Arizona, USA, 2011-2016, including introduced syntopic at, and introduced non-syntopic (INS) Abert's squirrels near the MG site. Separate values for seeds reflect a difference in cones targeted by red and Abert's squirrels (i.e., Abert's squirrels were not observed processing spruce cones).

	<i>n</i>	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		mean	SD	mean	SD
MG red	110	-18.93	0.80	3.90	1.23
SF red	10	-19.41	0.57	3.51	0.76
WM red	17	-19.45	0.52	6.50	2.13
Abert's	71	-19.34	0.85	4.02	1.08
INS Abert's	7	-19.86	0.64	6.6	1.43
SF Abert's	14	-19.80	0.41	7.41	1.07
WM Abert's	6	-18.83	0.43	4.55	0.64
Red seeds	12	-21.37	1.59	0.55	1.01
Abert's seeds	6	-20.34	1.12	0.41	0.74
Truffles	10	-24.45	1.25	5.88	2.45
Mushrooms	16	-23.93	1.62	-0.23	1.97
Phloem	19	-25.68	1.14	-1.17	2.92
Pollen	8	-26.05	0.62	-1.37	1.08

Table B2. Proportional contributions to diet (mean and 95% credible intervals) of 3 diet items/groups based on posterior estimates from Bayesian stable isotope mixing models of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in hairs of red and Abert's squirrels (a) in fall at Mount Graham (MG) in the Pinaleno Mountains, San Francisco Peaks (SF) and White Mountains (WM), Arizona, USA, 2011-2016, including introduced syntopic and introduced non-syntopic (INS) Abert's squirrels near the MG site, and (b) in summer at MG, SF, and WM (Abert's squirrels were not sampled in summer at the SF, WM, or INS sites).

a)

Fall mean (95% CI) diet estimates							
Squirrel _{year}	<i>n</i>	Seeds		Truffles		Mushrooms/phloem	
MGRS ₂₀₁₁₋₁₄	85	0.66	(0.60,0.71)	0.25	(0.21,0.29)	0.09	(0.04,0.15)
Abert's ₂₀₁₁₋₁₄	46	0.52	(0.47,0.57)	0.31	(0.27,0.35)	0.17	(0.12,0.23)
INS Abert's ₂₀₁₄	7	0.27	(0.11,0.41)	0.62	(0.46,0.78)	0.11	(0.01,0.28)
SF red ₂₀₁₄₋₁₅	5	0.58	(0.33,0.83)	0.17	(0.03,0.35)	0.25	(0.04,0.50)
SF Abert's ₂₀₁₆	14	0.27	(0.17,0.36)	0.68	(0.59,0.78)	0.05	(0.00,0.14)
WM red ₂₀₁₄₋₁₅	5	0.30	(0.04,0.59)	0.60	(0.19,0.89)	0.10	(0.00,0.37)
WM Abert's ₂₀₁₄	6	0.55	(0.41,0.69)	0.32	(0.20,0.45)	0.13	(0.01,0.29)

b)

Summer mean (95% CI) diet estimates

Squirrel year	<i>n</i>	Seeds		Truffles		Mushrooms/pollen	
MGRS 2013-14	25	0.60	(0.49,0.73)	0.09	(0.03,0.16)	0.31	(0.19,0.42)
Abert's 2011-14	25	0.30	(0.22,0.38)	0.24	(0.17,0.32)	0.46	(0.37,0.55)
SF red 2015	5	0.51	(0.24,0.79)	0.19	(0.05,0.35)	0.30	(0.05,0.56)
WM red 2014	12	0.42	(0.29,0.56)	0.51	(0.36,0.65)	0.06	(0.00,0.20)

Table B3. Proportional contributions to diet (mean and 95% credible intervals) of 3 diet items/groups based on posterior estimates from Bayesian stable isotope mixing models of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in hairs of Mount Graham red squirrels in fall before and after removal of Abert's squirrels from the Pinaleno Mountains, Arizona, USA in 2012 (a) and 2014 (b), and summer before and after removal in 2014 (c).

a)

Removal 1 Fall mean (95% CI) diet estimates							
Period-Area	<i>n</i>	Seeds		Truffles		Mushrooms/phloem	
Before-Control	9	0.67	(0.54,0.79)	0.28	(0.18,0.39)	0.05	(0.00,0.16)
Before-Treatment	13	0.67	(0.57,0.77)	0.29	(0.21,0.37)	0.04	(0.00,0.12)
After-Control	11	0.70	(0.54,0.86)	0.16	(0.06,0.26)	0.15	(0.02,0.29)
After-Treatment	15	0.78	(0.65,0.91)	0.14	(0.04,0.24)	0.08	(0.00,0.20)

b)

Removal 2 Fall mean (95% CI) diet estimates

Period-Area	<i>n</i>	Seeds	Truffles	Mushrooms/phloem
Before-Control	10	0.58 (0.41,0.77)	0.12 (0.02,0.23)	0.30 (0.13,0.45)
Before-Treatment	9	0.58 (0.40,0.78)	0.18 (0.03,0.34)	0.24 (0.05,0.44)
After-Control	11	0.43 (0.30,0.56)	0.45 (0.35,0.56)	0.12 (0.01,0.25)
After-Treatment	7	0.45 (0.26,0.64)	0.35 (0.22,0.50)	0.21 (0.03,0.39)

c)

Removal 2 Summer mean (95% CI) diet estimates

Period-Area	<i>n</i>	Seeds	Truffles	Mushrooms/phloem
Before-Control	5	0.70 (0.45,0.92)	0.10 (0.01,0.24)	0.20 (0.02,0.43)
Before-Treatment	8	0.63 (0.42,0.86)	0.11 (0.01,0.23)	0.26 (0.05,0.47)
After-Control	3	0.43 (0.06,0.83)	0.15 (0.01,0.43)	0.42 (0.05,0.80)
After-Treatment	9	0.52 (0.31,0.74)	0.10 (0.01,0.23)	0.38 (0.17,0.58)

Table B4. Models estimated to explain variation $\delta^{13}\text{C}$ of Mount Graham red squirrels (a) and introduced Abert's squirrels (b) in the Pinaleño Mountains, Arizona, USA, 2011-2014. We used linear models with conifer seed production (*c*), mushroom production (*m*), season (*s*), group (*g*), area (*a*), sex (*sx*), and an interaction between group and area as predictors of variation in $\delta^{13}\text{C}$ of Mount Graham red squirrels (*M*), and Abert's squirrels (*A*). We estimated a full model with an interaction between group and area, and removed non-significant predictors until only the diet items and significant predictors remained. We then used AIC to select the top model.

a)

Model	<i>K</i>	AIC	ΔAIC	AICwt
<i>c+m+s+g</i>	6	211.35	0.00	0.54
<i>c+m+s+g+a</i>	7	212.56	1.22	0.29
<i>c+m+s+g+a+sx</i>	8	214.64	3.29	0.10
<i>c+m+s+g+a+sx+(g*a)</i>	9	215.70	4.36	0.06

b)

Model	K	AIC	Δ AIC	AICwt
$c+m+s+g+a$	7	134.31	0.48	0.48
$c+m+s+g+a+sx$	8	135.53	0.26	0.74
$c+m+s+g$	6	136.20	0.19	0.93
$c+m+s+g+a+sx+(g*a)$	9	138.12	0.07	1.00

Table B5. Estimates from final linear model of the effect of seed and mushroom availability on the carbon isotope values of Mount Graham red squirrels (a) and introduced Abert's squirrels (b) in the Pinaleño Mountains, Arizona, USA, 2011-2014. Additional covariates were included to help explain variation in the main response.

a)

	Estimate	SE	<i>t</i> -value	<i>P</i> -value
(Intercept)	-19.127	0.101	-190.113	0.000
Conifer seeds	0.230	0.143	1.608	0.111
Mushrooms	-0.845	0.134	-6.293	0.000
Group	0.449	0.171	2.622	0.010
Season	0.368	0.158	2.329	0.022

b)

	Estimate	SE	<i>t</i> -value	<i>P</i> -value
(Intercept)	-19.605	0.155	-126.550	< 0.001
Conifer Seeds	0.583	0.198	2.952	0.004
Mushrooms	-0.304	0.171	-1.785	0.079
Group	0.604	0.298	2.027	0.047
Sex	0.400	0.152	2.632	0.011
Season	-0.706	0.152	-4.651	< 0.001

Figure B1.

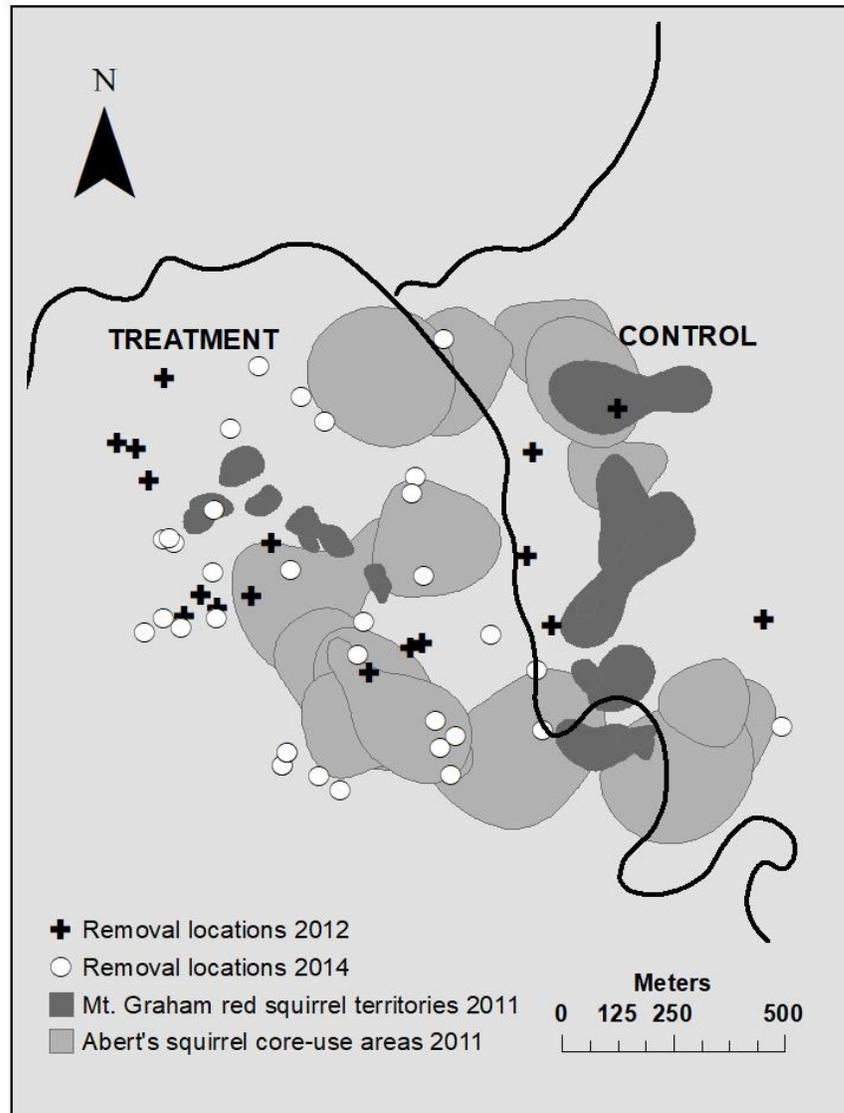


Figure B2.

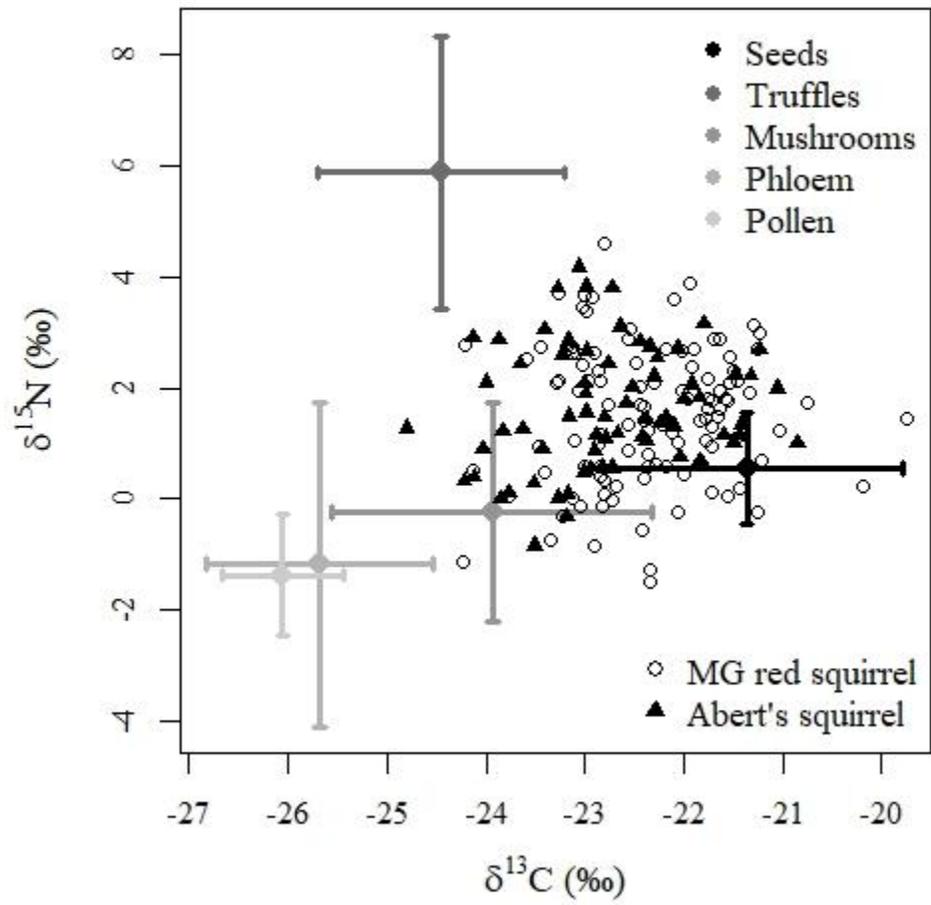


Figure B3.

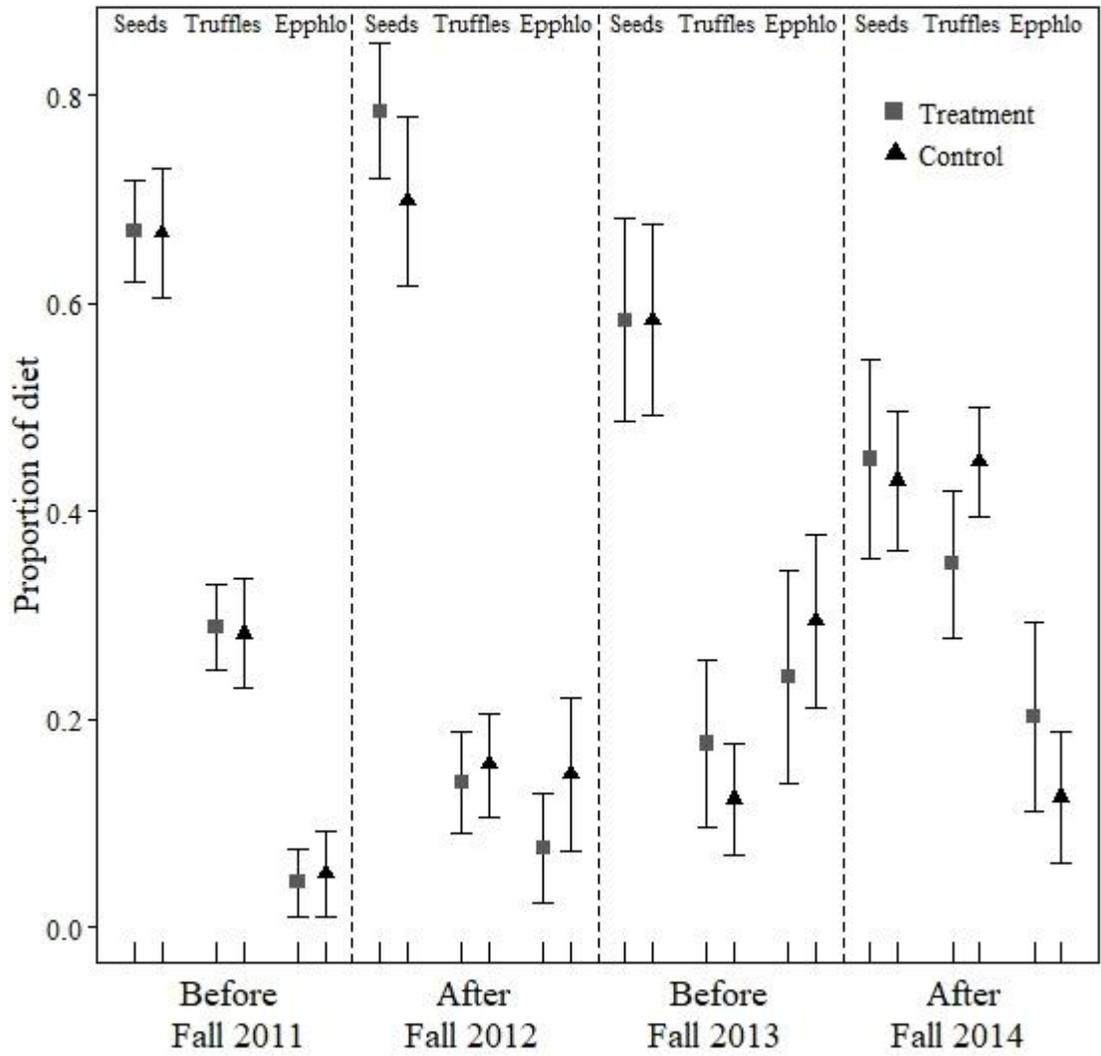
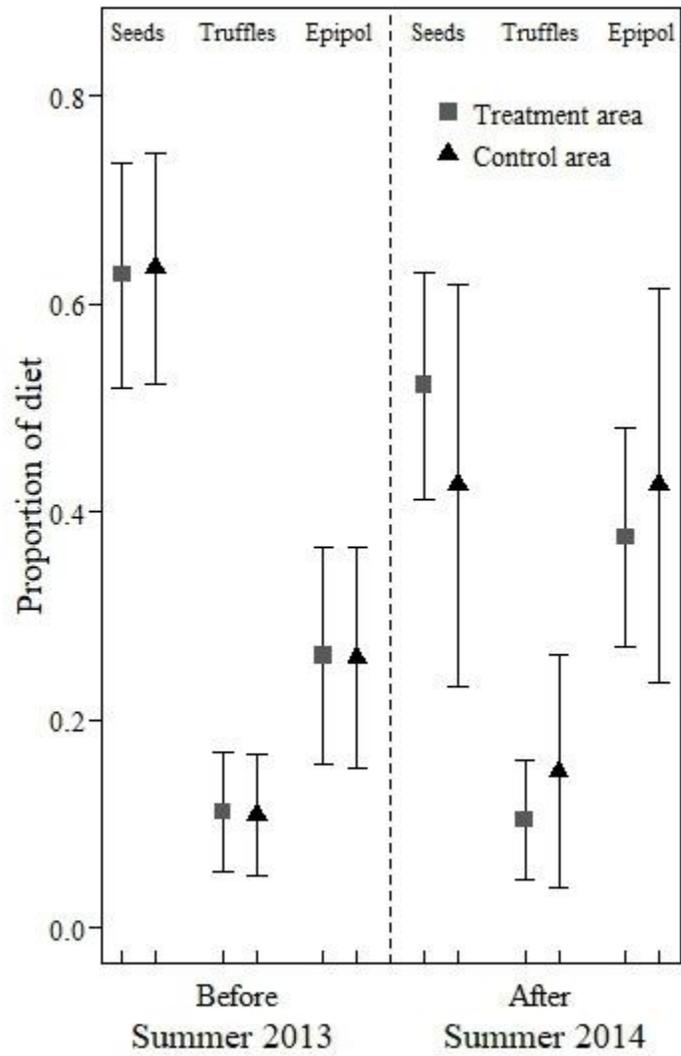


Figure B4.



APPENDIX C: UNFAMILIARITY BREEDS CONTEMPT:
TERRITORIAL BEHAVIOR VARIES AMONG ISOLATED
POPULATIONS IN NATURAL AND INVASIVE INTERACTIONS
WITH AN ECOLOGICALLY SIMILAR COMPETITOR

Jonathan J. Derbridge and John L. Koprowski

(In the format of *Biological Conservation*)

Unfamiliarity Breeds Contempt: Territorial Behavior Varies Among Isolated Populations in Natural and Invasive Interactions with an Ecologically Similar Competitor

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Abstract

Territorial behavior promotes fitness where animals can gain exclusive access to limited resources. However, this selective advantage could be reduced where native territorial animals are forced to protect their niches from ecologically similar invaders. Invasion-related competition may pose particularly urgent threats to the persistence of small, isolated populations, and behavior studies that explore how invasion alters trade-offs of territoriality can provide valuable information for conservation of imperiled territorial species. The Mount Graham red squirrel (MGRS; *T. fremonti grahamensis*) occurs in a small, isolated population in syntopy with an introduced population of Abert's squirrels

(*Sciurus aberti*) in Arizona, USA. In other mountain ranges, Fremont's squirrels (*T. fremonti*) and Abert's squirrels co-occur naturally. We simulated territorial intrusions to determine if introduced Abert's squirrels alter fitness trade-offs in territorial Fremont's squirrels (hereafter, red squirrels). We conducted tests at red squirrel larderhoards, known as middens, at sites of natural and anthropogenic syntopy with Abert's squirrels across three Arizona mountain ranges. We quantified movement and vocal responses as proxies of territoriality. We found that MGRS moved and vocalized significantly more than other red squirrels regardless of test. We detected no evidence of specific behavioral responses to introduced Abert's squirrels, but comparisons with naturally syntopic populations revealed MGRS were more aggressive overall, which may alter the economic defendability of their territories. Forest management for MGRS habitat could reduce the risk of invasion effects, but climate change may ultimately favor Abert's squirrel habitat. Small, isolated populations are commonly at risk from multiple anthropogenic factors and the ecological shock of sudden competition from invaders could reduce the advantage of territorial aggression and propel native territorial species further along the road to extinction. The threat from invasive species is a globally increasing phenomenon and conservation behavior studies like ours can rapidly provide critical information for management of imperiled species.

Keywords: behavior, conservation, endangered, experiment, invasive, territoriality

1. Introduction

Territorial behavior promotes fitness where animals can gain exclusive access to limited resources (Schoener 1983), and the vigor with which territories are defended likely reflects this selective advantage (Brown 1964). Under natural conditions, the trade-offs

for territoriality are clear, but where invasive species occupy the same niche, such trade-offs may be disrupted because native territorial animals are forced to expend more energy to defend the same resources. Some aspects of economic defendability trade-offs (Brown 1964), including the relationships between available resources (Fretwell and Lucas 1969), types of food caching (Andersson and Krebs 1978), and levels of territorial defense (Smith and Reichman 1984) are well documented. The effect of competition from ecologically similar invaders on the trade-offs for territorial behavior is less well understood. However, evidence suggests competing native or introduced species may be forced to adjust their ecological niches. For example, northern spotted owls (*Strix occidentalis caurina*) exhibited spatial avoidance of agonistic interactions with invasive barred owls (*S. varia*) by enlarging territory sizes (Wiens et al. 2014), and invasive American mink (*Neovison vison*) shifted their temporal niche to limit competition with river otters (*Lutra lutra*) and polecats (*Mustela putorius*) following recovery of these native species (Harrington et al. 2009). Field experiments on native species interactions have also provided insight; in the mouse genus *Peromyscus*, regardless of species, intruders tended to lose to territory owners, suggesting trade-offs for territoriality exist on a gradient from territorial centers (Wolff et al. 1983).

Territoriality is especially important for larder-hoarding animals as they maintain and defend single, centrally located food caches (Vander Wall 1990). Where ecologically similar species co-occur, natural selection may favor behaviors that make larder hoarding an economically defendable strategy. For example, larder-hoarding species may reduce caching rates in the presence of pilferers to minimize raids (Dally et al. 2006) and avoid the energetic cost of aggressive interactions with scatter-hoarding

competitors (Clarke and Kramer 1994). However, sudden competition from species introductions may negatively affect native larder hoarders (Minckley 1968) because they are unable to repel novel kleptoparasites or waste energy through excessive territorial behavior (Dally et al. 2006). Such ecological ‘shocks’ may be particularly unsustainable for species that have evolved in isolation (Blackburn et al. 2004) and research on ecological interactions of peripheral populations with introduced species is key for effective management (Hampe and Petit 2005). Genetic distinctiveness also gives peripheral populations high conservation value (Vucetich and Waite 2003) but isolation increases vulnerability to invasions. For example, westslope cutthroat trout (*Oncorhynchus clarki lewisi*) are threatened by hybridization with introduced rainbow trout (*Oncorhynchus mykiss*) in the US and Canada (Taylor et al. 2003).

Here, we compared the territorial behavior of a native species in response to an ecologically similar intruder in natural and invasive contexts, and considered the conservation implications for species under invasion globally. Fremont’s squirrels (*Tamiasciurus fremonti*) are larder-hoarding rodents that defend a single food cache or midden (Steele 1998), and variation in territorial behavior is related to intruder pressure (Shonfield et al. 2012) and resource availability (Smith 1968). Throughout summer and fall, Fremont’s squirrels (hereafter, red squirrels) harvest, store, and purposefully rearrange conifer cones and fungi at middens, which they aggressively defend through energetic territorial vocalizations and uninhibited repelling of intruders (Smith 1968). At its southern extent, red squirrel range overlaps with the northern distribution of Abert’s squirrels (*Sciurus aberti*) (Fig. C1), a scatter-hoarding tree squirrel associated with ponderosa pine (*Pinus ponderosa*) forest (Nash and Seaman 1977). Resource

competition occurs in sympatry, but red squirrels tend to repel Abert's squirrels from their middens (Hall 1981; Hutton et al. 2003).

A critically endangered red squirrel subspecies, the Mount Graham red squirrel (MGRS; *T. f. grahamensis*), occurs in a single population in the Pinaleño Mountains, Arizona, USA, at the southern periphery of the species' range. The MGRS is classified as endangered in part from concern over the potential for competitive exclusion by an introduced population of Abert's squirrels (U.S. Fish and Wildlife Service 1993). Although native to Arizona, Abert's squirrels were introduced to the Pinaleño Mountains in the 1940s (Davis and Brown 1988) and now occur throughout MGRS' range (Hutton et al. 2003). In addition to spatial overlap, both species primarily consume conifer seeds, and epigeous and hypogeous fungi (Nash and Seaman 1977; Steele 1998). Abert's squirrels may forage at red squirrel middens wherever the species co-occur, and the cost of excessive or minimal territory defense against the invasive species may have negative implications for the MGRS' survival. The occurrence of red squirrels and Abert's squirrels as natural and anthropogenic competitors in isolated mountain ranges, therefore, constitutes an useful system to test hypotheses on territorial behavior and conservation of endangered species.

In a separate study, data from remote cameras at middens and nearby random points showed Abert's squirrels were common within 50 m of MGRS middens but rare within 10 m, whereas at a site of natural syntopy, Abert's squirrels were relatively common within 10 m of middens (Derbridge and Koprowski, unpublished results). This suggests that MGRS are more active defenders of their middens than other red squirrels, potentially expending more energy to keep Abert's squirrels >10 m from middens. Such

costs are impacts of interference competition because they are incurred through direct interactions over scarce resources (Maurer 1984). Quantifying MGRS responses at the moment of intrusion could reveal evidence for invasion-related costs incurred when Abert's squirrels are repelled. Alternatively, observations could reveal that MGRS fail to respond to the potential threat of resource competition with Abert's squirrels. Reduced territory defense by MGRS compared to other red squirrels may make middens more vulnerable to pilferage (Smith 1968), leading to negative effects on individual fitness through exploitation competition (Petren and Case 1996).

To test the hypothesis that territorial behavior could increase MGRS vulnerability to interference or exploitation competition with introduced Abert's squirrels, we conducted a simulated intrusion experiment on MGRS and two red squirrel populations in Arizona that were naturally syntopic with Abert's squirrels. The interference competition hypothesis predicted greater territorial aggression during direct interactions between MGRS and introduced Abert's squirrels compared to interactions between naturally syntopic red squirrels and Abert's squirrels. The exploitation competition hypothesis predicted relatively reduced territorial aggression in MGRS, from which we would infer greater resource opportunity for introduced Abert's squirrels.

2. Methods

2.1. Study area and species

Approximately 300 MGRS (Arizona Game and Fish Department, 2015) inhabited Mount Graham (MG; 3267 m), Graham County, Arizona. This single population occurred in stands of Englemann spruce (*Picea engelmanni*) and corkbark fir (*Abies lasiocarpa* var. *arizonica*) at >3000 m elevation, and mixed conifer stands dominated by Douglas-fir

(*Pseudostuga menziesii*) and southwestern white pine (*Pinus strobiformis*) at 2800-3000 m elevation (Hutton et al. 2003). Our 100-ha MG study site was in the lower mixed conifer area where introduced Abert's squirrels were common (Fig. C1). Two further 100-ha study areas were in the San Francisco Peaks (SF), Coconino County, and the White Mountains (WM), Greenlee County, Arizona. The SF site was at 2500 m elevation, and was dominated by Douglas-fir and ponderosa pine. Our WM site was at 2800 m elevation, and was dominated by Douglas-fir and corkbark fir. We commonly observed Abert's squirrels and their sign (e.g., feeding remains and nests) at the SF site. We used Abert's squirrel sign and remote camera evidence from a separate study (Derbridge and Koprowski, unpublished results) to confirm presence at the WM site.

2.2. Apparatus

We constructed a vehicular squirrel model with a mounted Abert's squirrel arranged in an upright alert posture (hereafter, robot). We attached the robot to a Vaterra Slickrock 1/18th Rock Crawler (Model: VTR01003) radio-controlled vehicle (RCV). We attached a Memtek[®] I-Venstar Bluetooth[®] waterproof portable speaker (Model: CI092) to the hood of the RCV (Fig. C2). We used an Apple iPod Touch[®] (Model: MC540LL/A) and the Bluetooth[®] speaker to play an Abert's squirrel alarm vocalization, which we previously recorded with a Sony[®] Linear PCM Recorder (Model: PCM-D50) from an adult Abert's squirrel 10 m up in a southwestern white pine \approx 1 km northeast of the MG study site.

2.3. Data collection

We collected all data between Sep-Dec 2015. At the MG site, the endangered red squirrel population has been intensively monitored for >15 years by the University of Arizona Mount Graham Red Squirrel Monitoring Program and most territory-owning

adults were already marked with colored ear-tags for identification (Goldstein et al. 2017). At MG, we randomly selected 5 middens from 22 currently occupied by marked adult MGRS. At SF and WM, we located ≥ 10 active middens and randomly selected 5 middens ≥ 50 m from each other. In all cases, we verified middens were occupied by checking that middens contained > 2 m diameter of loosely piled conifer cone scales, and abundant feeding sign, and by observing the territory owner engaged in typical territorial behaviors, such as barking. Although we did not mark individuals at SF and WM sites, > 1 squirrel were never observed at a midden, and no SF or WM middens were abandoned during data collection.

At each site, we conducted three trials for the following tests at each midden ($n = 9$ trials per midden): 1) control, where only the RCV was used, 2) Test 1 (robot alone) where the RCV and Abert's squirrel mount were used, and 3) Test 2 (robot plus audio), where the RCV and Abert's squirrel mount were used, and our pre-recorded Abert's squirrel vocalization was played through the speaker. To minimize any habituation effect on tests, we conducted trials at least 5 days apart. We randomized the order of test across trials for focal squirrels.

Throughout the experiment, the same 2 observers (ADB and JJD) quietly approached to ≈ 10 m from a midden, and waited until the territory owner appeared to have clear sight of the stimulus. Before each trial, we placed forest duff over the vehicle to further camouflage the non-squirrel components of our robot. Observer 1 used the remote controller to drive the stimulus as directly as possible to the midden while observer 2 observed behavior of the focal animal and used a Sony® Linear PCM Recorder (Model: PCM-D50) to record descriptions of all behaviors. On reaching the

midden, observer 1 tapped the RCV controller for a few seconds at 30-second intervals to cause slight on-the-spot movements intended to mimic animal movement from the robot. In Test 2 (robot plus audio) we played a 30-second loop of our pre-recorded Abert's squirrel vocalizations through the speaker. Each round lasted for 3 minutes.

We recorded behaviors in binomial (yes/no) form during 20-second intervals. We recorded 3 response behaviors of red squirrels to intruders. The proximity response measured the closest distance (m) that the focal red squirrel approached to the stimulus. The travel response measured the difference between a focal red squirrel's distance from the stimulus at the start and the end of a trial. Finally, we measured whether and for how long a focal red squirrel vocalized during a trial, recording rattles, a strictly territorial vocalization, and barks, typically considered a response to predator presence (Smith 1978). These response measurements allowed us to examine not only how willing they were to approach an intruder (likely correlated with perceived urgency of threat), but also to estimate the amount of relative energy they spent moving around to track the intruder, and as time spent emitting energetically expensive high amplitude vocalizations.

2.4. Statistical analysis

As our interest was in the between-group rather than within-group differences, we used generalized estimating equations (GEE) to estimate the effects of our simulated intrusions on the 3 response variables: proximity, travel, and vocalization. We adjusted the 'sandwich' variance to control type I error rate due to small sample size (Wang and Long 2011). This adjustment has been shown to achieve robust results when all individuals have completed all tests, as in our case. We used the "geepack" package in R 3.3 (R Core Team 2016) and started with a full model, including site, test, and all environmental

covariates (i.e., temperature, cloud cover, wind, and time of day), and interactions. We used a backwards elimination process to remove non-significant covariates one at a time until models contained only significant variables. All results reported here are 2-tailed and significance level was set as $p < 0.05$.

3. Results

3.1. Field data

Mean temperatures during data collection were 13.0°C (SD = 2.3), 6.4°C (SD = 4.3), and 13.2°C (SD = 2.4) at MG, SF, and WM sites, respectively. Of 45 repeats, red squirrels failed to respond once at a MG site, and during 4 and 2 trials at SF and WM sites, respectively. Mean proximity to the stimulus across all tests was 6.5 m (SD = 5.7), 12.4 m (SD = 5.2), and 10.9 m (SD = 3.1) at MG, SF, and WM sites, respectively. Mean travel during trials across all tests was 4.5 m (SD = 5.0), 4.0 m (SD = 3.3), and 2.0 m (SD = 4.5) at MG, SF, and WM sites, respectively (Table C1). Red squirrels barked and rattled in response to our simulated intrusions at all sites (Table C2).

3.2. Predictors for territorial behavior responses

Rattle data were insufficient for our GEE analysis so only bark data were used to model vocal response. No environmental variables or interaction terms were significant predictors. In our final models, site was a significant predictor of proximity, travel, and vocal response. Red squirrels at the MG site approached much closer to our model stimuli compared to red squirrels at SF ($p < 0.001$) and WM ($p < 0.001$) across all tests (Fig. C3a). For the travel response, MG moved significantly more compared to WM red squirrels ($p = 0.021$; Fig 3b) and red squirrels moved more during Test 2 (robot plus audio) across sites ($p = 0.023$). For our vocal response tests, site was a significant

predictor of red squirrel barks, more barking by MGRS than SF ($p = 0.01$) and WM ($p = 0.03$) red squirrels (Fig. C4). Vocal response did not show a test response (Table C3).

4. Discussion

We examined the behavioral responses of a territorial species to an ecologically similar intruder in native and invasive contexts. We used a simulated intrusion experiment to test hypotheses that territorial behavior could increase the vulnerability of MGRS to interference or exploitation competition with introduced Abert's squirrels. The interference competition hypothesis was supported by greater vocal and territorial movement responses by Mount Graham red squirrels compared to other red squirrels. The greater movement measured during our second test (robot plus audio) suggested the addition of a pre-recorded Abert's squirrel vocalization represented a convincing threat to territorial red squirrels. In general, red squirrel responses did not differ between our experimental tests and the control. However, the indiscriminate nature of MGRS' proximity and bark responses suggest any intrusion would likely stimulate energetically expensive territorial behavior.

When native species compete with ecologically similar invaders, trade-offs for territoriality may be altered, leading to potentially unsustainable behavioral changes, as seen in northern spotted owls, whose avoidance of invasive barred owls forces them to maintain inefficient territory sizes (Wiens et al. 2014). Regardless of stimulus, MGRS showed higher levels of territorial aggression in response to simulated intrusions than red squirrels at WM and SF sites. Increased aggression may help resident species persist through invasion, and idiosyncratic territorial behavior could be a successful strategy in repelling ecologically similar invaders. In a separate study, cameras commonly recorded

Abert's squirrels at 50 m from, but not within 10 m of MGRS middens, which suggests the relatively intense territorial behavior of MGRS effectively prevented loss of stored food via pilferage (Derbridge and Koprowski, unpublished results). Despite possible advantages of intense territoriality, native species must still incur the costs of interference competition that may eventually lead to reductions in reproductive fitness and population health (Hixon 1980).

Natural selection may favor behaviors that promote economic defendability of limited resources. However, such strategies may incur fitness costs if inefficient in dealing with sudden competition from introduced species, particularly where native species have evolved in isolation (Blackburn et al. 2004). Compared to other red squirrel populations, MGRS home ranges are 3-10 times larger (Koprowski et al. 2008), and the cost of defense in time and energy must also be greater on average (Smith 1968). Although we did not directly measure energy expenditure, theory predicts vigilance and defense against intruders is energetically taxing and impedes foraging time (Schoener 1983). Reduced foraging time could result in energy deficits and lower reproductive output (Hixon 1980), and low survivorship is expected for red squirrels whose middens are not well stocked during the previous summer and fall (Gurnell 1984). Vigorous territoriality not only induces excessive energy expense but also attracts avian predators, which are the major cause of mortality for MGRS (Schauffert et al. 2002). Thus, MGRS territorial behavior may contribute to its unusually low survivorship (Goldstein et al. 2017) by wasting energy, reducing foraging time, and elevating predation risk. While aggressive territorial behavior may be effective at repelling Abert's squirrels, hyper-vigilance and elevated aggression may contribute to long-term MGRS decline.

Variation in resource defense behaviors from core populations is further evidence that peripheral populations face distinct conservation challenges. Assumptions that management strategies can be generalized from core to peripheral populations could be wrong (Hampe and Petit 2005), and our study adds behavioral evidence to recent demographic evidence (Goldstein et al. 2017) that conservation of endangered isolated species requires population-specific planning. One option for long-term conservation of isolated populations is assisted migration, which involves translocating individuals of threatened populations to new areas of habitat. The potential risks of this conservation strategy have been much debated (Hewitt et al. 2011; Ricciardi and Simberloff 2009) and our results caution against assuming peripheral populations are behaviorally identical to other populations or closely related species.

As human activities cause species ranges to contract and become increasingly fragmented, altered habitat can provide resource opportunities for introduced species (Shea and Chesson 2002), which are often ecological generalists, and territorial species may decline where heterogenous environments limit the trade-offs for ecological specialists (Futuyma and Moreno 1988). Habitat management may be an effective way to restore trade-offs for specialist species. For example, the protection of old growth forests in northwestern USA is the primary conservation tool for northern spotted owls where they are outcompeted by the invasive and more generalist barred owl (Wiens et al. 2014). Once considered obligates of ponderosa pine forest (Keith 1965), evidence now suggests Abert's squirrels are generalist invaders; numerous introduced populations have been established (Davis and Brown 1988), they have colonized new mountain ranges unaided (Davis and Brown 1989), and they occur in mixed-conifer forest where

ponderosa pine is uncommon (Hutton et al. 2003). Therefore, management for homogenous spruce-fir stands may be a necessary long-term strategy for maintaining economically defensible MGRS habitat (Koprowski et al. 2005).

Global climate change projections mean widespread changes to habitat of forest obligate species are inevitable (Allen et al. 2010). Temperatures in the southwestern US will warm 2-4°C by 2050 (Kunkel et al. 2013), and coniferous forest cover is predicted to decline as greater frequency of severe droughts leads to slower growth and higher mortality of trees, bark beetle outbreaks, and wildfires (Williams et al. 2010). Isolated peripheral populations like the MGRS face extirpation in such scenarios. By contrast, introduced Abert's squirrels are at the center of the species' geographic range, and increasingly mild winters will likely eliminate the challenges faced in finding food during prolonged periods of deep snow (Stephenson and Brown 1980). If scatter-hoarding becomes a permanently viable option, the adaptive advantages of larder-hoarding may be lost, and Abert's squirrels could ultimately replace MGRS. Conservation of peripheral populations is considered a priority as they often possess unique genetic and demographic characteristics (Lesica and Allendorf 1995; Vucetich and Waite 2003); our study suggests unique behavior is also an important consideration for conservation of such populations under invasion.

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References

- Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259, 660-684.
- Andersson, M., Krebs, J., 1978. On the evolution of hoarding behaviour. *Animal Behaviour* 26, 707-711.
- Blackburn, T.M., Cassey, P., Duncan, R.P., Evans, K.L., Gaston, K.J., 2004. Avian extinction and mammalian introductions on oceanic islands. *Science* 305, 1955-1958.
- Brown, J.L., 1964. Evolution of diversity in avian territorial systems. *The Wilson Bulletin* 76, 160-169.
- Clarke, M.F., Kramer, D.L., 1994. Scatter-hoarding by a larder-hoarding rodent: intraspecific variation in the hoarding behaviour of the eastern chipmunk, *Tamias striatus*. *Animal Behaviour* 48, 299-308.

- Dally, J.M., Clayton, N.S., Emery, N.J., 2006. The behaviour and evolution of cache protection and pilferage. *Animal Behaviour* 72, 13-23.
- Davis, R., Brown, D.E., 1988. Documentation of the transplanting of Abert's squirrels. *The Southwestern Naturalist* 33, 490-492.
- Davis, R., Brown, D.E., 1989. Role of post-pleistocene dispersal in determining the modern distribution of Abert's squirrel. *The Great Basin Naturalist* 49, 425-434.
- Fretwell, S.D., Lucas, H.L., 1969. On territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* 19, 16-36.
- Futuyma, D.J., Moreno, G., 1988. The evolution of ecological specialization. *Annual Review of Ecology and Systematics* 19, 207-233.
- Goldstein, E.A., Merrick, M.J., Koprowski, J.L., 2017. Functional semelparity drives population dynamics and endangers a peripheral population. *Biological Conservation* 205, 52-59.
- Gurnell, J., 1984. Home range, territoriality, caching behaviour and food supply of the red squirrel (*Tamiasciurus hudsonicus fremonti*) in a subalpine lodgepole pine forest. *Animal Behaviour* 32, 1119-1131.
- Hall, J.G., 1981. A field study of the Kaibab squirrel in Grand Canyon National Park. *Wildlife Monographs* 75, 3-54.
- Hampe, A., Petit, R.J., 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* 8, 461-467.
- Harrington, L.A., Harrington, A.L., Yamaguchi, N., Thom, M.D., Ferreras, P., Windham, T.R., MacDonald, D.W., 2009. The impact of native competitors on an alien invasive: temporal niche shifts to avoid interspecific aggression. *Ecology* 90, 1207-1216.

Hewitt, N., Klenk, N., Smith, A.L., Bazely, D.R., Yan, N., Wood, S., MacLellan, J.I., Lipsig-Mumme, C., Henriques, I., 2011. Taking stock of the assisted migration debate. *Biological Conservation* 144, 2560-2572.

Hixon, M.A., 1980. Food production and competitor density as the determinants of feeding territory size. *The American Naturalist* 115, 510-530.

Hutton, K.A., Koprowski, J.L., Greer, V.L., Alanen, M.I., Schauffert, C.A., Young, P.J., Jones, C.A., 2003. Use of mixed-conifer and spruce-fir forests by an introduced population of Abert's squirrels (*Sciurus aberti*). *The Southwestern Naturalist* 48, 257-260.

Keith, J.O., 1965. The Abert squirrel and its dependence on ponderosa pine. *Ecology* 46, 150-163.

Koprowski, J.L., Alanen, M.I., Lynch, A.M., 2005. Nowhere to run and nowhere to hide: Response of endemic Mt. Graham red squirrels to catastrophic forest damage. *Biological Conservation* 126, 491-498.

Koprowski, J.L., King, S.R.B., Merrick, M.J., 2008. Expanded home ranges in a peripheral population: space use by endangered Mt. Graham red squirrels. *Endangered Species Research* 4, 227-232.

Kunkel, K.E., Stevens, L.E., Stevens, S.E., Janssen, E., Sun, L., Wuebbles, D., Redmond, K.T., Dobson, J.G., 2013. Regional climate trends and scenarios for the U.S. National climate assessment: Part 5. Climate of the southwest U.S. National Oceanic and Atmospheric Administration, Washington, D.C.

Lesica, P., Allendorf, F.W., 1995. When are peripheral populations valuable for conservation? *Conservation Biology* 9, 753-760.

- Maurer, B.A., 1984. Interference and exploitation in bird communities. *The Wilson Bulletin* 96, 380-395.
- Minckley, W., 1968. Possible extirpation of the spruce squirrel from the Pinaleno (Graham) Mountains, south-central Arizona. *Journal of the Arizona Academy of Science* 5, 110-110.
- Nash, D.J., Seaman, R.N., 1977. *Sciurus aberti*. *Mammalian Species* 80, 1-5.
- Petren, K., Case, T.J., 1996. An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology* 77, 118-132.
- Ricciardi, A., Simberloff, D., 2009. Assisted colonization is not a viable conservation strategy. *Trends in Ecology & Evolution* 24, 248-253.
- Schauffert, C.A., Koprowski, J.L., Greer, V.L., Alanen, M.I., Hutton, K.A., Young, P.J., 2002. Interactions between predators and Mt. Graham red squirrels (*Tamiasciurus hudsonicus grahamensis*). *The Southwestern Naturalist* 47, 498-501.
- Schoener, T.W., 1983. Simple models of optimal feeding-territory size: A reconciliation. *The American Naturalist* 121, 608-629.
- Shea, K., Chesson, P., 2002. Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17, 170-176.
- Shonfield, J., Humphries, M.M., Boutin, S., McAdam, A.G., Taylor, R.W., 2012. Territorial defence behaviour in red squirrels is influenced by local density. *Behaviour* 149, 369-390.
- Smith, C., Reichman, O., 1984. The evolution of food caching by birds and mammals. *Annual Review of Ecology and Systematics* 15, 329-351.

- Smith, C.C., 1968. The adaptive nature of social organization in the genus of three squirrels *Tamiasciurus*. *Ecological Monographs* 38, 31-64.
- Smith, C.C., 1978. Structure and function of the vocalizations of tree squirrels (*Tamiasciurus*). *Journal of Mammalogy* 59, 793-808.
- Steele, M.A., 1998. *Tamiasciurus hudsonicus*. *Mammalian Species* 586, 1-9.
- Stephenson, R.L., Brown, D.E., 1980. Snow cover as a factor influencing mortality of Abert's squirrels. *The Journal of Wildlife Management* 44, 951-955.
- Taylor, E., Stamford, M., Baxter, J., 2003. Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. *Molecular Ecology* 12, 2609-2622.
- U.S. Fish and Wildlife Service, 1993. Mount Graham red squirrel (*Tamiasciurus hudsonicus grahamensis*) recovery plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico, USA.
- Vander Wall, S.B., 1990. Food hoarding in animals. University of Chicago Press, Chicago.
- Vucetich, J.A., Waite, T.A., 2003. Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics* 4, 639-645.
- Wang, M., Long, Q., 2011. Modified robust variance estimator for generalized estimating equations with improved small-sample performance. *Statistics in Medicine* 30, 1278-1291.

Wiens, J.D., Anthony, R.G., Forsman, E.D., 2014. Competitive interactions and resource partitioning between northern spotted owls and barred owls in western Oregon. *Wildlife Monographs* 185, 1-50.

Williams, A.P., Allen, C.D., Millar, C.I., Swetnam, T.W., Michaelsen, J., Still, C.J., Leavitt, S.W., 2010. Forest responses to increasing aridity and warmth in the southwestern United States. *Proceedings of the National Academy of Sciences* 107, 21289-21294.

Wolff, J.O., Freeberg, M.H., Dueser, R.D., 1983. Interspecific territoriality in two sympatric species of *Peromyscus* (Rodentia: Cricetidae). *Behavioral Ecology and Sociobiology* 12, 237-242.

Figure Legends

Figure C1. The southern portion of red squirrel (*Tamiasciurus fremonti*) range where it overlaps with disjunct northern native range of Abert's squirrels (*Sciurus aberti*). We collected data on territorial responses of red squirrels to simulated Abert's squirrel intrusions at three study sites, Mount Graham, San Francisco Peaks, and White Mountains in Arizona, USA. The Mount Graham site was in the Pinaleño Mountains, where Abert's squirrels are an introduced species in competition with the endangered Mount Graham red squirrel (*Tamiasciurus fremonti grahamensis*).

Figure C2. Abert's squirrel mount used in simulated intrusion experiments at red squirrel middens at sites in the Pinaleño Mountains, San Francisco Peaks, and White Mountains, Arizona, USA. The professionally prepared mount was strapped to a Vattera Slickrock 1/18th Rock Crawler (Model: VTR01003) radio-controlled vehicle (RCV), along with a Memteq[®] I-Venstar Bluetooth[®] waterproof portable speaker (Model: CI092), which was used to play pre-recorded Abert's squirrel alarm vocalizations.

Figure C3. Box and whisker plots of data for (a) the shortest distance between territorial red squirrels and a robot stimulus after the robot entered the midden area at the center of a territory, and (b) the total distance moved by a territorial red squirrel during a three-minute trial. We collected data during simulated territorial intrusions at five middens during three repeats of three tests at sites on Mount Graham (MG), San Francisco Peaks (SF), and White Mountains (WM), Arizona, USA. Bars and asterisks indicate significant differences ($\alpha = 0.05$), NS indicates no significant difference in effect of study site from generalized estimating equations models using these data.

Figure C4. Mean proportion of trials across three repeats of three tests during which red squirrels barked in response to a simulated Abert's squirrel territorial intrusion. Endangered Mount Graham red squirrels occur with a population of introduced Abert's squirrels at the Mount Graham (MG) site. Red squirrels and Abert's squirrels co-occur naturally at sites in the San Francisco Peaks (SF), and White Mountains (WM), Arizona, USA. Bars and asterisks indicate significant differences ($\alpha = 0.05$) in effect of study site for each test from generalized estimating equations models using these data.

Table C1. Mean responses to three experimental tests used to test behavior of red squirrels during simulated territorial intrusions by Abert’s squirrels on Mount Graham (MG), San Francisco Peaks (SF), and White Mountains (WM). Responses are mean values from three repeats of each test at five red squirrel territories within each site. “Proximity” was the shortest distance (m) between the stimulus and a red squirrel during a test. “Travel” was the distance (m) between the location in which a red squirrel was alert to the stimulus and the closest point to the stimulus during a test.

Test	Response	MG		SF		WM	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
Control	Proximity	6.9	6.5	12.7	5.5	10.1	3.1
	Travel	4.1	2.9	1.9	2.4	1.5	3.6
Test 1	Proximity	6.2	5.0	12.1	5.3	11.7	2.4
	Travel	3.3	6.9	4.3	3.8	3.9	5.5
Test 2	Proximity	6.5	5.7	12.2	5.2	11.0	3.6
	Travel	5.9	4.4	5.9	2.0	0.8	3.8

Table C2. Proportion of all trials during which focal red squirrels responded, and mean and standard deviation of duration (seconds) of vocal responses (barks and rattles) during three experimental tests used to test their behavior during simulated territorial intrusions by Abert's squirrels on Mount Graham (MG), San Francisco Peaks (SF), and White Mountains (WM), Arizona, USA.

		Vocal response	Vocal duration (s)	
		Prop. trials	\bar{x}	SD
MG	Bark	0.31	22.09	41.68
	Rattle	0.11	0.40	1.23
SF	Bark	0.07	2.02	9.37
	Rattle	0.04	0.22	1.06
WM	Bark	0.07	1.33	5.05
	Rattle	0.20	0.73	1.76

Table C3. Results from Generalized Estimating Equations (GEE) models on the responses of territorial red squirrels to simulated intrusions at five middens at three sites in Arizona, USA. Models examined the additive effects of site and test on three response variables. Site was a significant predictor of each response. For the “Proximity” response, MG differed from the other sites; SF and WM squirrels stayed further from the simulated intrusion across tests. For the “Travel” response, MG squirrels moved more than WM squirrels across all tests, and squirrels moved more across sites during Test 2 compared to the control. For the “Bark” response, MG squirrels barked significantly more on average than squirrels at SF and WM, but no test effect existed.

Response	Predictor	Estimate	SE	Chi-sq	<i>P</i> -value
Proximity	Intercept	6.459	1.067	36.657	<0.001
	SF	5.814	1.141	25.973	<0.001
	WM	4.369	1.023	18.232	<0.001
	Test 1	0.185	0.933	0.039	0.843
	Test 2	0.064	0.964	0.004	0.947
Travel	Intercept	3.478	0.697	24.916	<0.001
	SF	-0.472	0.856	0.304	0.581
	WM	-2.406	1.043	5.317	0.021
	Test 1	1.344	0.889	2.284	0.131
	Test 2	1.572	0.693	5.143	0.023
Bark	Intercept	-0.90	0.48	3.49	0.06
	SF	-1.84	0.68	7.29	0.01
	WM	-2.24	1.06	4.49	0.03

Test 1	0.00	0.72	0.00	1.00
Test 2	0.27	0.55	0.23	0.63

Figure C1.

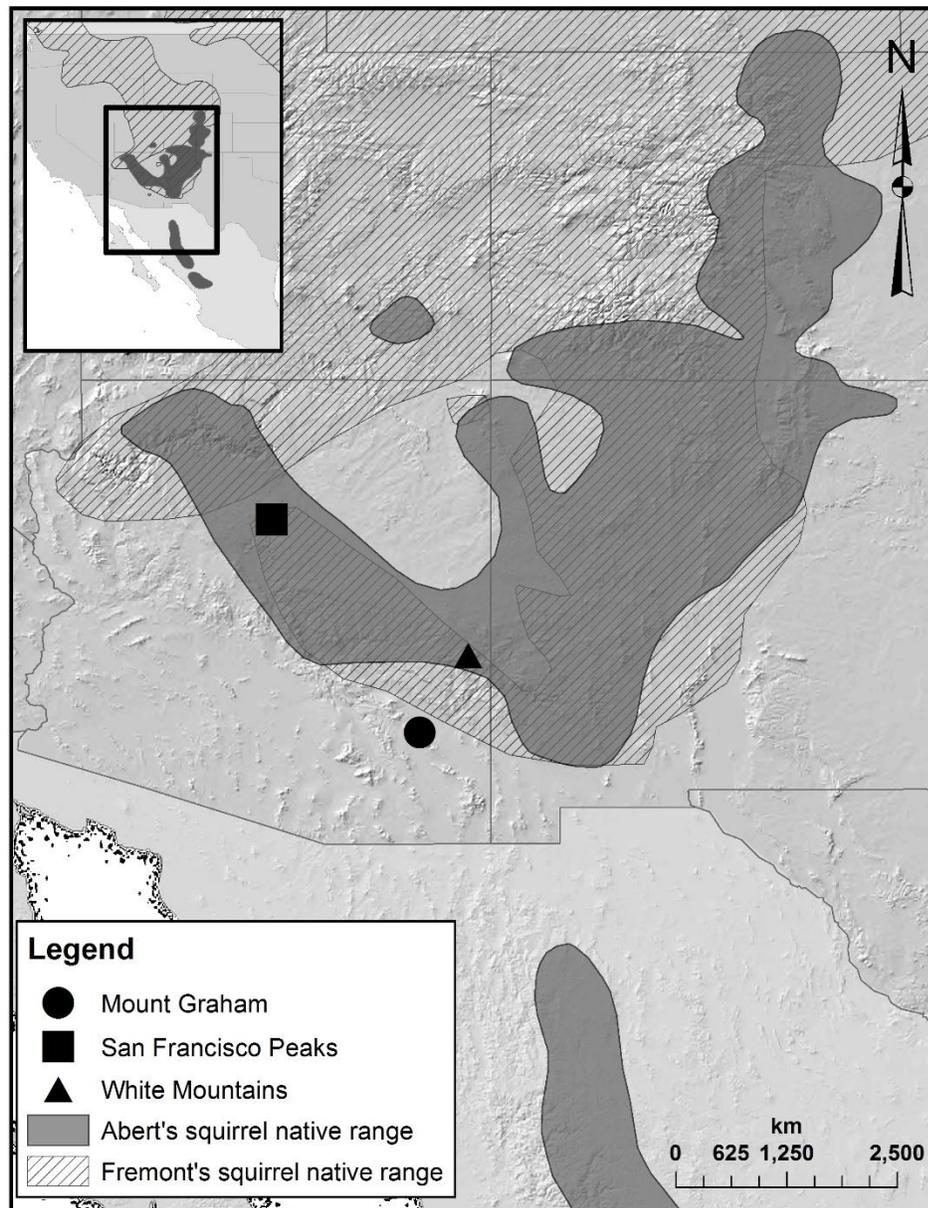
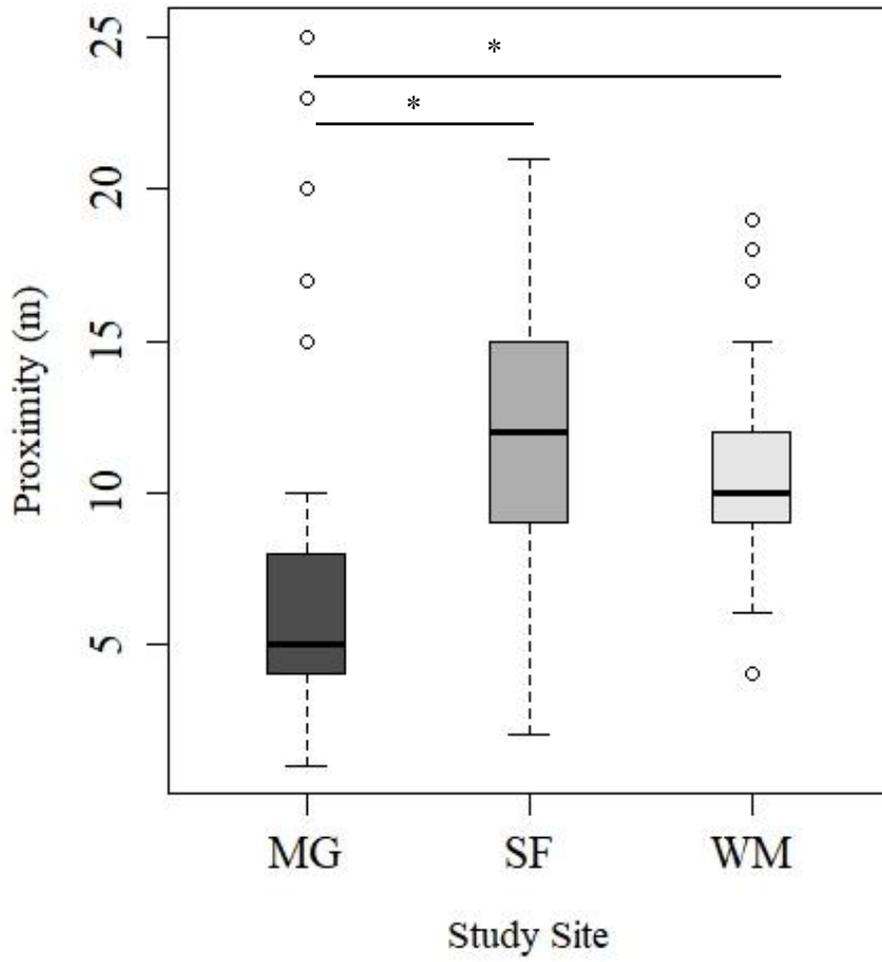


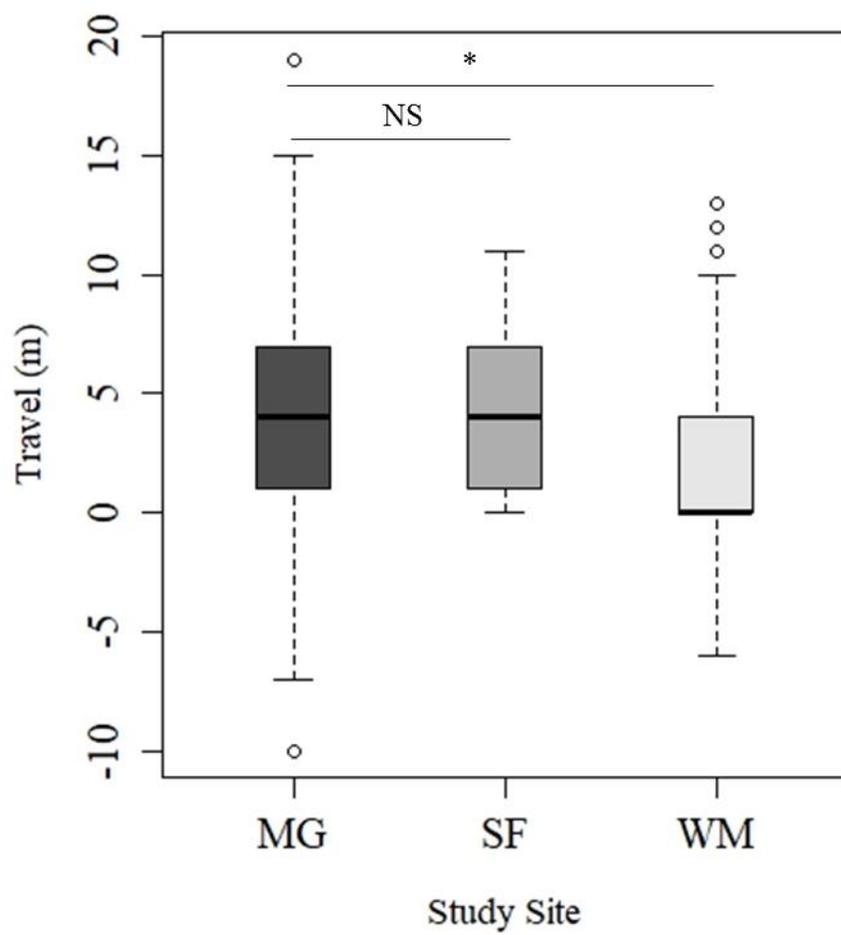
Figure C2.



Figure C3.



a)



b)

Figure C4.

