

FINAL REPORT
OFFICE OF SCIENCE, DEPARTMENT OF ENERGY
DE-FG02-05ER64023

HELLMANN, J. J., AND N. F. LOBO, Department of Biological Sciences, University of Notre Dame

OVERVIEW

The geographic range limits of many species are strongly affected by climate and are expected to change under global warming. For species that are able to track changing climate over broad geographic areas, we expect to see shifts in species' distributions toward the poles and away from the equator. A number of ecological and evolutionary factors, however, could restrict this shifting or redistribution under climate change. These factors include restricted habitat availability, restricted capacity for or barriers to movement, or reduced abundance of colonists due the perturbation effect of climate change.

This research project examined the last of these constraints – that climate change could perturb local conditions to which populations are adapted, reducing the likelihood that a species will shift its distribution by diminishing the number of potential colonists. In the most extreme cases, species' ranges could collapse over a broad geographic area with no poleward migration and an increased risk of species extinction.

Changes in individual species' ranges are the processes that drive larger phenomena such as changes in land cover, ecosystem type, and even changes in carbon cycling. For example, consider the poleward range shift and population outbreaks of the mountain pine beetle that has decimated millions of acres of Douglas fir trees in the western US and Canada. Standing dead trees cause forest fires and release vast quantities of carbon to the atmosphere. The beetle likely shifted its range because it is **not** locally adapted across its range, and it appears to be limited by winter low temperatures that have steadily increased in the last decades. To understand range and abundance changes like the pine beetle, we must reveal the extent of adaptive variation across species' ranges—and the physiological basis of that adaptation—to know if other species will change as readily as the pine beetle. Ecologists tend to assume that range shifts are the dominant response of species to climate change, but our experiments suggest that other processes may act in some species that reduce the likelihood of geographic range change.

In the first part of our DOE grant (ending 2008) we argued that the process of local adaptation of populations within a species range, followed by climatic changes that occur too quickly for adaptive evolution, is an underappreciated mechanism by which climate change could affect biodiversity. When this process acts, species ranges may not shift readily toward the poles, slowing the rate of species and biome change. To test this claim, we performed an experiment comparing core and peripheral populations in a series of field observations, translocation experiments, and genetic analyses. The papers in Appendix A were generated from 2005-2008 funding.

In the second part of the DOE grant (ending 2011) we studied which traits promote population differentiation and local adaptation by building genomic resources for our study species and using these resources to reveal differences in gene expression in

peripheral and core populations. The papers in Appendix B were generated from 2008-2011 funding.

This work was pursued with two butterfly species that have contrasting life history traits (body size and resource specialization) and occupy a common ecosystem and a latitudinal range. These species enabled us to test the following hypotheses using a single phylogenetic group.

H1: Species with traits that promote gene flow from the core to the periphery will demonstrate adaptations to central characteristics of their range. *Corollary:* Such species will benefit from warming conditions at their northern reaches as conditions under change become more characteristic of the core of their distribution.

H2: Species with life history traits that isolate populations and reduce gene flow will show a greater degree of adaptation to local climatic conditions at the edge of their range. *Corollary:* Such species will not benefit from climatic warming at the northern reaches of their geographic range, at least not until colonists tolerant of the new conditions arrive from more central locations or further evolution takes place.

We found little evidence for H1 in either of our study species, despite the fact that they strongly differ in life history traits and the amount of neutral genetic structure across their range (Zakharov & Hellmann 2008 Mol. Ecology). Instead, we found no evidence that poleward populations will increase under warming due to climate change, but for different reasons in the two species (Pelini et al. 2009 PNAS). Following this observation with a study of the genes involved in local adaptation, we found evidence for H2 in both species. Specifically, we found extensive genetic differentiation between core and peripheral populations (O'Neil et al. In review PNAS). This differentiation was more extensive for the species with less gene flow across its range, but it was pronounced for some key genes in the species with higher gene flow.

These results and others detailed in the Appendices A and B suggest that population differentiation is an important factor in species' tolerances to climate change, even in species that appear to be somewhat uniform across their range. This mechanism deserves greater consideration when predicting the extent of geographic range change of species under climate change, including changes in common species that affect albedo and carbon cycling

This research also enabled a number of other studies in oak-savanna ecosystems of British Columbia, Washington, Oregon, and California. The papers resulting from side projects are listed in Appendix C. Finally, we have synthesized our findings from this overall research program in a review article listed in Appendix D.

Hellmann and Lobo were the PI and co-PI on this grant, respectively, but we also recognize the contributions of two key collaborators: Brent Sinclair, Department of Biology, University of Western Ontario and Scott Emrich, Department of Computer Science and Engineering, University of Notre Dame. Students at both the University of Notre Dame and the University of Western Ontario were trained directly or indirectly by this grant.

PAPER TOTAL: 20

Appendix A. Papers arising directly from the studies proposed in the first (2005-2008) phase of our DOE research.

1. Pelini, S. L., J. D. K. Dzurisin, K. M. Prior, C. M. Williams, T. D. Marsico, B. J. Sinclair, and J. J. Hellmann. 2009. Translocation experiments with butterflies reveal limits to enhancement of poleward populations under climate change. *Proceedings of the National Academy of Sciences* 106: 11160-11165.

There is a pressing need to predict how species will change their geographic ranges under climate change. Projections typically assume that temperature is a primary fitness determinant and that populations near the poleward (and upward) range boundary are preadapted to warming. Thus, poleward, peripheral populations will increase with warming, and these increases facilitate poleward range expansions. We tested the assumption that poleward, peripheral populations are enhanced by warming using 2 butterflies (*Erynnis propertius* and *Papilio zelicaon*) that co-occur and have contrasting degrees of host specialization and interpopulation genetic differentiation. We performed a reciprocal translocation experiment between central and poleward, peripheral populations in the field and simulated a translocation experiment that included alternate host plants. We found that the performance of both central and peripheral populations of *E. propertius* were enhanced during the summer months by temperatures characteristic of the range center but that local adaptation of peripheral populations to winter conditions near the range edge could counteract that enhancement. Further, poleward range expansion in this species is prevented by a lack of host plants. In *P. zelicaon*, the fitness of central and peripheral populations decreased under extreme summer temperatures that occurred in the field at the range center. Performance in this species also was affected by an interaction of temperature and host plant such that host species strongly mediated the fitness of peripheral individuals under differing simulated temperatures. Altogether we have evidence that facilitation of poleward range shifts through enhancement of peripheral populations is unlikely in either study species.

2. Zakharov, E., N. R. Lobo, C. Nowak, and J. J. Hellmann. 2009. Historic introgression as a likely cause of mtDNA paraphyly in two allopatric skippers (Lepidoptera: Hesperiiidae). *Heredity* 102: 590-599.

Gene transfer between species during interspecific hybridization is a widely accepted reality in plants but is considered a relatively rare phenomenon among animals. Here we describe a unique case of mitochondrial DNA (mtDNA) paraphyly in the skipper genus, *Erynnis*, that involves well-diverged allopatric species. Using molecular evidence from both mitochondrial and nuclear genomes, we found high levels of intraspecific divergence in the mitochondrial genome within *E. propertius* (over 4% pair-wise sequence divergence) but no such differentiation in the nuclear genome. Sequence comparisons with related *Erynnis* suggest that past, but recent and infrequent introgression between *E. propertius* and *E. horatius* is the most reasonable explanation for the observed pattern of mtDNA paraphyly. This example of putative introgression highlights the complexity of mtDNA evolution and suggests that similar processes could be operating in other taxa that have not been extensively sampled. Our observations reinforce the importance of involving multiple genes with different modes of inheritance in the analysis of population history of congeneric taxa.

3. Prior, K. M., and J. J. Hellmann. 2009. The ecology and life history of *Erynnis propertius*, a threatened oak-feeding butterfly. *Canadian Entomologist* 141: 161-171.

We describe aspects of the life history of *Erynnis propertius* (Scudder and Burgess) (Lepidoptera: Hesperiiidae) by examining several populations over multiple years. We focused on peripheral populations of this species because they are isolated, are threatened by habitat loss, and may play an important role in driving poleward range expansion under increasing regional temperature. Our findings show that the annual larval growth rate does not vary directly with temperature, adult flight phenology and the timing of key resources respond to average daytime temperatures in spring, and population-density patterns among sites

are robust over years across a broad region near the species' northern range limit. In addition, we provide descriptions of all larval instars for this species. This fundamental information about the biology, timing, and abundance of this species will facilitate further experimental study and improved assessment of its conservation status.

4. Zakharov, E. V., and J. J. Hellmann. 2008. Genetic differentiation across a latitudinal gradient in two co-occurring butterfly species. *Molecular Ecology* 17: 298-305.

Genetic differentiation within a species' range is determined by natural selection, genetic drift, and gene flow. Selection and drift enhance genetic differences if populations are sufficiently isolated, while gene flow precludes differentiation and local adaptation. Over large geographical areas, these processes can create a variety of scenarios, ranging from admixture to a high degree of population differentiation. Genetic differences among populations may signal functional differences within a species' range, potentially leading to population or ecotype-specific responses to global change. We investigated differentiation within the geographical range of two butterfly species along a broad latitudinal gradient. This gradient is the primary axis of climatic variation, and many ecologists expect populations at the poleward edge of this gradient to expand under climate change. Our study species inhabit a shared ecosystem and differ in body size and resource specialization; both also find their poleward range limit on an island. We find evidence for divergence of peripheral populations from the core in both taxa, suggesting the potential for genetic distinctiveness at the leading edge of climate change. We also find differences between the species in the extent of peripheral differentiation with the smaller and more specialized species showing greater population divergence (microsatellites and mtDNA) and reduced gene flow (mtDNA). Finally, gene flow estimates in both species differed strongly between two marker types. These findings suggest caution in assuming that populations are invariant across latitude and thus will respond as a single ecotype to climatic change.

5. Hellmann, J. J., S. L. Pelini, K. M. Prior, and J. D. K. Dzirisin. 2008. The response of two butterfly species to climatic variation at the edge of their range and the implications for poleward range shifts. *Oecologia* 157: 583-592.

To predict changes in species' distributions due to climate change we must understand populations at the poleward edge of species' ranges. Ecologists generally expect range shifts under climate change caused by the expansion of edge populations as peripheral conditions increasingly resemble the range core. We tested whether peripheral populations of two contrasting butterflies, a small-bodied specialist (*Erynnis propertius*) and a large-bodied generalist (*Papilio zelicaon*), respond favorably to warmer conditions. Performance of populations related to climate was evaluated in seven peripheral populations spanning 1.2° latitude (160 km) using: (1) population density surveys, an indirect measure of site suitability; and (2) organismal fitness in translocation experiments. There was evidence that population density increased with temperature for *P. zelicaon* whose population density declined with latitude in 1 of 3 sample years. On the other hand, *E. propertius* showed a positive relationship of population density with latitude, apparently unrelated to climate or measured habitat variables. Translocation experiments showed increased larval production at increased temperatures for both species, and in *P. zelicaon*, larval production also increased under drier conditions. These findings suggest that both species may increase at their range edge with warming but the preference for core-like conditions may be stronger in *P. zelicaon*. Further, populations of *E. propertius* at the range boundary may be large enough to act as sources of colonists for range expansions, but range expansion in this species may be prevented by a lack of available host plants further north. In total, the species appear to respond differently to climate and other factors that vary latitudinally, factors that will likely affect poleward expansion.

6. Zakharov, E., J. J. Hellmann, and J. Romero-Severson. 2007. Microsatellite loci in the Propertius duskywing, *Erynnis propertius* (Lepidoptera: Hesperiiidae), and related species. *Molecular Ecology Notes* 7: 266-268.

Fifteen polymorphic dinucleotide microsatellite loci were characterized for *Erynnis propertius* using an enrichment protocol. The number of alleles varied from nine to 28 for a sample of 24 individuals. Observed heterozygosities ranged from 0.25 to 0.96. Homozygote excess was detected for 10 loci. Twelve markers successfully amplified in related *Erynnis* species and eight loci were polymorphic in at least one other species.

7. Zakharov, E. V., and J. J. Hellmann. 2007. Characterization of 17 polymorphic microsatellite loci in the Anise swallowtail, *Papilio zelicaon* (Lepidoptera: Papilionidae), and their amplification in related species. *Molecular Ecology Notes* 7: 144-146.

Fifteen polymorphic dinucleotide and two trinucleotide microsatellite loci were identified in the Anise swallowtail, *Papilio zelicaon*, from DNA genomic libraries enriched for simple sequence repeats. Allele numbers varied from eight to 29, with an excess of homozygotes observed for nine loci. This homozygosity is a feature of other lepidopteran microsatellites and is probably due to null alleles. Sixteen markers were amplified successfully in other representatives of *Papilio* with 11 loci retaining polymorphism in at least one species. These results suggest that the microsatellites reported here may be appropriate for measuring population genetic structure in a number of *Papilio* species.

Appendix B. Papers arising directly from the studies proposed in the second (2008-2011) phase of our DOE research.

1. O’Neil, S. T., J. D. K. Dzurisin, S. J. Emrich, N. F. Lobo, J. K. Higgs, J. M. Deines, C. M. Williams, R. D. Carmichael, E. Zeng, G. C. Wu, and J. J. Hellmann. In review. Related insects show extensive but differing localization of transcribed genes in response to climate. *PNAS*.

Population differences may determine geographic range shifts and adaptive evolution under climate change. Local adaptation in peripheral populations could preclude or slow range expansions, and populations with different genetic make-up could have distinct trajectories that produce complex spatial patterns of population change. To investigate the genetic extent of local responses to climate change, we exposed poleward-periphery and central populations of two Lepidoptera to reciprocal, common-garden climatic conditions and compared whole-transcriptome expression. We found extensive expression differences between populations in both species. Several thousand genes responded in a localized fashion in the species that exhibits greater population structure and phenotypic local adaptation, and a few genes responded in a strongly localized fashion in the species with higher gene flow, despite a lack of previously observed phenotypic local adaptation. Localized genes differed in identity and effect direction between species, suggesting that population differences do not generalize easily, even for related species living in the same climate. This is the first study demonstrating the genetic basis of climatic tolerance in a non-model species.

2. Williams, C. W., K. E. Marshall, H. A. MacMillan, J. D. K. Dzurisin, J. J. Hellmann, and B. J. Sinclair. In review. Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. *PLoS One*.

Increases in thermal variability elevate metabolic rate due to Jensen’s inequality, and increased metabolic rate decreases the fitness of dormant ectotherms by increasing consumption of stored energy reserves. Theory predicts that ectotherms should respond to increased thermal variability by lowering the thermal sensitivity of metabolism. We examined the thermal sensitivity of metabolic rate of overwintering *Erynnis propertius* (Lepidoptera: Hesperidae) larvae from a stable or variable environment reared in the laboratory in a reciprocal common garden design. Larvae that experienced variable temperatures had decreased thermal sensitivity of metabolic rate, and were larger than those reared at stable temperatures, which could partially compensate for the increased energetic demands. We then modelled overwinter energy use during the winters of 1973-2010 using meteorological data from either a stable or variable habitat to predict the energetic outcomes of metabolic compensation and phenological shifts. Even with depressed thermal sensitivity, the variable environment was more energy-demanding than the stable, with the majority of this demand occurring in autumn. Autumn phenology changes thus had disproportionate influence on energy consumption in variable environments, and variable-reared larvae were most susceptible to overwinter energy drain. Therefore the energetic impacts of the timing of entry into winter dormancy will strongly influence fitness of dormant ectotherms in northern temperate environments. We conclude that thermal variability appears to drive the expression of metabolic suppression in this species; that phenological shifts will have a greater impact on ectotherms in variable thermal environments; and that *E. propertius* will be more susceptible to shifts in phenology in autumn than in spring. We expect our findings to be generally applicable and that increases in overwinter thermal variability and/or extended, warm autumns, will negatively impact all non-feeding dormant ectotherms which lack the ability to suppress their overwinter metabolic thermal sensitivity.

3. Williams, C. M., J. J. Hellmann, and B. J. Sinclair In review. Lepidopteran species differ in susceptibility to winter warming. *Climate Research*.

Winter temperatures are changing rapidly, and although winter warming reduces cold stress for overwintering ectotherms, temperature-mediated increases in metabolic rate can decrease fitness by increasing consumption of energy reserves. This trait can vary among species, even within a taxonomic group. We exposed overwintering stages of the butterflies *Papilio glaucus*, *P. troilus* (Papilionidae), and *Erynnis propertius* (Hesperiidae) to winter temperatures approximately 4 °C above present conditions, and measured mass, water, lipid, protein and carbohydrate content throughout winter in all three species, and post-winter development time, adult size, and mortality in *E. propertius* and *P. troilus*. All three species lost mass over winter, and mass loss was higher during warm winters. *E. propertius* lost more mass than the *Papilio* species. In warm winter conditions, energy reserves significantly declined in *E. propertius*. Mortality and adult size were unaffected by warming in *P. troilus*, but development time was shorter after warmer winters. A decline in water content over winter in *E. propertius* and *P. troilus* suggested that the observed mass loss was predominantly due to water loss. We conclude that *P. glaucus* and *P. troilus* have low vulnerability to winter warming, while *E. propertius* shows some vulnerability. An understanding of the impact of winter warming on species from diverse taxonomic groups will enable the identification of species traits that predict vulnerability, and identify clades most at risk from winter warming.

4. Pelini, S. L., J. Keppel*, A. Kelley*, and J. J. Hellmann. 2010. Slow host plants prevent rapid insect response to climate change. *Global Change Biology* 16: 2923-2929.

We must consider the role of multitrophic interactions when examining species' responses to climate change. Many plant species, particularly trees, are limited in their ability to shift their geographic ranges quickly under climate change. Consequently, for herbivorous insects, geographic mosaics of host plant specialization could prohibit range shifts and adaptation when insects become separated from suitable host plants. In this study, we examined larval growth and survival of an oak specialist butterfly (*Erynnis propertius*) on different oaks (*Quercus* spp.) that occur across its range to determine if individuals can switch host plants if they move into new areas under climate change. Individuals from Oregon and northern California, USA that feed on *Q. garryana* and *Q. kelloggii* in the field experienced increased mortality on *Q. agrifolia*, a southern species with low nutrient content. In contrast, populations from southern California that normally feed on *Q. agrifolia* performed well on *Q. agrifolia* and *Q. garryana* and poorly on the northern, high elevation *Q. kelloggii*. Therefore, colonization of southern *E. propertius* in higher elevations and some northern locales may be prohibited under climate change but latitudinal shifts to *Q. garryana* may be possible. Where shifts are precluded due to maladaptation to hosts, populations may not accrue warm-adapted genotypes. Our study suggests that, when interacting species experience asynchronous range shifts, historical local adaptation may preclude populations from colonizing new locales under climate change.

5. O'Neil, S. T., J. D. K. Dzurisin, R. Carmichael, N. R. Lobo, S. J. Emrich, and J. J. Hellmann. 2010. Population-level transcriptome sequencing of the non-model organisms, *Erynnis propertius* and *Papilio zelicaon*. *BMC Genomics* 11: 310.

Background: Several recent studies have validated the use of Roche 454 sequencing technology for de novo transcriptome analysis. Low error rates and high coverage also allow for effective SNP discovery and genetic diversity estimates. However, genetically diverse data sets, such as those sourced from natural populations, pose challenges for assembly programs and subsequent analysis. Further, estimating the effectiveness of transcript discovery using Roche 454 transcriptome data is still a difficult task.

Results: Using the Roche 454 FLX Titanium platform, we sequenced and assembled larval transcriptomes for two butterfly species: the Propertius duskywing, *Erynnis propertius* (Lepidoptera: Hesperiidae) and the Anise swallowtail, *Papilio zelicaon* (Lepidoptera: Papilionidae). The Expressed Sequence Tags (ESTs) generated represent a diverse sample drawn from multiple populations, developmental stages, and stress treatments. Despite this diversity, > 95% of the ESTs assembled into long (> 714 bp on average) and highly covered (> 9.6x on average) contigs. For each species, we tested two assembly programs and two parameter sets; although CAP3 is commonly used for such data, the assemblies produced by Celera Assembler with modified parameters were chosen over those produced by CAP3. Still, clustering results and subsequent reassembly of clusters highlight some deficiencies in processing diverse data and suggest a pipeline for

refinement of assemblies. To estimate the effectiveness of transcript discovery, we compared the number of bases in the hit region of unigenes (contigs and singletons) to the length of the best match silkworm (*Bombyx mori*) protein—this novel “ortholog hit ratio” gives a lower bound on the amount of the transcript discovered relative to a model Lepidopteran genome. For unigenes with *B. mori* hits, greater than 20% had a ratio > 0.8, and 48% had a ratio > 0.5.

Conclusions: These are the second and third butterflies to have their transcriptomes reported, and the first hesperid (skipper). Ultimately, these assemblies and SNP data will be used to generate microarrays for ecoinformatics examining climate change tolerance of different natural populations. These studies will benefit from high quality assemblies with few singletons (less than 26% of bases for each assembled transcriptome are present in unassembled singleton ESTs), and effective transcript discovery (nearly half of our putative orthologs cover at least 50% of the corresponding model silkworm gene).

6. Williams, C. M., S. L. Pelini, J. J. Hellmann, and B. J. Sinclair. 2010. Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. *Biology Letters* 6: 274-277.

The hygric hypothesis postulates that insect discontinuous gas exchange cycles (DGCs) are an adaptation that reduces respiratory water loss (RWL), but evidence is lacking for reduction of water loss by insects expressing DGCs under normal ecological conditions. Larvae of *Erynnis propertius* (Lepidoptera: HesperIIDae) naturally switch between DGCs and continuous gas exchange (CGE), allowing flow-through respirometry comparisons of water loss between the two modes. Water loss was lower during DGCs than CGE, both between individuals using different patterns and within individuals using both patterns. The hygric cost of gas exchange (water loss associated with carbon dioxide release) and the contribution of respiratory to total water loss were lower during DGCs. Metabolic rate did not differ between DGCs and CGE. Thus, DGCs reduce RWL in *E. propertius*, which is consistent with the suggestion that water loss reduction could account for the evolutionary origin and/or maintenance of DGCs in insects.

Appendix C. Articles arising from side projects enabled for our DOE research program.

1. Marsico, T. D., and J. J. Hellmann. 2009. Dispersal limitation inferred from an experimental translocation of *Lomatium* (Apiaceae) species outside their geographic ranges. *Oikos* 118: 1783-1792.

Determining limitations on poleward range expansion is important for predicting how climate change will alter the distribution of species. For most species, it is not known what factors set their distributional limits and the role dispersal limitation might play if range-limiting factors were altered. We conducted a transplant study of three related and co-occurring *Lomatium* species at their northern range limits to test competing hypotheses of range limitation. We added seeds to experimental plots inside and outside the species' geographic range (a regional treatment) in a replicated design with vegetation intact and vegetation reduced (a disturbance treatment) and with herbivore access and herbivore exclusion (an herbivory treatment). Germination and reemergence were measured through two growing seasons, along with community-level variables. A fully-crossed linear mixed model revealed that *Lomatium* survivorship outside the current range was as good or better than survivorship within the range, at least when the vegetative community remained intact. This suggests that the species are dispersal limited. Germination often was improved in the presence of an intact vegetative community, but this potentially facilitative effect was absent in second-year reemergence. Plots exposed to herbivory had slightly, but significantly, reduced germination, though reemergence did not differ between herbivore treatments. *Lomatium dissectum*, a rare species, had significantly lower survivorship than its congeners, suggesting that range shifts in rare taxa may be particularly difficult. Seed additions beyond species' range limits may be a strategy for overcoming dispersal limitation and assisting species in poleward migrations.

2. Marsico, T. D., J. J. Hellmann, and J. Romero-Severson. 2009. Patterns of post-glacial pollen flow and seed dispersal in *Quercus garryana* (Fagaceae) as a baseline for understanding future responses to climate change. *Journal of Biogeography* 36: 929-941.

Aim We examined the genetic structure of *Quercus garryana* to infer post-glacial patterns of seed dispersal and pollen flow to test the hypotheses that (1) peripheral populations are genetically distinct from core populations and from one another; (2) genetic diversity declines towards the poleward edge of the species' range; and (3) genetic diversity in the chloroplast genome, a direct measure of seed dispersal patterns, declines more sharply with increasing latitude than diversity in the nuclear genome. We address our findings in the context of known historical oak distribution from pollen core data derived from previously published research.

Location Oak-savanna ecosystems from southern Oregon, USA (core populations/non-glaciated range) northward to Vancouver Island, British Columbia, Canada (peripheral populations/glaciated range).

Methods We genotyped 378 trees from 22 sites with five chloroplast and seven nuclear microsatellite loci. For both sets of markers, we estimated genetic diversity and differentiation using an analysis of molecular variance and generated Mantel correlograms to detect genetic and geographical distance correlations. For the nuclear markers, we also used a Bayesian approach to infer population substructure.

Results There was a large degree of population differentiation revealed by six chloroplast haplotypes, with little (≤ 3) or no haplotype diversity within sites. Peripheral island locations shared the same, maternally inherited chloroplast haplotype, whereas locations in mainland Washington had greater haplotype diversity. In contrast, genetic diversity of the nuclear markers was high at all locations sampled. Populations clustered into two groups and were significantly positively correlated over large spatial scales (≤ 200 km), although allele richness decreased significantly with latitude. Population substructure was observed between core and peripheral populations because rare alleles were absent in peripheral localities and common allele frequencies differed.

Main conclusions The observed pattern of chloroplast haplotype loss at the northern periphery suggests restricted seed dispersal events from mainland sites to peripheral islands. This pattern was unexpected, however, as refugial oak populations remained near the current post-glacial range even during the Last

Glacial Maximum. Using nuclear markers, we found high within-population diversity and population differentiation only over large spatial scales, suggesting that pollen flow is relatively high among populations.

3. Prior, K. M., and J. J. Hellmann. 2010. Impact of an invasive oak-gall wasp on a native butterfly: a test of plant-mediated competition. *Ecology* 91: 3284-3293.

Phytophagous insects commonly interact through shared host plants. These interactions, however, do not occur in accordance with traditional paradigms of competition, and competition in phytophagous insects is still being defined. It remains unclear, for example, if particular guilds of insects are superior competitors or important players in structuring insect communities. Gall-forming insects are likely candidates for such superior competitors because of their ability to manipulate host plants, but their role as competitors is understudied. We investigate the effect of invasive populations of an oak gall wasp, *Neuroterus saltatorius*, on a native specialist butterfly, *Erynnis propertius*, as mediated by their shared host plant, *Quercus garryana*. This gall wasp occurs at high densities in its introduced range, where we stocked enclosures with caterpillars on trees that varied in gall wasp density. Biomass production of butterflies was lower in enclosures on high-density than on low-density trees because overwintering caterpillars were smaller, and fewer of them eclosed into adults the following spring. To see if the gall wasp induced changes in foliar quality, we measured host plant quality before and after gall induction on 30 trees each at two sites. We found a positive relationship between gall wasp density and the percentage change in foliar C:N, a negative relationship between gall wasp density and the percentage change in foliar water at one site, and no relationship between the percentage change in protein-binding capacity (i.e., phenolics) and gall-wasp density. Additionally, there was a negative relationship between foliar quality and butterfly performance. Our results provide evidence for a plant-mediated impact of an invasive oak gall wasp on a native butterfly and suggest that gall wasps could act as superior competitors, especially when they occur at high densities.

4. Prior, K. T., and J. J. Hellmann. An experimental test of the enemy release hypothesis for a range-expanding insect. *Science*. To be submitted Jan. 2012

Species distributions are being altered at rapid pace. Species will not move in concert, however, and species interactions are likely to be disrupted. Enemies, for example, can lag behind range-changing species and may be slow to attack species in their new range. The enemy release hypothesis (ERH) suggests that this reduced enemy pressure will cause a demographic advantage for species in their new range and that this is a major explanation for why some introduced species become invasive. Despite the popularity of the ERH, most evidence comes from observations of enemy loss while release from enemy control is often inferred. We conducted a rigorous examination of the ERH for an invasive gall wasp, *Neuroterus saltatorius* that has undergone a poleward intra-continental introduction. We take a biogeographical approach to assess enemy loss and experimentally test if loss causes release. Comparisons of gall wasp abundance and parasitoid rates supported the ERH, that this species is outbreaking and that there are lower parasitoid rates in its introduced than in its native range. Contrary to predictions of the ERH, however, a parasitoid exclusion experiment revealed that enemy loss did not translate into release. Reducing parasitoid pressure had a larger effect on gall wasp survival in the introduced range compared the native range and this was driven by increased background survival in the absence of enemies in the introduced range. Our results suggest that some other factor, such as host plant suitability, limits *N. saltatorius* in its native range and largely contributes to its increased success in its introduced range. This study provides the first biogeographical experimental test of the ERH for an invasive animal species and offers an interesting contrast to species that have been transported over long distances. Our results suggest that species that move over short distances, such as those undergoing climate-driven range expansions, have the potential to become invasive, but contrary to popular expectations not solely because of enemy release.

5. Prior, K. M., and J. J. Hellmann. In review. A review of the enemy release hypothesis as an explanation for the success of invasive species in multiple trophic levels and

ecosystems. Invasive Species in a Globalized World, Keller, R. M., M. Cadotte, and G. Sandiford, eds. University of Chicago Press, Chicago, IL.

- Invasion success occurs when populations of species are introduced into new locations and have increased fitness or demographic rates compared to native populations
- The enemy release hypothesis (ERH) is a leading hypothesis of invasion success, and it posits that introduced species lose enemies and are released from enemy control
- Despite the popularity of this hypothesis and its implications for biological control, our literature survey reveals that it has only been properly evaluated in limited contexts
- Rigorous tests of the ERH are needed for invasive species in higher trophic levels and in aquatic systems to reveal the general importance of this hypothesis of invasion success
- A fundamental understanding of what causes invasion success is essential to apply effective control strategies for the management of invasive species

6. Stewart, J. M., T. D. Marsico, D. Burge, and J. J. Hellmann. In review. Largest known *Quercus garryana* clone discovered on a steep slope at the boundary of Larrabee State Park, Washington, USA. *International Oaks*.

The occurrence of clonal growth in deciduous trees is fairly common, particularly for regeneration after stem damage. Within the genus *Quercus* there are many species that have been described as possessing the ability to reproduce vegetatively, but the discovery of large clones is limited. Here we describe a largest known clone of *Quercus garryana* Douglas ex Hook. produced by extensive suckering. The *Q. garryana* clone described is located on a steep, west-facing slope at the boundary of Larrabee State Park, Washington, USA. Twenty-eight twig samples within the stand were collected and processed for genetic analysis. The samples were analyzed using seven nuclear microsatellites, and the results showed that all individual samples were genetically identical. The combined canopy of this clone covers an estimated 383m² and is ~37m long (N – S) and ~20m wide (W – E) at its largest dimensions. In this case, we propose that the mechanism of continual cloning is a result of the perception of fallen stems due to gravitational pull on a steep slope. It is not yet known if this stand of *Q. garryana* is one of many large clones or unique, but it highlights the need for research into the mechanisms driving clonal growth to understand rates of vegetative biomass accumulation and possible trade-offs between sexual and asexual reproduction in woody plants.

Appendix D. Review article that synthesizes our DOE research.

1. Hellmann, J. J., K. M. Prior, S. L. Pelini. 2012. The influence of species interactions and local adaptation on geographic range change under climate change. *The Year in Ecology and Conservation*. In press.

The fossil record tells us that many species shifted their geographic distributions during historic climate changes, but this record does not portray the complete picture of future range change in response to climate change. In particular, it does not provide information on how species interactions will affect range shifts. Therefore, we also need modern research to generate understanding of range change. This paper focuses on the role that species interactions play in promoting or preventing geographic ranges shifts under current and future climate change, and we illustrate key points using empirical case studies from an integrated study system. Case studies can have limited generalizability but are critical to defining possible outcomes under climate change. Our case studies emphasize host limitation that could reduce range shifts and enemy release that could facilitate range expansion. We also need improvements in modeling that explicitly consider species interactions, and this modeling can be informed by empirical research. Finally, we discuss how species interactions have implications for range management by people.