Sabbatical Report, Fall 2018
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DEVELOPMENT OF FACULTY/STUDENT RESEARCH IN EVOLUTIONARY AND APPLIED ECOLOGY

I. Summary of Purpose and Goals

The purpose of this sabbatical leave request was to provide faculty an opportunity to refocus research agenda and for retraining in emerging technology and analytical methodology necessary for scholarship, publication, and instructional improvement.

For over twenty years I have sustained an interest in the evolution and diversification of land plants using the techniques of comparative DNA sequencing (the study of variations in the genetic material among related species) and molecular phylogenetics (the use of statistical analyses to determine the rates and patterns of change occurring in DNA sequences to reconstruct evolutionary relationships among organisms). My previous work focused on questions that were global in scope, involving the use of nuclear markers to study the early divergence of basal plant lineages and the evolution of plant cellular housekeeping genes. This work is labor-intensive, requiring massive amounts of DNA sequence data, and is collaborative, involving scientists in many laboratories. Continuing innovations in high-throughput and whole-genome sequencing technologies and associated computational data analysis methods have changed dramatically the scope of work required for peer-reviewed publication in this field, demanding increasingly large data sets and computational power. Maintaining my currency and productivity in this type of research has been challenging because my work requires research infrastructure and instrumentation and time not available due to severe limitations in laboratory facilities, absence of master's level students, and my focus on teaching and service. Moreover, I served as chair of the biology program from 2008 to 2017, since my first semester as a tenured associate professor. During my term as chair, the biology program experienced a three-fold increase in majors, FTES, and faculty, creating administrative responsibilities that effectively prevented me from participating in any collaborative research projects and isolated me somewhat from former colleagues. As my chair service drew to a close, it was critical that I re-establish a faculty/student research program to support our students, the university's mission, and my own professional development. The field of molecular phylogenetics develops rapidly and has a steep learning curve; my sabbatical leave was intended provide a necessary opportunity for me to update my skills in new technologies and analytical tools, explore research collaborations, and interact productively with other scientists in my field. I also planned to take advantage of time spent with colleagues to reconsider my research focus with the goal of moving my scholarship from large, genome-scale projects to smaller, local, and more tractable questions.

At the time the leave was proposed, I planned extended visits to the laboratories of two colleagues, Dr. Scott Armbruster at the University of Portsmouth in the United Kingdom, and Dr. Steffi Ickert-Bond at the University of Alaska Fairbanks to develop small research

projects, each exploring a different aspect of lineage diversification in regional plant populations. My goal was to redirect my scholarship from larger-scale projects that are not feasible given time and resource constraints at CSU Channel Islands and establish two distinct but thematically related research projects that are a better fit with our teacher/scholar model, appealing to students, relevant to current questions in the field of evolutionary biology, achievable, and ultimately publishable.

II. Summary of Outcomes

The outcomes of the sabbatical as granted were not the completion of a specific project, rather, the leave was to allow me to spend extended, uninterrupted time with colleagues in my field who are engaged in productive plant biogeography and evolutionary ecology research, for the purpose of updating and refocusing my research specialization and developing a realistic research plan for the future.

a) Honeybee disease ecology

An unexpected personnel emergency in the University of Portsmouth laboratory required me to postpone my visit and plan to update strategies to address local plant diversification issues (*e.g.*, speciation resulting from the adaptive specialization of mainland organisms to island habitat in Channel Islands species *Solanum wallacei* and *Leptosyne gigantea*). Despite this disappointment, I maintain an interest in plant island-mainland genetic structure relationships and will continue this work in the future. Fortunately, a new research prospect was presented to me: biology colleague Dr. Ruben Alarcón approached me with a potential collaborative project in applied honeybee ecology.

Populations of bees around the world are exhibiting declines, which are the result of multiple interacting factors, including viral pathogens. More than 24 viruses have been identified in western honeybees (*Apis mellifera*) to date. Viral infections can result in a range of symptoms, from no obvious phenotype to rapid death and colony loss, depending on the viral species, physiological state of the host, and presence of other stressors. Despite the importance of bees as pollinators of flowering plants in agricultural and natural landscapes, including Ventura County, and the importance of viruses to bee health, our understanding of bee viruses is surprisingly limited. Bee viral ecology is particularly complex, since many viruses seem to be shared across diverse bee species. Several studies have demonstrated that viruses can spill-over from managed *A. mellifera* or bumble bee (*Bombus spp.*) colonies to wild bee populations, with increased viral prevalence in areas with increased density of infected managed colonies. Viruses found to be pathogenic in *A. mellifera* have also been found to be pathogenic in wild bee species, meaning that infected managed colonies may not only reduce pollination efficiency and crop productivity in our area, but they can also result in adverse impacts to bees in wild local ecosystems.

For several years Dr. Alarcón has an ongoing study of a nutritional supplement, developed by local professional beekeepers, that appears to help honeybee colonies repel parasitic mites and recover from viral infections. In addition to field trials at neighboring ranches currently underway, Dr. Alarcón and his students have begun experiments with caged bees to quantify

the effects of the supplement on parasitic load, and he proposed that I develop a simple genetic scan for the presence of RNA viruses in local bees. The goal is a fast polymerase-chain reaction (PCR) test to identify up to 10 strains of virus known to infect honeybee colonies that could be carried out easily by students within the constraints of limited molecular diagnostic equipment available at CSUCI.

To date, candidate primer-pairs for PCR amplification and specific identification of 10 common strains have been developed based on published virome sequences[1]. Dr. Alarcón has begun accumulating dead bee samples for the purposes of primer development, so there is an abundance of template DNA available for initial testing. Ongoing laboratory space issues required that I vacate my lab space during my sabbatical so that incoming biology faculty could begin research, and I have not yet been able to begin testing these on bee samples, however, once new lab space is completed for newly-hired faculty, confirmation of the efficacy of the initial primer set for amplification should be quickly confirmed. Additional fine-tuning of primer sequences may be required to confirm that there is no cross-amplification between primer sets, ensuring that the test can accurately discriminate between all viral strains, as well as correctly indicate infections of a single bee host by more than one virus. The goal is to have enough data by spring 2020 to present at the Pacific Branch of the Entomology Society of America meeting.

b) Training in emergent techniques for molecular phylogeography

My sabbatical proposal included collaboration with Professor Steffi Ickert-Bond at the University of Alaska Fairbanks, investigating evolutionary diversification and historical biogeography in two closely allied *Rhododendron aureum* populations on either side of the Bering Strait. Dr. Ickert-Bond shares my appreciation of arctic plant biodiversity and has considerable expertise in the most recent molecular phylogeographic analytical techniques.

Together we developed a project is compatible with my long-term interest in historical biogeography and the response of arctic plant populations to climate change, however, instead of focusing on large-scale postglacial plant evolution and migration, Dr. Ickert-Bond and I are using a single Beringian plant species as a model system in which to study range shifts following Pleistocene climate oscillations. Unglaciated Beringia was a Quaternary refugium for plants and higher genetic diversity is expected within those areas of a species' current distribution that were refugial. Beringia is therefore key to understanding how arctic biodiversity has been shaped by historical factors and how this flora will respond to future changes in climate and human population expansion. When I was on the faculty of the University of Alaska, my biogeography research involved molecular investigations of multiple arctic species to elucidate postglacial colonization patterns across the entire arctic floristic spectrum. This type of broad-based study is not practical at CSUCI, however, the study of historical population demography individual species using molecular markers as well as morphological variation will contribute to the growing body of knowledge regarding the potential of arctic species to adapt to climate warming. Due to proximity to multiple populations for sampling and access to on-site core DNA sequencing facilities at UAF, the bench work for this project will be completed in Alaska, however, with training I received in

[1] Galbraith, D.A., *et al.* 2018. Investigating the viral ecology of global bee communities with high-throughput metagenomics. *Nature/Scientific Reports* 8:8879 | DOI:10.1038/s41598-018-27164-z

emerging analytical techniques for molecular phylogeography (see below), I will collaborate on analysis of the dataset when it is completed, and more importantly, now have the analytical tools to direct students in similar biogeographic studies of local plant species.

In evolutionary biology, similarity among organisms or species is attributable to inheritance from a common ancestor. A phylogeny is a hypothesis that reconstructs the historical relationships, and in some cases the timing, of events that occur as species diversify. Phylogenies provide the analytical framework for all questions of species adaptation, specialization, and diversification. They are generated using specialized computer applications that infer process from pattern: the process of organismal evolution is deduced from patterns of DNA variation. Although many software programs continue to be developed and improved, their reliability is dependent upon the size and composition of the particular molecular data set under analysis. The merits and shortcomings of these various methods of phylogenetic inference and interpretation are subject to much scientific debate, because the danger of generating incorrect results is greater in computational molecular phylogenetics than in many other scientific disciplines. It is critical, therefore, that the user understand what the analytic method is actually doing with the data. While my CSUCI faculty colleagues are well-versed in many of the same molecular and evolutionary biology bench techniques as me, because they apply their data to different types of questions, they use different analytical programs and tools. This is why it was so important for me to update my analytical toolbox using real experimental data, under the direction of experts well-versed in the theoretical underpinnings of phylogenetic methodology. Through direct analysis of data generated during my sabbatical, as well as in weekly journal club discussions, I received training in the application of several new tests and software packages for phylogenetic analysis (RASP: Reconstruct Ancestral State in Phylogenies[2], and BEAST: Bayesian Evolutionary Analysis Sampling Trees[3]) which I will continue to use in my research and in teaching (BIOL 303-Evolutionary Biology, BIOL 406-Biogeography, BIOL 319-Plant Systematics & Identification, BIOL 494-Independent Study, BINF 500-DNA and Protein Sequence Analysis).

In sum, my sabbatical leave provided me with an experience not possible during my normal workload assignment due to constraints in time, instrumentation, and the availability of local expertise. During my leave I acquired new technical and analytical proficiency required to maintain currency in my field and generated data for future publication, teaching, and extramural funding. It also allowed me to begin a small collaborative project (Ickert-Bond) which will maintain my currency in arctic biogeography and ecosystems, and to begin work with a CSUCI colleague (Alarcón) by expanding into more applied biology developing tools for disease detection in honeybees. Tangible outcomes of this sabbatical are: 1) background study and literature search, research plan, and first set of PCR primers developed for

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[2] Yu, Y., Harris A.J., Blair C., He X.J. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution*. 87: 46-49

[3] Bouckaert, R., *et al*. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology*, 10(4), e1003537, doi:10.1371/journal.pcbi.1003537

honeybee virus screening project, which, when up and running will be largely student-driven, 2) data generated and analytical methods learned for one future collaborative publication on Beringian plant biodiversity, 3) appropriate methods learned for future research and teaching, and 4) real data sets converted into learning exercises for students in BIOL 303 (Evolution), BIOL 311 (Plant Biology), BIOL 319 (Plant Systematics & Identification), and BINF 500 (DNA and Protein Sequence Analysis. The skills I learned are relevant far beyond my applications to plant evolution – these techniques are used across the biological subdisciplines of biomedicine, molecular biology, conservation biology, bioinformatics, and genomics. My leave also provided an extended opportunity for me to interact with my scientific peers on a level that I could not otherwise. This informs my teaching in an important way by allowing me to share with my students, both undergraduate, and OLLI, applied and theoretical research ideas and the newest data and results.