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

Activities

Our overarching objective for this research program is to quantify factors influencing population size and distribution of a native California leaf beetle. This insect (*Chrysomela aeneicollis)* lives at high elevations (2,800-3,700 meters) in the Sierra Nevada mountains of central California. We are utilizing these native California beetle populations to develop a mechanistic understanding of genetic, ecological and environmental factors affecting population persistence in a changing environment.

Objective 1- *Characterize genetic composition of populations of known abundance over time, using **microsatellite, mitochondrial, and nuclear** markers. We will test hypotheses about the genetic composition of populations and evolutionary forces maintaining genetic variation. Data from putatively neutral genetic markers will compared to allozymes such as phosophoglucose isomerase (PGI), a marker of temperature adaptation.*

Note- We originally proposed to develop and characterize microsatellite markers, and use these as markers of rapid, non-selective evolutionary change, and compare microsatellite frequency variation with that of PGI, a gene that appears to be under natural selection in Sierra willow beetle populations. This past year, we shifted our approach, to incorporate a broader comparison among other nuclear genes, cytochrome c oxidase, and microsatellite variation. Objective 1 has been modified accordingly.

- Over 60 populations in 5 drainages (North Fork Big Pine Creek, South Fork Bishop Creek, North Fork Bishop Creek, Pine Creek and Rock Creek) in the Eastern Sierra Nevada were re-surveyed during Summer 2010. Adult beetles were collected from about 50 of these populations, including from several populations for which beetles have been absent for a number of years; Population sizes were compared with earlier years (see Findings). Genetic analysis of these samples will be conducted this year. Students from SCU and SSU worked as a team to achieve this extensive sampling effort.
- We analyzed variation at 6 allozyme loci: adenylate kinase (AK), mannose phosphate isomerase (MPI), Malic enzyme (ME), isocitrate dehydrogenase (IDH), phosphoglucose mutase (PGM), and phosphoglucoase isomerase (PGI) for 500 beetles sampled in 2009. These were samples for which we analyzed microsatellite loci last year (see Findings).
- DNA sequence variation for around 600 base pairs of the mitochondrial cytochrome oxidase II region (COII) was determined for about 100 individuals collected in 2009 along a broad latitudinal gradient in the Eastern Sierras- the S. Fork King's River, Big Pine Creek, South Bishop Creek, North Bishop Creek, Pine Creek and Rock Creek. These results were compared with geographic variation observed at COII in 2001, using Genepop and Arequin (see Findings). We are currently filling in geographic gaps in this dataset by sequencing 36 additional individuals collected in 2009.
- We further optimized microsatellite analysis. We have 5 microsatellite loci that are working very well. They are in agreement with variation at the 6 allozyme loci and are in HWE. We have 4 other microsatellite loci that require additional optimization of PCR conditions, and we have almost completed this process. These regions also required us to design new primer pairs, assisted by SSU molecular biologist Jim Christmann. Use of these primer pairs revealed heterozygotes not found in the first survey. We are in the process of constructing new multiplex PCR protocols, which we will add to analysis of

2009 samples and apply to 2003 samples (below).

- Beetles were dissected on dry ice and DNA extracted from head and thorax tissue of 400 individuals collected in 2003 for analysis of genotypic variation at microsatellite loci and other DNA markers. We selected 2003 to compare with 2009 because there was significant population contraction between 2003 and 2009, whereas there was expansion between 2009 and 2010, a cool, wet year. Analysis of variation at three allozyme loci (PGI, PGM, IDH) using beetle abdomens is underway.
- We sampled populations of *C. aeneicollis* at sites across Western North America where beetles were found in 1996 and 2001. Survey sites included coastal northern California (Gualala and Point Arena), Coastal Oregon (Cannon Beach), Cascade Range (Mt. Adams- no beetles), Glacier National Park (no beetles) and 4 sites in montane Colorado (Independence Pass, Granite, Lake Windsor, and Timberline Lake trail; beetles only found at highest elevations sites). We collected 20-100 beetles per population found.
- We have started to determine sequence of the PGI gene, in collaboration with Christopher W. Wheat, University of Helsinki. The purpose of this is to investigate differences and homologies in structure among PGI electromorphs, and map relevant amino acid substitutions responsible for functional differences among allozymes onto the quaternary structure of the enzyme. Second, it will allow us to screen current and past populations for sequence-based genetic variation at PGI and to compare this with other genes. Wheat's group is making cDNA and using degenerate primers to PCR and sequence PGI and 3 other central metabolic genes (triose phosphate isomerase, glucose phosphate dehydrogenase, enolase) from *C. aeneicollis* and other beetles.

Objective 2- Determine factors that influence changes in beetle populations over space and time. We will use temperature loggers deployed at each site to measure air temperature and snow cover. We will also obtain allozyme genotypes of beetles from each population and develop a statistical model relating environmental and genetic variation to beetle population persistence.

- Abundance of beetle populations at over 60 different sites in the Sierra Nevada was quantified multiple times during summer 2010; tissue samples were collected for genetic analysis. Continued to survey regions (higher and lower elevations, adjoining drainages) where new populations may colonize after long extirpation.
- Aligned past, current and future sampling localities with past localities using GIS software. In cases where sampling locality has shifted or was renamed, agreed upon uniform site and name, site and GPS code, and assembled this master list in a complete database that includes survey-only sites, logger sites, and weather station sites. Site localities available to team members and the public on line at WMRS website.
- Data from temperature and humidity loggers launched in 2009 at over 60 sites in 5 drainages in the Eastern Sierra Nevada (North Fork Big Pine Creek, South Fork Bishop Creek, North Fork Bishop Creek, Pine Creek and Rock Creek) were downloaded in Spring 2010. Data were downloaded from over 160 loggers, and combined with logger records downloaded in Spring and Fall of 2009. Data were filtered and placed it into 4 separate logger databases, one for each logger location or type: CUP- logger placed in a plastic thermal shield about 1 meter up in willow; BASE- logger placed at base of same willow; SOIL- logger placed 6-10" in soil beneath willow; PRO- highly sensitive, expensive loggers that measure temperature and humidity. This data, along with raw data files, are available upon request on the WMRS web site (see Products).
- In June 2011, we installed 11 meso-climate weather stations in our three main study

drainages (Northern Big Pine Creek, Northern and Southern Bishop Creek, Rock Creek). Most sites are located within the John Muir Wilderness Area, Inyo National Forest. The United States Forest Service approved installation locations and design. Weather stations were installed below the beetles' current elevational range, in the middle of it, and above it, up to 3,800 meters. Undergraduate student teams conducted installations. First set of data was downloaded from these weather stations in September 2010. These data are available on the WMRS site.

- Abundance and logger data are archived as part of a master database that is stored (using Filemaker web serving functionality) on a WMRS office computer and shared via a ftp site on the WMRS web server ("weatherbeetle"). Both are backed up in various secure locations. The database includes all previous field data from the project dating back to 1998. It includes raw data files from willow in-canopy temperature loggers, filtered data stored in comma-delimited text files, and cleaned and complied filtered data in a Filemaker relational database. The database also includes beetle count data from the same time span of years.
- Used temperature logger data to compare differences in microclimate between locations on the host plant, as well as examine differences in lapse rate along elevation gradient between microclimate temperature (loggers) and weather station data.
- Used Hoboware software to conduct initial analysis of summer 2010 temperature logger data measured during larval development and fecundity experiments (see Objective III). Conducted analysis to determine "degree day," a measure of thermal sum, and mean daily maximal, minimal and average temperature at each site.
- The willow beetle project website is updated regularly, and provides news, background information, site descriptions, publications resulting from the research, links to Dahlhoff and Rank's home research pages, and other content important for project participants and other parties:http://www.wmrs.edu/projects/willow%20beetles/default.htm,

Objective 3- Identify mechanisms contributing to changes in population size by measuring key components of population growth and persistence in field experiments.

- In July 2010 (July 9-25), measured differences in mating frequency and fecundity of natural populations of beetles collected in Bishop Creek and outplanted (in mesh bags) along replicate elevational gradients in Big Pine Creek, Bishop Creek and Rock Creek, using published methods (Dahlhoff et al. 2008). Adult beetles were collected in early July and mating pairs were placed onto host plants at three elevations per drainage, corresponding to the upper and lower limits to the historical range of beetles in that drainage (2800-3200 m). Thirty-five mating pairs were placed at each site, resulting in 105 observations per drainage. Mating frequency and number of eggs laid was measured every 1-2 days until egg-laying ceased. At end of experiment, beetles were collected from mesh bags, flash-frozen on dry ice, and stored at -80°C. Analysis of allozyme genotypes is ongoing.
- From June to late August 2010, we investigated larval development rate in nature at 16 sites located in Rock Creek, Bishop Creek and Big Pine Creek. At each site, an hour-long census was conducted in which the number of beetles in each life stage was recorded (adult, eggs, 1st, 2nd 3rd instar larvae, pupae, new adults). By the end of the field season, sites had been visited a total of 76 times (4-7 visits per site). To analyze effects of climate variation on larval development rate, each beetle was assigned a number ranging from 0 to 6 (0 = overwintered adult, 1 = egg, 2 = 1st instar, etc) and average life stage was calculated as the mean of these values. The mean development

rate at each site was calculated by regressing average developmental stage at the site versus number of days since the first census, and using the slope value as an estimate of the rate of change (c.f. Rank 1994, Rank et al. 1998). Temperature logger data were used (Objective II) to find average minimum and maximum daily temperatures at the site over the observation period.

- We began investigating mechanisms of cold tolerance in overwintering adult beetles. First, we have established a collaboration with Dr. Brent SInclair, University of Western Ontario, and one of his graduate students (Evelyn Boychuk). In January 2011, Rank visited the Sinclair laboratory with adult beetles that had been overwintered under laboratory conditions at SCU since October 2010. Preliminary analyses of diapause metabolic rate (indexed by CO_2 production) and super-cooling point were conducted. In addition, laboratory over-wintered adults are being sub-sampled for tissue analyses for presence of cryoprotectants (e.g. glycerol, sorbital, glycogen), which will be conducted later this year.
- Investigated structure of beetle tracheal system using confocal microscopy, as a preliminary study to see whether its morphology depends on populations or genotype as is the case in other insects. Discussing a collaboration with Dr. James Marden (Pennsylvania State University) to further develop this project.

MENTORING PLAN

This grant is a collaborative award shared by two RUI institutions. As such, undergraduates conducted almost all of this work. Based on conversations with our program officer, we felt it would be useful to briefly describe our undergraduate mentoring plan.

Student Expectations

It is expected that students will maintain excellent performance in their academic coursework. Students who are not able to maintain academic performance may be asked to leave our program, as we have more interested students than we do spaces in our labs. However, we do try to be flexible, and work within the constraints of students' other academic demands.

It is expected that student will work 8-12 hours per week during the academic year, close to full time if they participate in the summer. They are also expected to attend lab meetings and read the relevant primary literature.

Students are expected to develop their own research project (SCU Honor's students, SSU BS Biology students), or take ownership of some aspect of the NSF-funded activity. Students are expected to present this work at a local, regional or national meeting at some point in their development, usually during their Senior year or shortly thereafter.

Student Support

We recruit students into our programs who have strong academic records and are interested in research. We also work within our respective programs to attract students from under-represented groups to our program. We do not discriminate on the basis of background, ethnicity, or desired academic outcome (i.e. pre-meds are welcome to join, and many do).

At our home universities, we have group lab meetings about once a week, for 1-1.5 hours. At these lab meetings, we either review a research article (discussion led by students), or discuss logistics, experimental plans, and the like.

We each meet our own students personally at least 1-2 times per term, to talk about student's research project in more detail than can be covered during lab meeting. During these meetings, we also discuss summer activities, future career goals, graduate and medical school applications, and letters of recommendation.

Dahlhoff and Rank co-supervise each other's students, attend lab meetings, hang out together at scientific meetings (and make last minute edits on presentations!), and otherwise are highly interactive with students from each other's programs.

Each spring, we have an "all hands" meetings to prepare the group for summer activity, and to introduce new SSU and SCU students to each other, and go over expectations and plans for the coming field season.

We are in the process of developing a detailed field protocol notebook, to facilitate field training and development.

We have created a Facebook group, "Team Beetle", for current and former members of our research team. This is really useful for maintaining contact with former students (co-authorship on papers, letters of recommendation, career tracking, etc) and setting up events for current lab members.

We have added mandatory intensive wilderness and basic first aid training for new research students who work with us in the Sierra Nevada.

Papers, presentations, posters, symposia and grants

MANUSCRIPTS

Dick*, C. A., N. E. Rank, D. Hollis* and E. P. Dahlhoff. 2010. Effects of thermal stress on running and mating in a scramble competition mating system: mating frequency of willow beetles varies among phosphoglucose isomerase genotypes; *Functional Ecology,* submitted.

Dahlhoff, E. P. and N. E. Rank. Physiological adaptation to climate change in a montane insect. Paper from the symposium: A synthetic approach to the response of organisms to climate change: the role of thermal adaptation*. Integrative and Comparative Biology*, in preparation.

INVITED SYMPOSIA AND SEMINARS

Dahlhoff*, E. P. and N. E. Rank. Physiological adaptation to climate change in a montane insect. *A synthetic approach to the response of organisms to climate change: the role of thermal adaptation.* Society of Integrative and Comparative Biology, Salt Lake City, Utah, USA (2011).

Rank*, N. E. 2011. Enzyme polymorphisms and population biology in a montane insect. Department of Biology, University of Western Ontario, London, Ontario.

Dahlhoff*, E. P. 2011. How are native populations responding to climate change? The story of a little bug. Department of Biology, Santa Clara University, Santa Clara, CA.

Dahlhoff*, E. P. 2010. Physiological consequences of genetic variation may be buffered by heat shock protein expression. Department of Biology, Ecology and Evolutionary Biology Group, Dartmouth College, Hanover, NH.

Rank*, N. E. 2010 Is PGI a plasticity gene?. Bay Area Biosystematists Meeting, Rohnert Park, CA.

CONTRIBUTED PAPERS AND POSTERS

Dahlhoff, E. P., C. A. Dick*, D. Hollis*, M. McCarthy*, D. A. Bruce, S. McWeeney, N. E. Rank. 2010. Effects of temperature on performance and reproductive success in a Sierra willow beetle- implications for populations facing climate change. American Physiological Society, Intersociety Meeting: Global Change and Global Science: Comparative Physiology in a Changing World Westminster, Colorado (paper).

Dahlhoff, E. P., C. A. Dick*, D. A. Bruce, N. E. Rank. 2010. Effects of temperature on performance and reproductive success of a native willow beetle experiencing climate change. Society for the Study of Evolution Annual Meeting, Portland, OR (paper).

Heidl, S, P. Mardulyn, E. P. Dahlhoff, J. T. Smiley and N. E. Rank. 2010. Concordance between geographic variation at phosphoglucose isomerase and mtCOII in a montane leaf beetle. Society for the Study of Evolution Annual Meeting, Portland, OR (paper).

Heitkamp*, R. A., R. M. Neal*, **C. A. Macko*,** N. E. Rank and E P. Dahlhoff. 2010. Phosphoglucose isomerase (PGI) alleles vary along an environmental temperature gradient in California populations of the willow beetle *Chrysomela schaefferi.* Society for the Study of Evolution Annual Meeting, Portland, OR (poster).

Heitkamp*, R. A., R. M. Neal* and **C. A. Macko*** (N. E. Rank and E P. Dahlhoff). 2010. Phosphoglucose isomerase (PGI) alleles vary along an environmental temperature gradient in California populations of the willow beetle *Chrysomela schaefferi.* West Coast Biological Sciences Undergraduate Research Conference, Santa Clara, CA (poster).

Lewis*, C. and S. Reano* (N. E. Rank and E P. Dahlhoff). 2010. Effects of color pattern on

reproductive activity of the willow beetle *Chrysomela schaefferi.* West Coast Biological Sciences Undergraduate Research Conference, Santa Clara, CA (poster).

Rank, N. E., S. Heidl, J. T. Smiley, P. Mardulyn and E. P. Dahlhoff. 2010. Variation in nuclear and mitochondrial genes important for energy metabolism along a climatic gradient in montane populations of a leaf beetle. American Physiological Society, Intersociety Meeting: Global Change and Global Science: Comparative Physiology in a Changing World Westminster, Colorado (Poster).

*Undergraduate author. Presenting author(s) in **bold**.

GRANTS

Abercrombie*, Margaret M. 2010. Determining the roles of genetic and environmental variation on reproductive success, survival and fecundity of a native California insect using a laboratory-reared beetle colony. Claire Booth Luce Fellowship ($9.000); funded.

Boychuk, Evelyn, University of Western Ontario. Overwintering Physiology through the different life stages of a montane willow leaf beetle, *Chrysomela aeneicollis***.** Graduate Student Minigrant Program, White Mountain Research Station, University of California, San Diego ($1,290); in review.

Dahlhoff, Elizabeth. REU Supplement to DEB 08044406. Ecological and evolutionary responses to environmental change in Sierra Nevada populations of a montane willow beetle. ($7,500); funded.

Heidl, Sarah, Sonoma State University. Larval development rates and the effects of environmental temperature and population genetics on development rate in natural populations of a willow leaf beetle (*Chrysomela aeneicollis).* Graduate Student Minigrant Program, White Mountain Research Station, University of California, San Diego ($1,740); funded.

Heidl, Sarah, Sonoma State University. Larval development rates and the effects of environmental temperature and population genetics on development rate in natural populations of a willow leaf beetle (*Chrysomela aeneicollis).* Graduate Student Minigrant Program, White Mountain Research Station, University of California, San Diego ($2,000); in review.

Heidl, Sarah, Sonoma State University. Effects of environmental and genetic variation on larval performance in a California native montane insect; California Desert Research Fund ($2,405); in review.

Lynn, Julie. 2010. Undergraduate Research Award from Sonoma State University Instructionally Related Activities Fund. Support to present findings at West Coast Undergraduate Research Conference in Tacoma, WA ($750); funded.

Rank, Nathan. RET Supplement to DEB 0844404. Ecological and evolutionary responses to environmental change in Sierra Nevada populations of a montane willow beetle. ($30,000); funded.

Shortz, Savannah. 2010. Undergraduate Research Award from Sonoma State University Instructionally Related Activities Fund. Support to purchase allozyme reagents for senior thesis project ($750); funded.

Zavala, Nicholas and Elizabeth P. Dahlhoff, Santa Clara University. Temperature adaptation in a mitochondrial metabolic gene in Sierra Nevada populations of a willow beetle; Santa Clara University Grant Program ($5,882); in review.