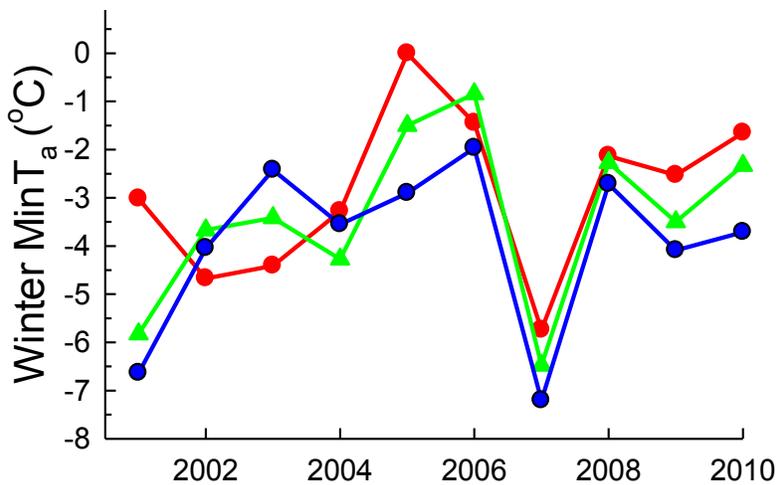
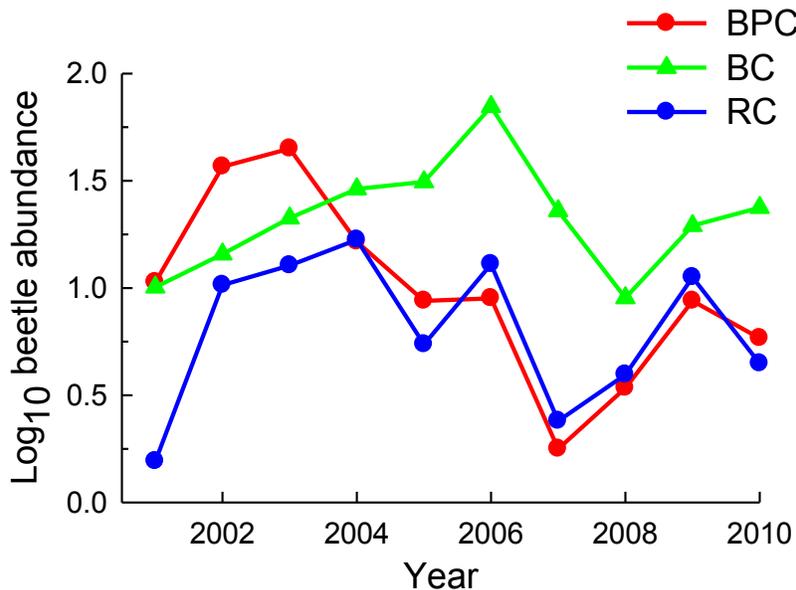


Findings- Ecological and evolutionary responses to environmental change in Sierra Nevada populations of a montane willow beetle.

Changes in maximal adult *C. aeneicollis* abundance in the past decade. Data reported are log maximal number of adult individuals observed per year per site.

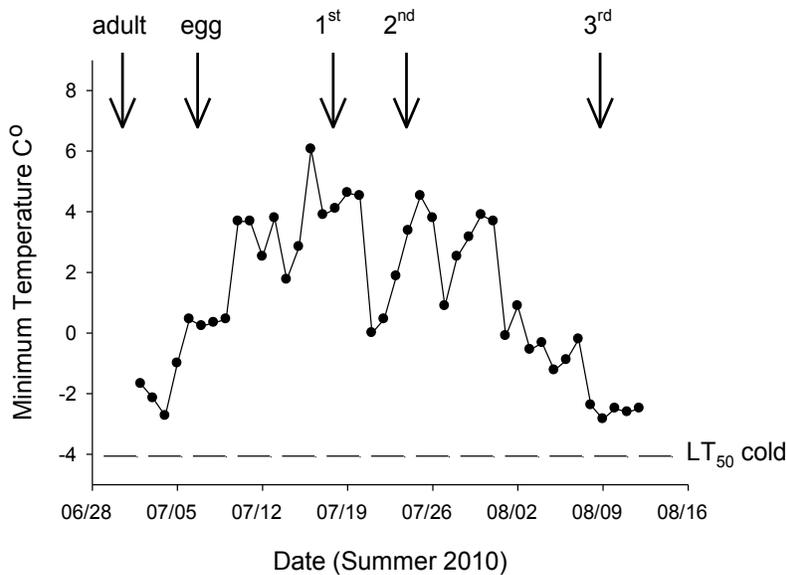


The winter of 2009-2010 was a relatively wet one, with extensive snow cover at high elevation sites until July. As in past years, the late snow melt resulted in warm average temperatures at our Sierra Nevada field sites, because willows and dataloggers were not exposed to the air until winter night time temperatures were relatively mild. This is in contrast to conditions in 2007, where lack of snow cover resulted in low minimum temperatures and extensive frost damage on plants in June.

Beginning of season censuses revealed that populations continued to increase at high elevation sites in Big Pine Creek, but declined at lower elevations, resulting in a slight decline compared to 2009. A similar pattern was found in Rock Creek, where fluctuations in abundance continued to track changes observed in Big Pine Creek. In Bishop Creek, surveys suggested an ongoing increase in population size.

Preliminary analysis revealed up to 10°C differences between afternoon temperatures measured with physiologically relevant inverted cups (which allow radiation to strike the logger from below) and fully shielded air temperature loggers. These results also highlight the great differences that may exist in- and outside of willow canopies, and the importance of microhabitat measures for modeling effects of climate on native insects and other vulnerable species.

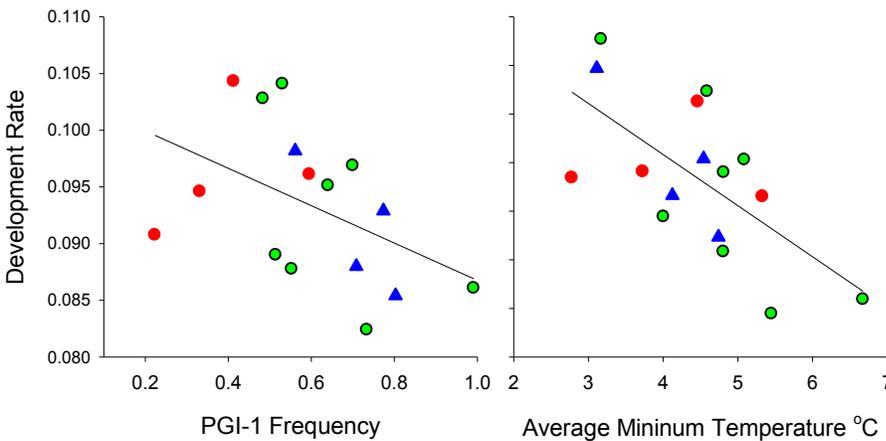
Larval development rate of *C. aeneicollis* in the field during summer 2010. Data shown are minimum daily temperatures recorded at any of 16 sites during June-Aug 2010 and the date on which most beetles made a transition from one life stage to another.



This was a relatively mild summer in the eastern Sierra Nevada. There were few nights where minimum temperature dipped below 0°C and ambient temperatures never reached minimum values that cause beetle mortality in the laboratory. There was also no evidence that beetles experienced mortality due to cold, in contrast to prior field seasons (Rank and Dahlhoff 2002). Our study of larval development rate spanned the transitions to the final larval instar in early August.

At the end of summer 2010, surveys revealed that adult beetles were found in high and low elevation localities where they had not been observed for several years, suggesting that adults disperse after eclosion from pupae in late summer, rather than after snowmelt in the spring.

Larval development was analyzed using a multiple regression that included topographic



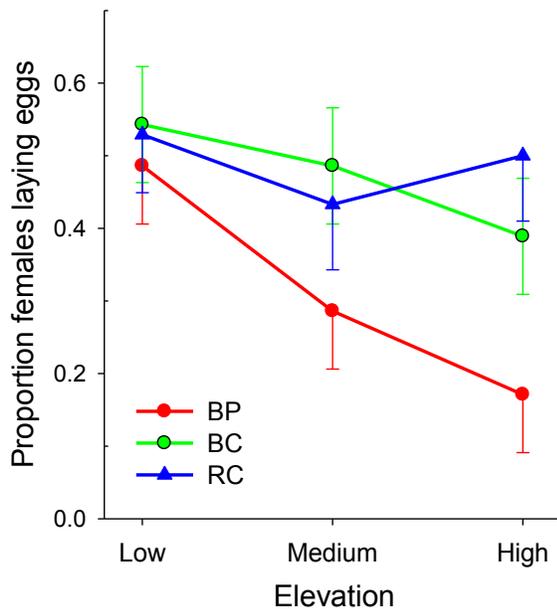
variables, summer temperature data, and PGI allele frequencies at each locality in 2009. The best regression model based on the Akaike Information Criterion included PGI-1 frequency and average minimum temperature.

Results suggested that development tended to be slower in populations where

PGI-1 frequency was high ($F=3.5$, $P = 0.083$). It also shows that as the average minimum temperature increased, rate of development decreased ($F = 9.6$, $P = 0.009$). These results indicate an unexpected finding; development rates were fastest in the coolest habitats. We hypothesize that beetles in cooler habitats experienced torpor at night, which reduces metabolic rate and energy expenditure relative to beetles in warmer habitats. As temperatures rose during the day, these beetles may have had more energy to expend on growth than beetles in warmer habitats.

The negative relationship between PGI frequency and larval development is consistent with prior studies using transplanted beetles. McMillan et al. (2005) also found that development was slowest for larval groups that had the highest frequency of PGI-1. A possible explanation for this finding is that individuals with the PGI-1 allele, which express more heat shock proteins at moderate temperatures than individuals with the PGI-4 allele, pay a growth cost in nature for their more labile heat shock response. We plan to follow up on these observational findings with an outplant experiment during summer 2011.

Fecundity of *C. aeneicollis* in the field during summer 2010. Data shown are mean proportions of females who laid eggs during the 14 day experiment. Overall, 42% of females ($n = 271$) laid eggs, a lower proportion than in previous studies. Conditions were often rainy and cold during this period, and this might have reduced fecundity. Nonetheless, some clear patterns were evident.



Beetles in Big Pine Creek laid fewer eggs than in Bishop or Rock Creek (logistic regression, binomial response variable; $G = 8.8$, $df = 2$, $P = 0.01$). In general, beetles laid fewer eggs at high elevations than at low elevations (logistic regression, binomial response variable; $G = 6.9$, $df = 2$, $P = 0.03$). The pattern at Rock Creek appears somewhat different than the other two drainages, but the interaction between drainage and elevation was not significant. When we complete genotyping at allozyme loci, we may predict that enzyme genotype at PGI will also relate to fecundity.

Comparison of genetic differentiation at allozyme and microsatellite markers in 2009.

Population genetics analysis was conducted for beetles occurring along a south-north transect from Taiboo Pass to Rock Creek in 2009. Genotypes were obtained from all individuals for

Drainage	Subpops	Individuals	mtDNA	
Taboose Pass (TP)	3	45	13	five microsatellite loci and six allozyme loci, including PGI. Samples from all populations have been collected in prior years and archived in an ultracold freezer. A subset of beetles from this sample were sequenced at mtCOII, a locus that was sequenced for a sample from the same study area from 2001.
Big Pine Creek (BP)	4	60	14	
South Bishop Creek (SL)	7	139	43	
Tyee Lakes (TL)	3	55	-	
Piute Pass (PP)	3	45	-	
Pine Creek (PC)	2	40	9	
Rock Creek (RC)	2	39	13	

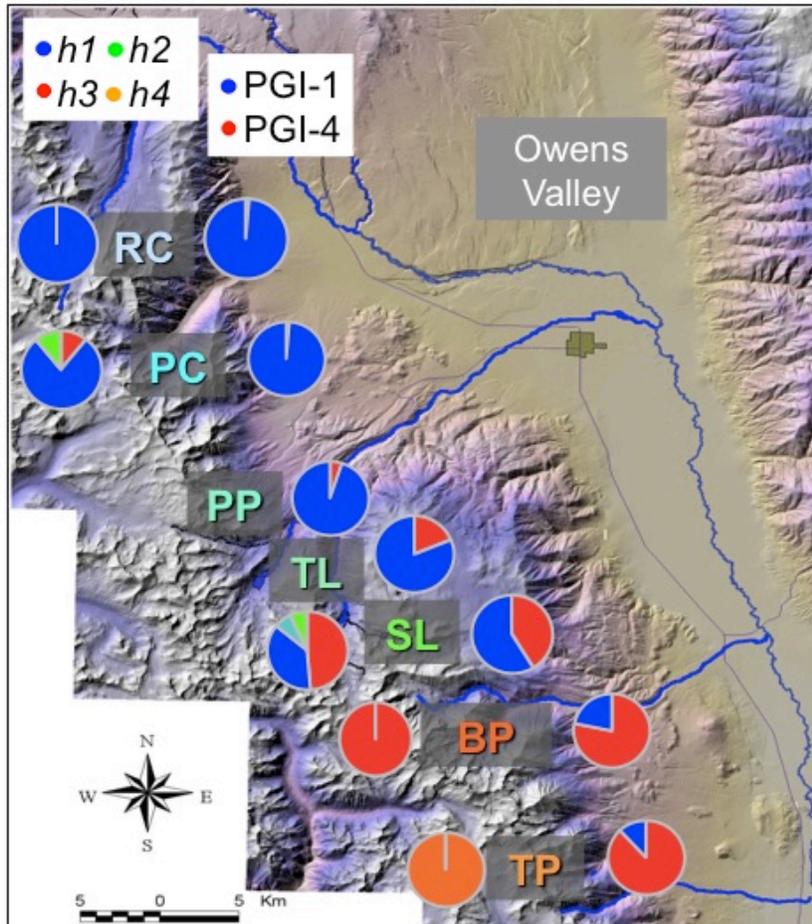
Levels of genetic differentiation among these populations at five microsatellite loci show overall

Allozyme	# alleles	F_{st}	Microsatellite	# alleles	F_{st}
<i>ak-1</i>	2	0	di1	10	0.029
<i>idh-2</i>	4	0.006	di5	11	0.062
<i>me-1</i>	3	0.019	di3	34	0.026
<i>mpi-1</i>	3	0.032	tri38	36	0.044
<i>pgm-1</i>	6	0.048	tri29	6	0.082
Overall		0.024	Overall		0.045

concordance with estimates based on five allozyme loci, even though the number of alleles per locus was generally much greater at the microsatellite loci. Genetic differentiation among populations was significant but not so high as to suggest substantial, long-term reproductive isolation.

Comparison of genetic differentiation at PGI and mtDNA markers in 2009.

In contrast, differentiation at the allozyme locus PGI and the mitochondrial COII locus is substantial along the same transect. In Taboose Pass, the PGI-4 allele is nearly fixed, but in northern populations PGI-1 is nearly fixed. The main transition between PGI-1 and PGI-4 occurs within Bishop Creek, between the southern sub-drainages (SL) and the northern subdrainage (PP). Within Bishop Creek, there is no evidence for linkage disequilibrium between the mitochondrial haplotypes and PGI genotypes, but at the larger geographic scale associations between genotypes at these loci are evident.



In Taboose Pass, unique haplotypes are found that do not occur in other populations (including Montana, Colorado, and Oregon). In Big Pine Creek, haplotypes occur that are also found in Bishop Creek at lower frequency but are rare or not present in more northern drainages. We are currently sequencing additional individuals from the Tye Lakes and Piute Pass subdrainages of Bishop Creek to obtain greater resolution on the transition in mtCOII haplotype frequencies in this critical region. Finally, haplotypes detected in 2009 were the same as haplotypes detected in the same populations in 2001, indicating that little or no genetic exchange occurs between Taboose Pass and the other study drainages.