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Conservation and Management

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CHAPTER TWENTY-ONE

Effects of Climate Change on Nutrition and Genetics of White-tailed Ptarmigan

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Abstract. White-tailed Ptarmigan (*Lagopus leucura*) are well suited as a focal species for the study of climate change because they are adapted to cool, alpine environments that are expected to undergo unusually rapid climate change. We compared samples collected in the late 1930s, the late 1960s, and the late 2000s using molecular genetic and stable isotope methods in an effort to determine whether White-tailed Ptarmigan on Mt. Evans, Colorado, have experienced recent environmental changes resulting in shifts in genetic diversity, gene frequency, and nutritional ecology. We genotyped 115 individuals spanning the three time periods, using nine polymorphic microsatellite loci in our genetic analysis. These samples were also analyzed for stable carbon and nitrogen isotopic composition. We found a slight trend of lower heterozygosity through time, and allelic richness values were lower in more recent times, but not significantly using an alpha of 0.05 ($P < 0.1$). We found no changes in allele frequencies across time periods, suggesting that population sizes have not changed dramatically. Feather $\delta^{13}C$ and $\delta^{15}N$ values decreased significantly across time periods, whereas the range in isotope values increased consistently from the late 1930s to the later time periods. Inferred changes in the nutritional ecology of White-tailed Ptarmigan on Mt. Evans relate primarily to increased atmospheric deposition of nutrients that likely influenced foraging habits and tundra plant composition and nutritional quality. Future work seeks to integrate genetic and isotopic data with long-term demographics to develop a detailed understanding of the interaction among environmental stressors on the long-term viability of ptarmigan populations.

Key Words: climate change, genetics, *Lagopus leucura*, stable isotopes, temporal variation.

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All major ecosystems are predicted to experience alterations resulting from climate change (Intergovernmental Panel on Climate Change 2007). Alpine ecosystems may be particularly susceptible to warming because their existence is partially affected by low temperature conditions, which are expected to rise (Armstrong and Halfpenny 2001). Projected changes in alpine systems will likely be detrimental to vertebrate species inhabiting these ecosystems since alpine habitats are analogous to islands, separated by expanses of low-lying, warmer habitats. Alpine vertebrates are highly specialized in their habitat requirements (Armstrong and Halfpenny 2001); as treeline is expected to advance in elevation and plants from lower altitudes invade, concomitant changes to alpine environments may influence the viability of endemic species (Price 1997).

Changes in climate over the past century have been shown to influence many aspects of avian population biology (Crick 2004, Wormworth and Mallon 2006). Advancing trends in breeding phenology (e.g., egg-laying and hatching dates) are the most widely observed response to increasing spring temperatures (Crick 2004, Lyon et al. 2008); however, few studies have investigated how climate-induced responses in avian breeding phenology have impacted species at the population level. This is due in part to a general paucity of long-term data sets that include demographic information in addition to data on nesting activities. Ecosystem changes mediated through anthropogenic impacts or climate change have the potential to directly affect demographic parameters, but studies are needed that address other potentially relevant responses, such as genetic and nutritional responses, to gain a more comprehensive understanding of alterations at the population and individual levels.

The White-tailed Ptarmigan (*Lagopus leucura*) is endemic to alpine habitats at or above timberline throughout the mountain west of the U.S. and Canada (Braun et al. 1993). Unlike other avian species breeding in alpine habitats, White-tailed Ptarmigan remain at high altitudes throughout the year and, with few exceptions, spend almost their entire life history above treeline (Hoffman 2006). The species is well adapted to harsh environments found in the alpine and has developed several behavioral and physiological traits that allow it to survive under such conditions. In cold weather, for example, ptarmigan choose microclimates that are sometimes 7°C above ambient temperatures, and they have a tendency to walk rather than fly, both traits that help ptarmigan maximize metabolic efficiency (Braun et al. 1993, Martin et al. 1993). The thermoneutral zone of this species is broad, allowing it to survive in a wide range of temperatures without having to expend excess energy (Johnson 1968). In Colorado, the primary food of this species throughout the non-breeding season (September through April) includes the buds, twigs, and leaves of *Salix* species, with forbs and berries becoming highly important during the breeding season (May through August) (May and Braun 1972). Insects are vital components of chick diets the first three weeks after hatching (May 1975).

White-tailed Ptarmigan on Mt. Evans, Colorado, have been studied annually since 1966 (major findings reviewed by Hoffman 2006), representing the longest-running data set known for the species. The overall focus of this research was to investigate demographic patterns and included annual observations of individually marked birds, allowing estimation of survival and breeding success, various morphometric measurements (i.e., mass, primary feather length, tarsus length, etc.), and locations and occupancy of territories. Such long-term data sets are extremely valuable for investigating a species' response to environmental change. While the alpine ecosystem may be less likely to be directly impacted by human disturbance, it is thought to be particularly vulnerable to environmental change due to climate warming (Price 1997, Baron et al. 2000a) and atmospheric deposition of airborne pollutants (Baron et al. 2000b).

Investigation into the interaction between ecological and evolutionary responses to environmental change is an important aspect of ecosystem-based studies. Such responses can be examined using molecular genetic and stable isotope techniques (Kelly 2000, Kelly et al. 2005, Reusch and Wood 2007, Valenzuela et al. 2009). Documenting shifts in genetic allele frequencies and changes in levels of genetic diversity can provide clues as to past demographic patterns, movement among populations, and the ability of a species to adapt (Avise 1994). Similarly, stable isotope analysis of consumer tissues can document shifts in foraging ecology, which may be associated with changes in landcover or landscape biogeochemistry (Hilton et al. 2006, Inger and Bearhop 2008). We conducted a temporal comparison spanning the last 70 years to begin to investigate whether White-tailed

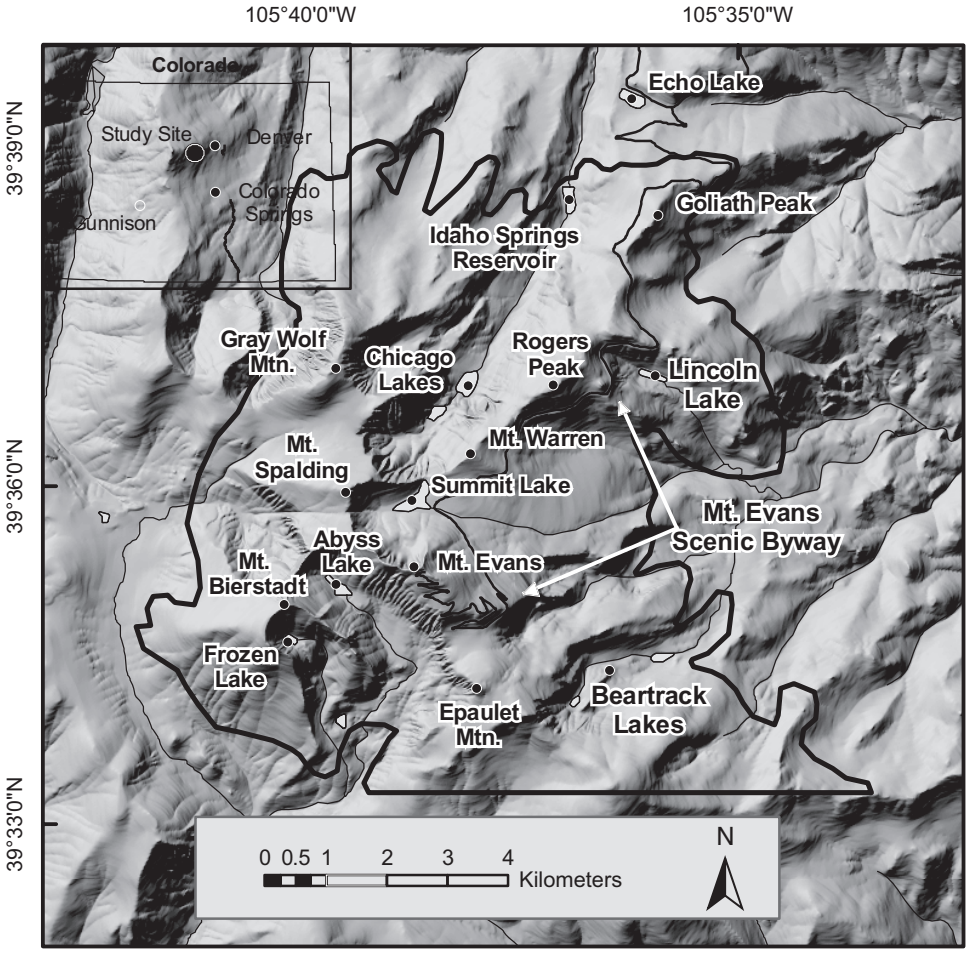

Figure 21.1. Study area for White-tailed Ptarmigan at Mt. Evans, Colorado.

Ptarmigan on Mt. Evans have experienced environmental changes resulting in shifts in genetic diversity, gene frequency, and nutritional ecology. This study represents a preliminary examination into whether selected responses of ptarmigan to environmental change could be detected. Our specific objectives were to (1) assess whether microsatellite allele frequencies have changed, (2) document changes in genetic diversity, and (3) detail potential shifts in the nutritional ecology of White-tailed Ptarmigan on Mt. Evans.

METHODS

Study Site

A population of White-tailed Ptarmigan was studied in the Mt. Evans Wilderness Area from 1966 to 2008 (herein Mt. Evans, 39°35′ N, 105°38′ W; Fig. 21.1). The study area is ~16 km southwest of Idaho Springs, Colorado, and ranges in elevation from 3,500 to 4,350 m at the summit, covering 13.2 km^2 of alpine habitat. Vegetation is typical of alpine habitat in the southern Rocky Mountains and includes communities of cushion plants, *Kobresia* sedge meadows, and sedge–grass wet meadow and willow–sedge hummock stands (Braun and Rogers 1971). Ptarmigan are typically found in *Trifolium* cushion fellfields, *Carex* meadows, *Carex–Geum* rock meadows, and near receding snowfields in mid- to late summer (Braun et al. 1993). Maximum daily air temperature measured at the nearby Niwot Ridge Long-Term Ecological Research site (50 km north of Mt. Evans; 1964–2006) has averaged 12.6°C and –9.4°C for July and January, respectively (http://culter.colorado.edu/NWT/data/datmanaccess.html; accessed 4 October 2007). Daily precipitation for July and January has

averaged 4.6 and 2.4 mm, respectively, with annual precipitation averaging 1,153.4 mm. These data also suggest an increase in the number of days when maximum temperatures exceed 15°C from 1966 to 2008 ($r^2 = 0.19$, $P = 0.003$).

Tissue Collection

Ptarmigan were captured at the Mt. Evans study site from 1966 to 2008 using a noose design modified from Zwickel and Bendell (1967). In a typical year, captures occurred during two intensive field sessions in May–June, and again in August–September. In mid-May, ptarmigan were located by playing tape recordings of male territorial calls (Braun et al. 1973). Males on breeding territories were typically accompanied by hens, both of which were subsequently captured. Ptarmigan were located during the second capture session by playing tape recordings of chick distress calls. Feather samples were collected from birds during the 1966–1968 period (herein the late 1960s). Additionally, blood and feather samples were collected from birds captured in 2007 and 2008 (herein the late 2000s). Blood samples were collected by clipping a toenail and collecting 2–3 drops in a microfuge tube previously coated with EDTA (Brinkmann). Retrix and/or contour feathers were taken from individual birds. Contour feathers were collected from the body of captured birds, primarily on the upper breast, scapulars, and upper tail coverts. During both capture sessions, only contour feathers that were dark, and hence newly grown on breeding areas, were plucked from birds. Ptarmigan begin molting from their all-white basic plumage in April to darker, cryptic nuptial plumages, which persist throughout the summer and early fall (Braun et al. 1993). Hence, all contour feather samples were grown while on breeding areas. Blood samples and feathers were frozen at –20°C.

Additionally, we located nine White-tailed Ptarmigan collected specifically from Mt. Evans in 1937 (herein the late 1930s). These specimens are preserved as study skins at the Denver Museum of Nature and Science. Skin and feather samples from each of the nine specimens were obtained using clean techniques for use in this study.

Molecular Genetics

DNA was extracted from skin and feathers of nine museum specimens collected on Mt. Evans in the late 1930s using the GENECLEAN kit for Ancient DNA (Bio 101) following manufacturer's protocol using buffer dehybridization solution B, and including the proteinase K step. Extractions from museum samples were conducted in an isolated ancient DNA facility to avoid contamination from modern samples. The 42 feather samples collected in the late 1960s were extracted using the Promega Wizard DNA Purification Kit following the manufacturer's instructions (Promega Corporation) with the following modifications: The DNA precipitation step was allowed to incubate for 3 hr on ice with a subsequent 15 min centrifugation step. The DNA was rehydrated in 30 μL of the kit supplied DNA rehydration solution. DNA was extracted from 64 blood samples collected during the late 2000s with the GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences Corp.) using the manufacturer's instructions with modifications following Oyler-McCance et al. (2005).

All 115 White-tailed Ptarmigan samples were screened using nine nuclear microsatellite loci. Primer pairs for three of these loci (LLST1, LLSD4, and LLSD8) were originally designed for Red Grouse, *Lagopus lagopus scoticus* (Piertney and Dallas 1997). The forward primer for microsatellite locus LLSD4 was redesigned to make the PCR product smaller (LLSD4.2F CATGGTTGTCTTATCCTCTGAGAAAACTG). An additional microsatellite locus (SGCA11) was targeted using primers designed for Greater Sage-Grouse (*Centrocercus urophasianus*) by Taylor et al. (2003). Primers for this locus were also redesigned to shorten the locus (SGCA11.2F GAATATCTTTCTTTAACAGAATCC, SGCA11.2R CTACTGTTCTGTTGTGCAAGAC). Five new microsatellite loci that were isolated from Gunnison Sage-Grouse (Oyler-McCance and St. John 2010) were used in this study (SGMS06-8, MSP 7, MSP 18, SGMS06-2, and SGMS06-6). The Polymerase Chain Reaction (PCR) was used to amplify each microsatellite locus with a fluorescently labeled forward primer (Beckman Coulter). Thermocycler conditions for conventional one-step PCRs were as follows: Preheat at 94°C for 2 min, followed by 35 cycles of 94°C for 40 sec, anneal for 1 min (temperatures in Table 21.1), and 1-min extension at 72°C. The reaction concluded with a 5-min final extension at 72°C.

Additionally, a two-step or multiplex PCR procedure (Piggott et al. 2004) was performed to amplify samples that were homozygous for

TABLE 21.1

Nine polymorphic microsatellite loci assessed in a population of White-tailed Ptarmigan at Mt. Evans, Colorado, 1937–2008.

Microsatellite Locus	Annealing Temperature (C)	Allele Size Range
SGCA11.2	52	154–161
SGMS06.8	52	121–135
MSP7	55	137–139
MSP18	55	93–95
LLSD4.2	63	154–156
SGMS06.2	55	107–143
SGMS06 .6	58	127–154
LLST1	55	134–159
LLSD8	55	141–151

eight of the nine loci, for samples that would not amplify with a standard single PCR reaction consisting of 35 cycles, and for all the museum specimens. All two-step PCRs were repeated completely (both steps) at least twice. Additionally, museum specimens that were genotyped as homozygotes were amplified multiple times to minimize the chance for allelic dropout. In the multiplex procedure, step one involved the amplification of each sample with two separate pooled primer sets each consisting of six individual primer pairs. A second PCR was used to amplify each locus individually by using 2 μL from the first PCR in a 20-μL reaction. This second PCR consisted of 40 cycles with the parameters previously mentioned (Piggott et al. 2004). All PCR amplifications were performed with GoTaq Flexi (Promega) DNA polymerase. PCR products were run on the CEQ8000 XL DNA Analysis System following manufacturer's protocol (Beckman-Coulter). All samples included the size standard S400 (Beckman-Coulter) and were analyzed using the Frag 3 method of the CEQ Genetic Analysis Software Package (version 6.0). The resulting size fragment chromatograms were scored independently by two individuals.

Stable Isotopes

Feathers were cleaned in a 2:1 chloroform–methanol solution and allowed to air dry for 24–48 hours. Approximately 1 mg of vane material was clipped from the distal end of individual contour feathers and transferred into 5 × 9 mm tin capsules for stable isotope analyses. Only retrix feathers were available for all but three birds sampled in 1968; subsamples were obtained similarly to contour feathers. Samples were analyzed for stable carbon (C) and nitrogen (N) isotopic composition using an elemental analyzer (Carlo Erba, NC1500) interfaced to a micromass Optima mass spectrometer operated in continuous flow mode (Fry et al. 1992). Isotope values are expressed in delta (δ) notation:

$$\delta X = (R_{sample}/R_{standard}) - 1$$

where X represents ^{13}C and ^{15}N in parts per thousand (‰) deviation relative to a standard (monitoring) gas and R represents $^{13}C/^{12}C$ and $^{15}N/^{14}N$ for samples and the standard, respectively. Isotopic data were normalized to V-PDB and Air using the internationally distributed primary standards USGS 40 (–26.24‰ and –4.52% for $\delta^{13}C$ and $\delta^{15}N$, respectively) and USGS 41 (37.76‰ and 47.57% for $\delta^{13}C$ and $\delta^{15}N$, respectively). Analytical error was assessed through replicate measures of primary standards, which was less than 0.2‰ measured across all analytical sequences. A secondary standard (reagent grade keratin) was analyzed in duplicate within each analytical sequence and used as a quality control check; reproducibility was better than 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$. Accuracy was assessed by analyzing primary standards as unknowns and was within 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$.

Data Analysis

For all analyses, we assessed genetic and isotopic data for differences between the three sampling periods (late 1930s, late 1960s, and late 2000s). Microsatellite genotypes were tested for departures from Hardy–Weinberg equilibrium within each time period, using a Bonferroni corrected *P* value of 0.002, in the computer program ARLEQUIN 2.00 (Schneider et al. 2000). ARLEQUIN uses a Markov-chain random walk algorithm (Guo and Thompson 1992) that is analogous to Fisher's Exact test but extends it to an arbitrarily sized contingency table. We used 300,000 as the forecasted chain length and 5,000 dememorization steps for this analysis. Linkage disequilibrium for each pair of loci was evaluated in each time period in GENEPOP (Raymond and Rousset 1995; Markov chain parameters: 10,000 dememorization steps, 1,000 batches, 10,000 iterations per batch). Significance was determined using a Bonferroni corrected *P* value of 0.002.

The amount of genetic diversity per time period was documented several different ways. We calculated mean observed and expected heterozygosity levels per time period using ARLEQUIN. Allelic richness, which adjusts for discrepancies in sample size by incorporating a rarefaction method, was estimated in FSTAT 2.9.3.2 (Goudet 1995). We tested whether the allelic richness differed significantly between time periods using a Wilcoxon matched-pairs signed-ranks test and an alpha level of 0.05. In addition, mean number of alleles per locus per time period was calculated.

We used pairwise population F_{ST} significance tests with a Bonferroni corrected alpha level of 0.02 between each pair of time periods, using ARLEQUIN to examine whether allele frequencies had changed over time. We also tested for differences in allele frequencies based on gender using a pairwise population F_{ST} test with an alpha value of 0.05. Additionally, we used the software program STRUCTURE 2.00 (Pritchard et al. 2000) as an alternative approach to explore changes in allele frequencies. STRUCTURE uses a model-based clustering analysis that groups individuals into genetic clusters without regard to their original sampling locale. We estimated the number of genetic clusters (K) by conducting 10 independent runs each for $K = 1 - 5$ with 500,000 Markov chain Monte Carlo repetitions with a 500,000 burn-in period using the model with admixture, correlated allele frequencies, and no prior information on sampling locales (popinfo = 0).

We tested for gender and capture date effects on stable isotope values using two sample t-tests. Captures in the 1960s and 2000s were conducted in both spring (May–June) and summer (July–September). We used ANOVA to test for differences among the three time periods. Systat (version 8) was used for all statistical tests related to stable isotope data using an alpha of 0.05.

RESULTS

Molecular Genetics

One of the nine museum samples from 1937 failed to amplify in any microsatellite locus. The number of microsatellite alleles per locus across time periods ranged from two to nine. There were no significant departures from Hardy–Weinberg equilibrium and no two loci were found to be linked. The mean number of alleles per time period ranged from 3.1 in the late 1930s samples to 4.6 in the late 2000s samples (Table 21.2). Mean observed heterozygosity was highest for ptarmigan in the late 1930s and lowest in those

TABLE 21.2

Levels of genetic diversity among three different time periods for a population of White-tailed Ptarmigan at Mt. Evans, Colorado.

Time period	Sample size	Mean no. of alleles per locus (SE)	Mean observed heterozygosity (SE)	Mean expected heterozygosity (SE)	Allelic richness
1930s	8	3.1 (0.43)	0.52 (0.09)	0.60 (0.04)	2.5
1960s	42	4.4 (0.84)	0.51 (0.08)	0.51 (0.09)	2.1
2000s	64	4.6 (0.85)	0.49 (0.08)	0.52 (0.08)	2.1

sampled in the late 2000s. Allelic richness, which corrects for unequal sample sizes, was highest in the late 1930s (2.5) and similar in the late 1960s and 2000s (2.1 in both; Table 21.2), although not significantly different among any pair of time periods.

There were no significant differences in allele frequencies among any of the time periods (pairwise F_{ST} tests). However, the small sample size for the late 1930s limited our power to detect differences. We found no differences by gender when all genetic data were pooled across years ($n = 110$). The most appropriate value of K in the STRUCTURE analysis given our data was 1, suggesting that all birds from all time periods represent one genetically distinct population with no detectable differences in allele frequencies through time.

Stable Isotopes

Gender differences were not statistically significant when all isotope data were pooled across years ($n = 113$). Isotopic differences between retrix and contour feathers were not obvious, and sample size was too limiting ($n = 3$ for contour feathers in 1968) to evaluate using parametric statistical procedures. Additionally, systematic differences between the two feather types were unlikely, based on the narrow 95% confidence intervals; gender and feather types were pooled to evaluate isotopic shifts over time. Statistically significant differences among capture seasons ($n = 49$) were observed for both $\delta^{13}C$ and $\delta^{15}N$. However, mean differences were approximately 0.3 and 0.7‰ for C and N, respectively, which were smaller than overall differences observed among the three time periods, so these data were pooled for subsequent analyses.

Carbon isotope values in feathers averaged –20.7‰ during the late 1930s and late 1960s, but declined significantly ($F_{2,114} = 151.9$, $P < 0.001$) to –22.2‰ during the late 2000s (Fig. 21.2A). The range in $\delta^{13}C$ within time periods increased from 0.7‰ in the late 1930s ($n = 9$) to 1.4‰ and 2.4‰ during the late 1960s ($n = 42$) and 2000s ($n = 66$). Nitrogen isotope values in feathers averaged 5.4‰ during the late 1930s and declined significantly to 4.8‰ and 3.7‰ in the late 1960s and 2000s, respectively ($F_{2,114} = 33.0$, $P < 0.001$; Fig. 21.2B). Similar to $\delta^{13}C$, the range in measured $\delta^{15}N$ values increased across time periods from 2.3‰ in the late 1930s to 3.9‰ and 4.5‰ in the late 1960s and 2000s.

DISCUSSION

All genetic diversity levels except mean number of alleles per locus were highest in the samples from the late 1930s (Table 21.2). Due to the low sample size from this time period ($n = 8$), it is not surprising that the mean number of alleles per locus was lower. When we corrected for differences in sample size (allelic richness), the samples from the late 1930s had the highest diversity (2.5 compared to 2.1 in both the 1960s and the 2000s), although not statistically significant using an alpha of 0.05 ($P < 0.1$). Both mean observed and mean expected heterozygosity were highest in the late 1930s and lower in the more modern sample periods. Loss of genetic diversity is typically associated with population decline (Cornuet and Luikart 1996). Although we documented a slight trend of lower diversity and heterozygosity, these changes are minimal compared to those associated with severe population bottlenecks in other species (Bellinger et al. 2002). Maintenance of genetic diversity is important, as it is relevant to the health and viability of a population and preserving its ability to adapt to and survive future environmental challenges (O'Brien and Evermann 1988, Quattro and Vrijenhoek 1989). While our data may suggest a trend toward loss of diversity, analysis of additional samples from the 1930s is needed to reliably verify this trend.

Our analysis also revealed that there have been no significant shifts in allele frequencies between the late 1960s and 2000s. We failed to detect significant differences between the modern samples and the late 1930s, but sample size was likely inadequate in the latter to make a robust assessment. Results from the STRUCTURE analysis also did not reflect a shift in allele frequencies, as all birds were considered to have come from one population, suggesting there have not been any detectable shifts in allele frequencies for this population over the last 70 years. Shifts in allele frequencies within a population over time can suggest significant changes in population size (Glenn et al. 1999, Bellinger et al. 2002) or that a population is small, isolated, and strongly affected by genetic drift (Hilfiker et al. 2004, Oyler-McCance et al. 2005). Either the Mt. Evans population is sufficiently large that genetic drift

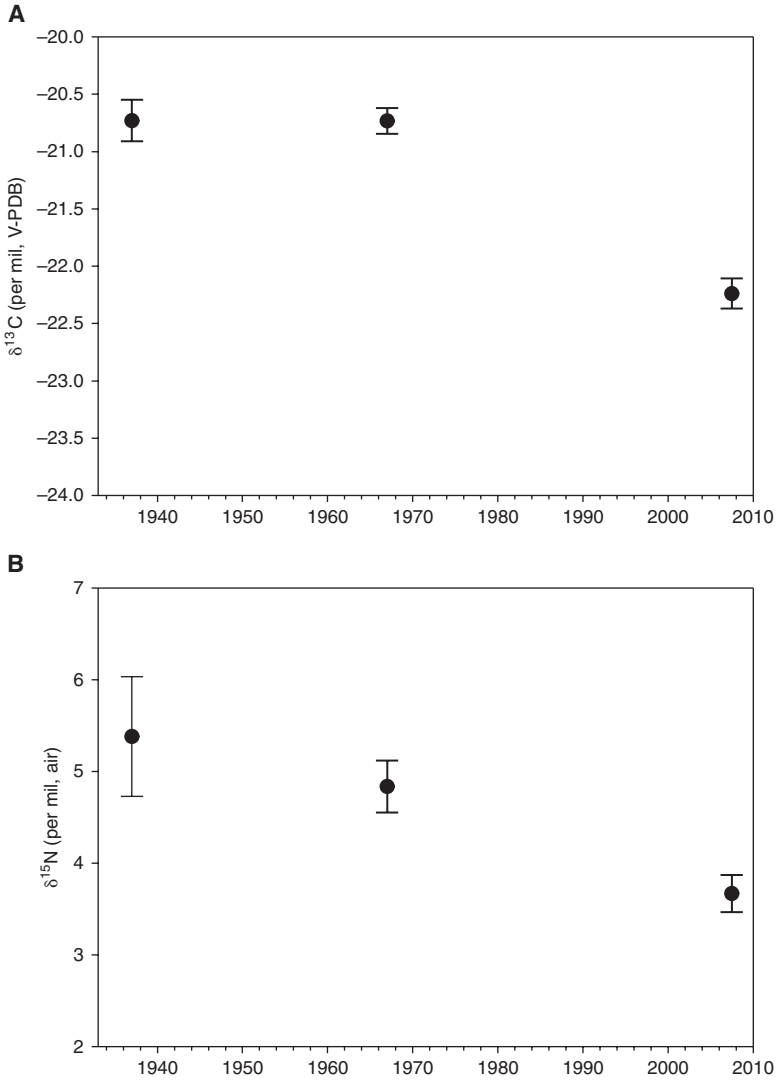

Figure 21.2. Average values for stable isotopes of (A) carbon and (B) nitrogen for feathers of White-tailed Ptarmigan collected at Mt. Evans, Colorado, during three discrete time periods (late 1930s, late 1960s, and late 2000s). Error bars indicate 95% CI.

is not a strong factor or this population may be loosely linked to other nearby populations (e.g., Hall Valley, Kenosha Pass, Square Tops, Argentine Pass, Montezuma Basin, Waldorf, and Loveland Pass). While birds captured at Mt. Evans appear to return to their breeding territories regularly (C. E. Braun, unpubl. data), this population moves to wintering areas comprised of birds from multiple breeding areas. At this point, the extent of mixing between populations remains unknown (but see Hoffman and Braun 1975).

A significant shift in carbon isotope values was observed only since the late 1960s, which is best explained by altered foraging ecology or changes in landcover during recent times. However, the carbon isotopic composition of atmospheric CO_2 has changed significantly since at least the late 1950s, with ~1‰ decrease in $\delta^{13}C_{CO2}$ for the period 1957 to 1995 (Keeling et al. 1979, Francey et al. 1995). Additionally, isotopic data for atmospheric CO_2 at Niwot Ridge, Colorado (http://gaw.kishou.go.jp/wdcgg/; accessed 14 March 2009) suggest ~0.2–0.3‰ decline since the mid-1990s. The apparent shift in feather $\delta^{13}C$ of approximately 1.5‰ since the late 1960s is in line with isotopic changes in atmospheric CO_2, suggesting

that alpine animals may be excellent sentinels for changes in atmospheric chemistry. However, changes in atmospheric chemistry do not fully explain the isotopic shift and range in feather data since the late 1960s, suggesting that altered CO_2 concentrations and increased deposition of nutrients such as nitrogen may be influencing the productivity, nutritional quality, and foraging habitats of White-tailed Ptarmigan in the Mt. Evans study area. For example, Baron et al. (2000a) predicted increased photosynthetic rates and enhanced water use efficiency of alpine tundra following a 2°C warming and doubling of atmospheric CO_2. While such conditions have not yet been expressed, tundra plant productivity may have changed since the late 1960s, and tundra responses are thought to be more tightly linked to winter and spring conditions than to summer (Baron et al. 2000a). Evidence for altered foraging habits is also supported by the increased range of $\delta^{13}C$ and $\delta^{15}N$ feather values across time periods. Isotopic variability within populations has been used as a proxy for niche width, with increased variability equating to a greater diversity of foraging habits (Bearhop et al. 2004, Newsome et al. 2007). It would appear the trophic niche breadth of Mt. Evans ptarmigan has expanded in recent times.

A linear decline in feather nitrogen isotope values was evident across all time periods. Historical records of N deposition in alpine ecosystems suggest an increase during the last century due to anthropogenic sources (Williams et al. 1996, Baron et al. 2000b, Wolfe et al. 2001). An increase in soil N stores due to atmospheric deposition would likely shift the isotopic composition of this nutrient pool, with concomitant shifts in primary consumer tissues. Recent work by Nanus et al. (2008) has shown that the $\delta^{15}N$ of atmospherically derived nitrate ranges from –6.6 to 4.6‰, with precipitation generally having lower values compared to surface waters. Similarly, declining $\delta^{15}N$ values of sedimentary organic matter since at least the 1950s has been demonstrated in alpine lakes of the southern Rocky Mountains (Wolfe et al. 2001). Collectively, these accounts are in agreement with changes in $\delta^{15}N$ of ptarmigan feathers over the last 70 years. Additionally, the increase in N deposition during the 20th century has enhanced the nutritional quality of alpine and subalpine plant species (Rueth and Baron 2002, Rueth et al. 2003). Bowman et al. (2006), through field experiments, showed changes in plant community composition and tissue nutrient concentrations, suggesting that alpine vegetation can potentially serve as a net sink for atmospherically derived N. Increased nutritional quality of available ptarmigan forage may lead to a positive feedback on population demographics, but appears to be at odds with observed declines in recruitment (C. E. Braun, unpubl. data). Currently, we are unable to resolve potential shifts in foraging ecology with changes in plant community composition and nutritional quality. Energetic costs to ptarmigan associated with a rapidly warming alpine may offset benefits associated with enhanced nutritional quality. More detailed studies are required to address these complex relationships.

Studies investigating the impacts of climate on alpine avian species are rare, although a recent study by Wang et al. (2002) of White-tailed Ptarmigan at nearby Rocky Mountain National Park suggests this species has been affected by warming temperature trends. Wang et al. (2002) reported the nesting phenology of this species advanced by ~15 days from 1975 to 1999. Hatch dates were found to be negatively related to average temperatures in May and June. A preliminary analysis of ptarmigan hatch dates from the Mt. Evans study area suggests similar trends, with advanced hatching of roughly 11 days from 1980 to 2008 ($P < 0.05$; G. T. Wann and C. E. Braun, unpubl. data). In addition, there is strong evidence that the Mt. Evans population is changing in its population–age structure. From 1966 to 2008 the age-class ratio of juveniles to adults has sharply declined ($P < 0.001$; G. T. Wann and C. E. Braun, unpubl. data), an indication that juvenile recruitment into the population has decreased. We speculate this may be a result of increased susceptibility of nests and young chicks to weather events that is expected to occur with earlier nesting, or to increased survival of adults because of decreased harvest rates over this period, but more work is needed.

Alpine species may be ideal sentinels for monitoring change in sensitive ecosystems, but long-term perspectives are generally lacking and difficult to unravel due to a suite of co-interacting factors. However, focused studies that seek to set demographic, genetic, and isotopic baselines are desperately needed to successfully identify future change/stressors and implement adaptive management strategies for the conservation of focal species

and habitats. Here we have shown subtle shifts in genetic diversity through time, concomitant with significant shifts in feather isotope values. Importantly, the genetic data collected in this study provide an excellent control for inferences regarding long-term changes in the nutritional ecology of Mt. Evans White-tailed Ptarmigan. Substantial long-term changes in allele frequencies could indicate movement of ptarmigan from neighboring populations that may have different isotopic signatures. Because such changes were not observed, we are confident that shifts in feather isotope values reflect environmental change specific to the Mt. Evans study area. Further, studies employing combined molecular and stable isotope techniques offer clear advantages in species-based investigations of long-term ecosystem change, since diverse aspects of life history and population ecology are explored simultaneously (Valenzuela et al. 2009).

Future research in the Mt. Evans alpine ecosystem will focus on assessing relationships between genetic and dietary shifts and long-term population, climate, and landcover data. Additionally, a better understanding of the genetic basis of phenotypes under selection may allow prediction and mitigation of the effects of climate change on population viability (Reusch and Wood 2007). Identifying genetic markers under selection and examining whether these markers can be correlated with environmental changes associated with climate change and population-level demographic changes should be a focal point. Moreover, there is great synergistic potential if ecologists work more closely with biogeochemists that have traditionally focused on changes in landscape nutrient cycling. In particular, a more detailed analysis of controls on plant species distributions in the alpine will be helpful in assessing potential changes in the foraging ecology of the Mt. Evans ptarmigan population and other alpine species. Finally, the role of ungulate grazing on plant productivity and nutrient cycling remains tenuous (Singer and Schoenecker 2003), although the seasonal abundance of elk (*Cervus elaphus*) and mountain goats (*Oreamnos americanus*) has increased substantially in the Mt. Evans area since the late 1960s (C. E. Braun, pers. obs.). Competition for seasonally nutritious forbs may be an important interaction among alpine species that may ultimately be expressed negatively on ptarmigan demography. Further, there is some evidence that increased N deposition may indirectly influence plant–insect interactions (Throop and Lerdau 2004), and herbivorous insects are known to be an important dietary resource for ptarmigan chicks (May and Braun 1972). Focused studies on sentinel animals in sensitive ecosystems, like the alpine, are certain to advance our understanding of climate change effects on ecosystems.

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