

SPECIATION IN PATAGONIA: ESTABLISHING SUSTAINABLE INTERNATIONAL COLLABORATIONS IN EVOLUTION, ECOLOGY, AND CONSERVATION BIOLOGY

PROJECT DESCRIPTION

Prior NSF Support (see reference section for publications resulting from prior support)

Jerald B. Johnson – No prior NSF support.

Keith A. Crandall – **The Phylogenetic Relationships among the Freshwater Aegliidae (Decapoda: Anomura)**. NSF DEB-0075600, \$220,000 2000-2003. NSF DEB-0073154, 0073155, 0206537 DDIGs for D. Posada, J. Fetzner, and M. Porter, respectively. This project has established phylogenetic relationships for all species of freshwater crabs in the family Aegliidae, identified conservation priorities for endangered Chilean freshwater ecosystems, and led to taxonomic revisions, including the description of new species from molecular data and morphological data. Fetzner's project examined the use of nested clade analysis in a linear habitat using a freshwater crayfish. Posada examined the relative performance of methods to detect recombination in sequence data. Porter examines opsin evolution in shrimp species across a spectrum of habitats.

Jack W. Sites, Jr. – **Population structure, nest-site fidelity, and conservation genetics in the Giant Amazon River Turtle (*Podocnemis expansa*; Pelomedusidae)**. NSF DEB-9815881, \$221,992; 1999-2003. **The Phylogeny of Xantusiidae, and its Placement within Scleroglossa (Reptilia: Squamata)**. NSF DEB-0132227, \$318,000, 2002-2006. **Species boundaries and phylogenetic relationships in chromosome races of the *Sceloporus grammicus* complex (Squamata: Phrynosomatidae)**. NSF DEB-0309103, \$11,953, 2003-2004. **Phylogeny of the genus *Microlophus* (Squamata: Tropiduridae): a history of colonization and dispersal**.

NSF DEB-0309111, \$11,970, 2003-2005. All of these projects involve extensive field research in Latin America, and included collection of tissues and museum vouchers, collaboration and co-authorship of papers and presentations with scientists and students from host countries, REU supplements and BYU support for extensive undergraduate mentoring, short-term and long-term training of graduate students and faculty from host countries, and support of US graduate students and post-doctoral associates. NSF DEB-9815881 – Blood samples & DNA collected from > 450 turtles representing 18 nesting beaches in Brazil, Colombia, Peru, & Venezuela; 9 microsatellite loci scored on these, & mtDNA control region scored from ~ 290 animals representing the same localities. Almost 1000 more hatchlings sampled (nondestructively) from multiple clutches at selected nesting beaches in BR and VE for paternity studies. Four Brazilians hosted in various capacities; two post-docs supported; several undergrads mentored.

NSF DEB-0132227 – Tissues/DNA collected from > 900 lizards representing: (1) all known species of the 'night lizard' genus *Xantusia* (including multiple individuals & localities of widespread taxa); (2) all but 2 species of *Lepidophyma*; & (3) the monotypic *Cricosaura* – this represents the life's work of Co-PI R.L. Bezy. Co-PIs J. Gauthier & J. Maisano are collecting osteological and CT-scanned data, post-doc J. Pramuk has optimized 7 nuclear gene regions for phylogenetic study, and we have developed 18 microsatellite loci for questions of origin of unisexual taxa. Argentinean couple M. Morando & L. Avila also linked studies of the lizard genus *Liolaemus* to this project, and came to the PI's lab on CONICET fellowships. This work produced a tissue/voucher specimen collection of > 1000 lizards, and multiple papers ranging from alpha taxonomy to conceptual issues in phylogeography in temperate South America. Two foreign visitors & many undergrads have been mentored in field and lab work.

NSF DEB-0309103 (DDIG, J. Marshall & J. Sites) – field work completed with undergrads from BYU and MX; genotypic data summarized from >650 lizards of the *Sceloporus grammicus*

complex, representing 8 recognized chromosome races, mtDNA sequences collected from ~ 450 of these, and nuclear sequences from ~ 90 individuals; Marshall now post-docs at Yale Univ. DEB-0309111 (DDIG, E. Benavides & J.W. Sites, Jr.) – field work completed in Galapagos & Peru; with additional material was borrowed from Galapagos, mainland Ecuador, & Chile. Several presentations have been made and a number of students mentored in lab and field; data collection & analyses are in progress, and Benavides should defend in Oct-Nov. 2005.

Leigh Johnson – Phylogenetic and evolutionary inference in the *Collomia-Navarretia* clade (Polemoniaceae). NSF DEB-0344837, \$302,276 2004-2006. This ongoing project is designed to test for sister relationships between all species of the plant genera *Collomia* and *Navarretia*. We use a multiple population approach to species sampling, and considerable field collection of material for DNA and morphological analyses. In our first year of funding, we have discovered one new species of *Collomia* and 3-4 new species of *Navarretia*, as well as clarified the monophyly of *Collomia* with respect to *Navarretia*. We have also developed novel nuclear markers for addressing species level relationships. Field work in Argentina will begin in 2005 to obtain the relevant South American species, work conducted in collaboration with R. Poznar at Darwinion. This is my first collaborative effort with South American colleagues.

Guillermo Ortí – CAREER: Molecular Systematics of Ray-finned Fishes. NSF-DEB-9985045, \$533,295 (including REU and RET supplements) 2000-2006. **RCN: DeepFin will Advance The Phylogeny of Fishes.** DEB-0443470; \$500,000; 2005-2010. **Paternity, cooperative breeding and genetic chimerism in callitrichids.** IBN-0417202; \$161,198; 2004-2006. The ongoing research funded by the CAREER award has involved several graduate and undergraduate students, a postdoctoral associate, and a local high-school teacher (some through REU and RET supplements). A comprehensive tissue collection with more than 900 specimens organized in a relational database has been compiled. Preliminary results of analysis of more than 400 mtDNA sequences and more than 200 nuclear gene sequences (RAG-1) have been presented at annual meetings during 2002. Two papers have been published and several are in preparation. The teaching component of the award has helped to support development of new courses in molecular systematics and molecular ecology at the University of Nebraska. As a result of coordinating activities with other labs around the world the project spawned the formation of an international consortium of systematic ichthyologists and theoreticians with expertise in the analysis of fish biodiversity (DeepFin), seeking to integrate knowledge of morphology, paleontology, molecular biology, and bioinformatics. A portal for internet resources has been created (www.deepfin.org) that contains a searchable directory and collaborative tools, and funding from NSF's RCN program has been secured for the next 5 years. Another active area of research with current NSF support involves a PhD student (C. Ross) and collaborator J. French (from UN-Omaha) and focuses on marmoset genetic chimerism, documented by microsatellite markers. Prior NSF support (1991-1993) was for **Phylogenetic Relationships of Major Lineages of Characiform Fishes.** DEB-9112367; DDIG for \$10,140. The doctoral dissertation award resulted in five high-profile publications on the phylogeny of characiform fishes.

INTRODUCTION

Over the past decade, scientists have made unparalleled progress in the use of DNA data to unravel some of evolutionary biology's most important puzzles (Avice 2004). One such puzzle is to understand how biological diversity is shaped by the abiotic processes of earth's history, including factors such as climate change, mountain building and erosion, and glacial cycles. The field of phylogeography has emerged to examine the relationship between a species' evolutionary past and its current distribution and abundance (Avice 2000). Molecular genetic data collected from populations within a species can be used to reconstruct several aspects of a species' history, including phylogenetic relationships among populations and their demographic histories (DeChine and Martin 2004; Hoffman and Blouin 2004; reviewed in Funk and Omland

2003) and historical patterns of gene flow over space and time (Jokush and Wake 2002; reviewed in Losos and Glor 2003; Weins 2004a,b). From these findings, biologists can then make inferences about the relative importance of historical factors on speciation, and on current patterns of genetic and phenotypic diversity *within* any given species. Yet, despite this progress, we still know remarkably little about the overall contribution of historical events on region wide biological diversity. Drawing such conclusions requires comparing the evolutionary pasts of *several* different species and species complexes that occupy the same geographic region, a field known as comparative phylogeography. Such research projects are typically beyond the expertise and capacity of any single scientist, so instead must be undertaken as a collaborative partnership among several researchers, each with unique taxonomic and technical expertise.

Patagonia—the southern-most area of South America (Figure 1)—is a part of the world where such an undertaking would yield particularly important results. Unlike temperate regions of North America and Europe where considerable effort has focused on phylogeny reconstruction within and among species (Riddle and Hafner 2005) and where *a priori* hypotheses can be constructed from independent geological and/or species co-occurrence data (Johnson 2002; Knowles 2001), very little is known about the phylogeography of temperate South America. More importantly, for the broad question posed here, no efforts have been made to compare the evolutionary histories of multiple Patagonian species simultaneously. Consequently, it is unclear how and if past historical events have shaped speciation and evolutionary diversification across temperate South America, and if shared historical events in this region have resulted in common patterns of biological diversification.

Two elements of the Patagonian landscape suggest that historical events may have played a crucial role in bio-diversification of southern Chile and Argentina. **First, the Andean mountain range** creates a prominent east-west barrier to contemporary movement and gene flow for many species. Hence, we expect that deep genetic fragmentation coincident with the first rise of the Andes in the lower Cretaceous and subsequent uplift events through the Miocene should be evident in the evolutionary pasts of many species (especially aquatic organisms) that now span this mountain range. For some species, Andean orogeny probably created corridors for east-west movement or created habits of mountain top islands. In these cases, we expect to find a pattern of fragmentation concordant with the peaks and valleys formed by the Andean uplift. **Second, glaciers covered much of Patagonia** at predictable times during the Pleistocene epoch (summarized in Clapperton 1993). Hence, we expect to see shared patterns of range expansion among several taxa—southward in Chile and south-southeast across Argentina—concomitant with glacial retreat at the end of the most recent Pleistocene glacial events (Avila et al. in review).

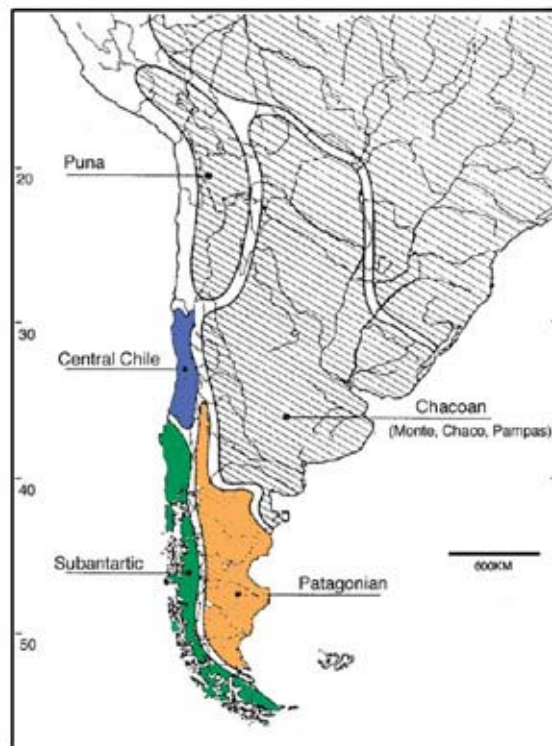


Figure 1. Map of Patagonia (colored area) showing three geographical provinces where we will sample for this study. We will attempt to collect individuals from each taxon from at least 30 common reference regions within Patagonia.

Table 1. A list of Co-PI's and senior personnel participating in this project, including their national and institutional affiliations and their taxonomic expertise.

Country	Institution	Scientist	Taxa
USA	Brigham Young University	Jerry Johnson	Fishes
		Jack Sites	Lizards Frogs
		Keith Crandall	Crabs
		Leigh Johnson	Plants
	University of Nebraska	Guillermo Ortí	Fishes
Canada	Dalhousie University	Daniel Ruzzante & Sandra Walde	Fishes
Argentina	Centro Nacional Patagonico	Mariano Morando & Luciano Avila	Lizards
	Universidad Nacional del Comahue	Victor Cussac	Fishes
	Instituto de Botánica Darwinion	Raul Poznar	Plants
Chile	Universidad de Concepcion	Evelyn Habit	Fishes
		Pedro Victoriano	Lizards
		Carlos Baeza & Eduardo Ruiz	Plants
		Carlos Jara	Crabs
	Universidad Austral de Chile	José Núñez	Frogs

We also expect to find common areas of refuge where both plants and animals must have persisted when ice covered regions that have now been re-colonized by several taxa (see Muellner et al. 2005). Where these refuges occurred is important to know, especially for conservation purposes, as they likely contain much higher levels of genetic diversity than more recently colonized regions.

Clearly, several important biological and conservation questions motivate a multi-taxa comparative phylogeography study of Patagonia. Yet, success in this endeavor will require a carefully orchestrated partnership among scientists with expertise in molecular phylogeography, phylogenetic reconstruction,

morphology and traditional taxonomy, and in the regional flora and fauna and natural history of Patagonia. Our project will bring together such expertise, drawing upon the skills of researchers from Chile, Argentina, Canada, and the United States (Table 1). An intended outcome of this project is that it will also serve as a vehicle to deliver long-term partnerships and training opportunities for scientists and students in each of the participating countries. In other words, our project is designed to promote the kind of cooperative cultural change in science that is needed in order solve some of our most pressing biological problems. Here, we describe our project in two parts; the first detailing our research objectives, hypotheses, methods, and anticipate outcomes, and the second describing how our educational plan will provide a framework for cooperative training during and beyond the five-year funding period from NSF.

RESEARCH PROGRAM

Research Objectives

To evaluate the general impact of historical events on evolutionary diversification in Patagonia, it is necessary to reconstruct the phylogeographic history of several distinct species, preferably from a wide range of taxonomic possibilities. Here, we propose to reconstruct the phylogeographic and demographic pasts of 16 species (or species complexes) that share a common geographic distribution (Table 2). In so doing, we will accomplish three fundamental research objectives:

- 1. Conduct coordinated field work across Patagonia.** We will make extensive standardized collections of voucher specimens, tissues, and digital images from selected groups of fish, lizards, frogs, freshwater crabs, and flowering plants endemic to and co-distributed across Patagonian habitats. We will catalog and collect this information now while Patagonia remains relatively pristine so that consequences of future environmental assaults might be fully evaluated.

Table 2. A list of taxonomic groups to be included in this study.		
Fish	Patagonian Silverside	<i>Odontesthes hatcheri</i>
	Trout Perch	<i>Percichthys trucha</i>
	Patagonian Catfish	<i>Hatcheria macreii</i>
Lizards	Andean lizards	<i>Liolaemus elongates-boulengeri-bibroni groups</i>
	Chilean lizards	<i>Liolaemus clades 1 & 2 (Schulte et al. 2000)</i>
Frogs	Chilean frogs	<i>Eupsophus calcaratus</i>
		<i>E. emiliopugini</i>
Crabs	Freshwater crab	<i>Aegla abtao</i>
	Freshwater crab	<i>A. laevis</i>
Plants	Desert thorn	<i>Lycium ameghinoi</i>
	Scarlet trumpet flower	<i>Collomia cavanillesii</i>

2. Implement molecular and morphological studies to determine patterns of Patagonian biodiversity across space and time. These data will be used to generate robust phylogenetic patterns within and among species, to evaluate testable hypotheses of species boundaries and historical distributions in the selected groups, and to test for co-phylogenetic congruence among co-distributed endemics.

3. Test general biogeographical hypotheses and formulate the next generation of specific hypotheses to explain biodiversity patterns in Patagonia. We will evaluate patterns of speciation and comparative phylogeography against a backdrop of the Andean mountain uplift and past cycles of Pleistocene glaciation. A novel feature of our study is that we will also produce a set of new or refined hypotheses to explain regional patterns of genetic diversity in Patagonia, an important contribution given the paucity of region-wide data currently available to generate such predictions.

Generating Hypotheses

As described above, Andean orogeny and Pleistocene glaciation likely have had pronounced effects on patterns of regional biodiversity and genetic variation in Patagonia. Below we highlight evidence from preliminary data on lizards and fishes that support this general assertion. Yet, geological and species co-distribution data alone remain insufficient to pose detailed ideas on specific historical factors responsible for speciation and the spread of species across Patagonia through space and time. Hence, we intend to use these studies to *generate* specific hypotheses that can be tested in a rigorous *a priori* framework using coalescent and co-phylogeny approaches (see Avila et al. *in review* for an example in *Liolaemus*). In this regard, our study will have the additional outcome of producing new hypotheses against which geologists and palaeo-climatologists can also evaluate their data; this 'regional biota' approach has proven useful in suggesting the presence of cryptic vicariant events that had not been obvious prior to molecular phylogenetic/phylogeographic studies (Patton et al. 2000; Riddle and Hafner 2005). What then is the most appropriate way to use genetic and morphological data to uncover the next generation of biogeographical hypotheses for Patagonia?

Nested clade analysis: a hypothesis generating technique – One statistical method that can very effectively be used to generate new data-driven hypotheses is the NCA (Templeton et al. 1995; recently extended as “nested clade phylogeographic analysis” [NCPA] to provide cross-validation of single locus inferences by using multiple unlinked genes [Templeton 2004]). The original NCA has been criticized for failing to consider multiple historical scenarios simultaneously, or to provide error terms for a particular causal factor, when inferring population histories (Knowles and Maddison 2002). Limited simulations suggest that the NCA inferences may mislead (Knowles and Maddison 2002; but see Templeton 2004), and while this is not the only population structure estimator susceptible to such error (Abdo et al. 2004), the application of NCA to single gene trees does offer the possibility for over-interpretation if appropriate caution is not applied (Knowles 2003 [table 1]).

Further progress will obviously depend on the incorporation of unlinked nuclear markers into phylogeographic analyses (Rosenberg and Nordborg 2002; Hey and Machado 2003), but another issue is the fundamental shift away from the traditional null hypothesis testing approach

in ecology and evolutionary biology to *a priori* model selection, in which several competing hypotheses are evaluated simultaneously (Johnson and Omland 2004). This is precisely the argument for the emergence of “statistical phylogeography” (Knowles and Maddison 2002), but implementation of this paradigm requires that it successfully confront two major issues: the stochasticity of coalescent histories of unlinked genes (Hudson and Turelli 2003), and the potentially complex histories of species and populations (including migration, admixture, divergence in isolation or with gene flow, population bottlenecks and expansions; Knowles, 2003). Conducting such a study requires that an investigator: (1) collect data for multiple gene genealogies (Wakeley and Hey 1998; Templeton, 2004), (2) specify a sufficient number of alternative historical hypotheses to approximate biological reality, but not to offer so many alternatives that spurious findings become likely (Johnson and Omland 2004), (3) make decisions about a model’s complexity (complex models may incorporate more biologically meaningful parameters, but at the expense of requiring more data to distinguish among alternative hypotheses; Knowles 2003); and (4) integrate external data, such as paleoecological or bioclimatic information (Hugall et al. 2002).

Although this is an ideal approach, its use in most phylogeographic studies is limited because independent nuclear genetic markers remain unavailable for many organisms, and the cost for their development may be non-trivial (Avice 2004; Morin et al. 2004). Second, statistical phylogeographic models that both accurately represent the genealogical histories (and need to incorporate a wide array of potential processes), and in a manner in which alternative hypotheses can be distinguished statistically, are not yet developed (Knowles, 2003; p. 4). Finally, in many poorly-studied groups of organisms from poorly-sampled regions, there may be little or no usable external data from which to develop plausible *a priori* hypotheses necessary for this approach. In these cases, the NCPA null model approach is a good place to begin, and the mtDNA locus often will have to be used at least as the ‘first pass’ marker to assess general patterns of population variation, and to formulate more specific hypotheses that can be further tested with other classes of markers. On the positive side, this locus should track recent population splits with higher fidelity than a single nuclear marker under many biologically plausible scenarios, and mtDNA phylogeographic analyses have become increasingly sophisticated as the limitations of the single-gene approach are better understood (Funk and Omland, 2003; Ballard and Whitlock 2004). For example, the assumption of neutral evolution is now routinely tested, and cross-validation of NCPA inferences is possible by a number of independent criteria (Morando et al. 2004; Carstens et al. 2004). In this context, what has been viewed as a weakness of NCPA in an inferential framework is in fact its primary strength when used as a *post-hoc* analysis tool—that is, it provides the best supported explanation for the roles of various historical events that could have shaped the distribution of genetic variation within a species, or among closely related species, over space and time.

In this project we will use NCPA to generate preliminary hypotheses for poorly-studied groups (plants, frogs, and most fishes), and for the better-studied groups (crabs, lizards) we will use previously generated NCPA hypotheses as null models for more sophisticated tests. All of these can also be used as *a priori* predictions for species not included in this study, or when considering several species simultaneously in comparative phylogeography tests (see below).

Methods

Study Area, Field Collections, & Tissue Acquisition – In this study, we define Patagonia as the southernmost region of South America extending from approximately 38 ° S to the tip of Tierra del Fuego (about 54 ° S); this geographic boundary coincides with The Nature Conservancy and World Wildlife Fund’s southernmost freshwater eco-region. Within Patagonia, collections will be made at up to 48 different sites. To facilitate comparisons among taxa, we have identified 25 reference locations (Figure 1); efforts will be made to collect each species as close as possible to these reference sites in order to ensure comparable geographic coverage

for each taxon. At each location, digital photographs and tissue samples will be taken from up to 48 individuals and up to 50 whole individuals will be kept for museum voucher specimens at the host country's museums/herbaria. All field collected animal tissues will be preserved immediately in 95% EtOH and plant tissues in desiccating silica preparatory to laboratory analysis. Species will be collected as follows: fishes will be collected by electrofishing, seine nets, or gill netting; lizards will be collected by hand, noose, or rubber bands; frogs will be collected by hand and seine nets, crabs will be collected by hand, dipnet, and/or trapping; and plants will be gathered from leaves, with whole plants or segments significant for identification pressed for use as vouchers.

Our target species (Table 2) were selected based on several criteria. First, we chose species with maximum geographic overlap in Patagonia; this ensures our ability to carry out the multi-species analyses described below. Second, we focused on endemic taxa and/or taxa that were likely to be impacted by Patagonian historical events. Third, where possible, we chose taxa for which preliminary data were available, and where sampling expertise existed among our Patagonian collaborators. Fourth, we chose taxa that overlapped in areas where they could be most easily collected (e.g., Andean lizards and plants in similar terrestrial environments, and fishes and crabs in similar aquatic environments). Using these criteria, we are confident that we have identified a representative group of species able to reflect any shared effects of common historical events on speciation and evolutionary divergence in Patagonia.

Molecular Laboratory Protocols – Laboratory protocols and genetic markers to be used will vary depending on the species under investigation; however, for each species we will collect both mitochondrial and nuclear DNA data (Table 3). All of our protocols take advantage of the high-throughput capacity and cost-efficiency of the BYU DNA Core Sequencing Facility, the Dalhousie University Gene Probe Laboratory, and the UNL Core facility. For each taxon, genomic DNA will be extracted from preserved tissues using Qiagen DNEasy 96-well plate kits. PCRs for sequencing and for microsatellites will also be conducted in 96 well plates using standard protocols optimized for each specific gene or microsatellite region (references for

Table 3. A list of genes and molecular markers to be targeted by taxonomic group for use in this study.			
Taxa	Mitochondrial / Chloroplast	Nuclear	References for primers and protocols
Fish	cyt <i>b</i> , d-loop	s7 ribosomal, RAG-1 intron several nuclear genes microsatellites	Johnson et al. 2004 Hassan et al. 2002 Behrengaray & Sunnocks 2000
Lizards	cyt <i>b</i> , ND4, 12s	several nuclear genes microsatellites	Morando et al. 2003, 2004; Avila et al., <i>in review</i>
Frogs	12s, 16s	several nuclear genes microsatellites	Pauly et al 2004 Pröhl et al. 2002
Crabs	COI, 16s	microsatellites	Perez-Losada et al. 2004
Plants	trnS-G, trnD-T	IDH microsatellites	Shaw et al. 2005 Weese & Johnson 2005

primers and protocols listed in Table 3). Reactions will be cleaned using the Millipore Montage 96-well vacuum system. Our laboratories regularly carry out 1/16th and 1/32nd cycle sequencing reactions using the ABI Dye Terminator chemistry (Perez-Losada et al. 2004) allowing us to cut our costs considerably

over standard reaction concentrations. Cycle sequencing reactions will be cleaned using hydrated sephadex in a 96 well plate format. Both sequencing and microsatellite reactions can be run on the ABI 3730xl and 3100 automated sequencers housed in the BYU core facility or on the LI-COR, CEQ and FMBIO automated sequencers housed in the Dalhousie core facility (these machines and a full-time technical support staff funded by BYU and Dalhousie, respectively, will be available to receive samples from all participants on the project, regardless of institutional affiliation). Similar equipment is available in Orti's lab at UNL.

Molecular Data Analyses – All sequence data will be coarsely aligned and pruned using Sequencher (GeneCodes Corp.) and finely aligned using Clustal X (Thompson et al. 1997) and adjusted by hand if necessary. Microsatellite data will be scored using binning algorithms constructed in the ABI fragment analysis software package, Genotyper. Once these sequences

and microsatellite results are gathered, we will evaluate our data at two levels: (1) as single species; and (2) as a collective set of co-distributed species.

Single species analyses: We will examine each group independently to evaluate species boundaries, to reconstruct the spatial and demographic history of populations within a species, and to identify associations between intraspecific diversity and past historical events. For the lizard, crab, and fish (one species) groups, sufficient data have been collected to conduct preliminary NCPA analyses resulting in a set of *a priori* hypotheses that can be evaluated with independent data (see lizard example below in *Preliminary Data and Progress to Date* section). In the remaining groups, we will need to first conduct a set of preliminary analyses to make *a posteriori* inferences about population histories and phylogeographic inferences. This will be done as follows. To facilitate traditional phylogenetic analyses, we will use the AIC score in ModelTest (Posada and Buckley 2004) to identify the model of molecular evolution that best explains variation in DNA sequences within each species. With this model, we will reconstruct phylogenetic hypotheses of haplotypes using both maximum likelihood (Felsenstein 1981) and Bayesian analyses (Huelsenbeck et al. 2001). These methods are effective at determining deeper evolutionary splits within and among species. We will use statistical parsimony network reconstruction techniques (Clement et al. 2000) to resolve relationships within shallow haploclades, which will serve as the basis for NCPA using the GeoDis program (Posada et al. 2000). These results will be interpreted using the most recent inference key (Templeton 2004) to generate *a posteriori* explanations about the historical processes that best explain the distribution of genetic variation across a contemporary landscape. Once such hypotheses are generated, they become *a priori* hypotheses to test with subsequent analyses and subsequent organismal groups. Here we will analyze the relevant molecular data using statistical approaches that allow the estimation of key parameters such as migration rates and levels of genetic diversity. Such methods are extensive as are their relative strengths and weaknesses. We have recently reviewed these methods (Pearse and Crandall 2004) and we propose to use a suite of these approaches to further analyze the data (see below for an example and details on specific methods).

Multispecies analyses: We will also conduct sets of comparative phylogeographic analyses using data from all aquatic and then terrestrial species. These analyses will allow us to test for significant patterns of correlated evolutionary divergence among different taxa (e.g., one lizard species versus another, or lizards versus plants, etc.) that provide evidence of shared histories. Once such patterns are detected, we can then identify features of earth history that could most likely account for congruent multi-species patterns of divergence. We will use two techniques to test for common effects of shared historical events. The first is a co-phylogeny approach, an event-based modification of Brooks Parsimony Analysis of (Ronquist 2003), with significance testing of phylogeographic congruence among repeated taxon pairs assessed by Bonferroni correction of the means. The second technique described by Lapointe and Rissler (resubmitted), has the desirable property of being able to detect common phylogeographic patterns among taxa with only partially overlapping distributions. Accordingly, multiple, independent area trees (phylogeographies) are tested for congruence by generating a maximum agreement subtree (MAST) for each pair of source trees. The size of the MAST is used as a congruence index (the larger the MAST, the more congruent the two area trees are). Comparisons of different sized trees are normalized by dividing MAST size by the number of areas common to the two trees. Significance of congruence between pairs of area trees is determined by randomization of trees with the same number of areas and computing their MAST score to determine if congruence of the original pair of area trees is greater than chance alone. Area trees that are statistically congruent are then combined using matrix representation with parsimony (MRP with triple fit, as it is not biased by shape of input trees [Wilkinson et al., in press]). The supertree summarizes geographic areas that correspond to lineage breaks in multiple taxa with unique ecological and life history characteristics, providing strong evidence for

a shared history of vicariant isolation or strong selection. Influential geophysical factors that may have played a role in generating such lineage breaks, such as climate variables (i.e. humidity, precipitation, radiation, temperature, etc.,) will be tested via multiple analyses of variance (MANOVA). Molecular divergence dates across multiple taxa will be tested for coincidence with Tectonic (Andean uplift) and glaciation events.

We demonstrate the utility of this MAST supertree approach below (see *Preliminary Data and Progress to Date*) using data from three Chilean *Liolaemus* species with partially overlapping distributions. We are aware that there are alternative approaches to integrating phylogenetic and phylogeographic studies of co-distributed taxa to retrieve shared historical patterns and identify areas of endemism (Brooks 2004; Riddle and Hafner 2004, 2005; Szumik and Goloboff 2004), and given the development of new techniques we acknowledge that approaches we ultimately use at the end of this project will not likely be what we describe here. Nevertheless, the combined approaches described above are currently most compatible with our needs to integrate molecular data with morphological and microsatellite data to both delimit species in many groups, and to link phylogenetic and phylogeographic inferences methods across species and species complexes with only partially overlapping distribution. Further, because Pleistocene glaciation cycles have likely had a large impact on current distributions of many of our taxa, phylogenetic comparative methods that do *not* include a means to distinguish historical from recent demographic histories are not likely to be suited to many of the methods described in the above reviews (Losos and Glor 2003).

Morphological Data Analyses – Morphological data provide an important complement to the molecular data in this study. Such data have long been used as the basis for taxonomic description in Patagonia (Arratia et al. 1983; Ringuélet et al. 1975; Avila 2003; Avila et al. 2003, 2004; Ruzzante et al. 1998, 2003). Hence, we are interested in the degree to which morphological and molecular data give consistent results in species delineation. Gross differences between the two kinds of data could alert us to the instability of our current estimates of biological diversity in Patagonia. Hence, where appropriate, we will test morphological taxonomic boundaries against our molecular hypotheses, as recently demonstrated by Johnson et al. (2004). We will rely primarily on multivariate assessments of meristic characters where these can be applied (lizards, fishes), and also use geometric morphometric techniques (Baylac et al. 2003) to quantify morphological variation in shape within each of the species (or putative species) included in this study. This technique has proven to be an effective independent test of species boundaries (Johnson et al. 2004). In brief, two-dimensional morphological landmarks will be scored from digital images of each of the individuals collected in this study; these data serve as inputs to the program TPSREGR (F.J.Rohlf 2002, <http://life.bio.sunysb.edu/morph>) which generates a consensus shape of the entire sample of landmark points. The program then computes two measures of shape variation for each individual (uniform components and partial warp scores) that can be used as inputs in a multivariate regression analysis to test for differences in shape among populations or among samples.

Preliminary Data and Progress to Date

A Priori Hypotheses - Analyses of Patagonian Lizards

SPECIES BOUNDARIES - Broad surveys of mtDNA variation in several complexes of *Liolaemus* from Argentina have been completed (Morando et al. 2003, 2004; Avila 2003, Avila et al. 2003, 2004, in review), and these studies offer new hypotheses about species boundaries and evolutionary vs. demographic histories in several widely distributed groups. For example, Morando et al. (2003) identified 10 “candidate” species from a complex of three nominal species (*L. elongates*, *L. buergeri*, and *L. petrophilus*), on the basis of mtDNA trees and a NCPA inference of historical fragmentation, and concordance of this pattern with morphological differences (color, body size, etc.). Such results provide *a priori* hypotheses of species boundaries that will be tested in a quantitative framework (reviewed by Sites and Marshall,

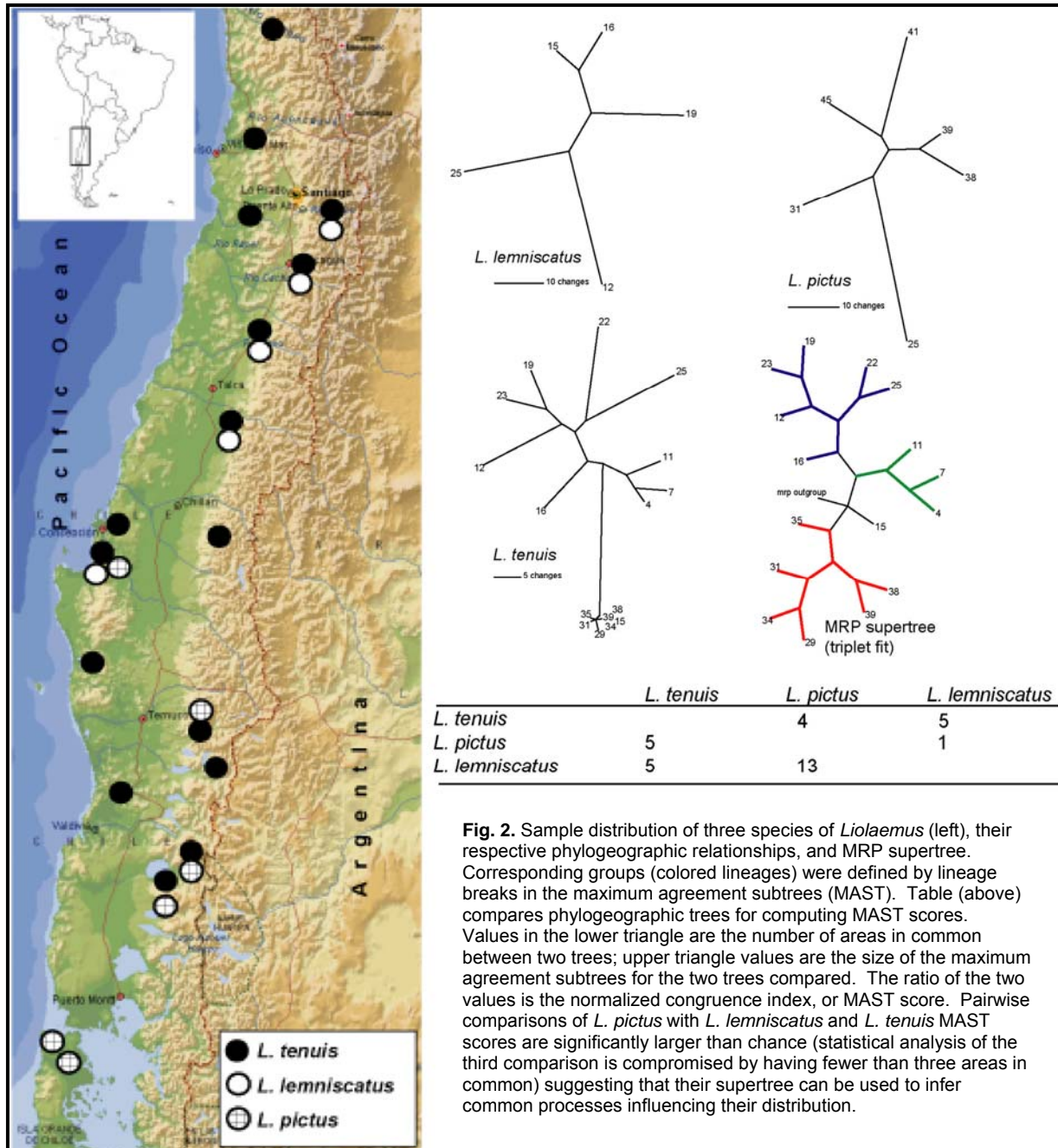
2003, 2004). Here we will test *Liolaemus* species boundaries using geometric morphometric methods as implemented by Johnson et al. (2004), and with microsatellite data. Newer gene flow estimators do not assume equilibrium (Whitlock and McCauley, 1999; methods reviewed by Pearse and Crandall, 2004), and some (individual-based assignment tests) appear to provide especially good estimates of dispersal and gene flow (validated using long-term mark-recapture data) while being robust to violations of some assumptions about mutation mechanisms (Berry et al. 2004). We will also use model-based clustering methods (as recently applied in our Amazon river turtle studies; Pearse et al., in prep. and by Ruzzante et al. to Atlantic cod) implemented in the software packages STRUCTURE (Prichard et al. 2000) and BAPS (Corrander et al. 2003); these likelihood and Bayesian approaches estimate the number of discrete populations in a sample. We also used individual-based assignment tests as alternative indicators of population differentiation and gene flow among populations, including the Bayesian estimator of migration rates implemented in the program BAYESASS+ (Wilson and Rannala 2003), which estimates migration among all populations based on the proportion of individuals sampled in each population that assign to another population. In the turtles all methods give the same picture of population structure across the Amazon basin (Pearse et al., in prep.), and we interpret concordant results obtained from analyses of the same data with a variety of methods as evidence that the results accurately reflect the signal present in the data and are not influenced by violations of method-specific assumptions (Jones et al. 2004). For the target taxa of this project, we will take a similar approach, and infer species limits where independent data (Templeton 2001) and methods show the highest degrees of concordance.

DEMOGRAPHIC HISTORIES - In these same studies we have previously inferred from NCPA the biological causes of intraspecific patterns of genetic variation in *Liolaemus* (Morando et al. 2004; Avila et al., in review). Each of these inferences can be treated as a hypothesis to be evaluated using additional tests. For examples of continuous range expansions inferred for several clades in the *L. darwini* complex (clades 1-10, 2-1, 2-2), or restricted gene flow with isolation-by-distance (clades 2-8 [?] and 3-3; see Morando et al. 2004), we will employ the graphical methods of Hutchison and Templeton (1999) which provides statistical power to distinguish between equilibrium (= restricted gene flow/isolation-by-distance) from nonequilibrium (= continuous expansion) populations (both of which could show 'gene flow' signatures by cluster and assignment tests described above). Historical fragmentation events (candidate speciation events) should also be reflected in divergence across multiple loci, including nuclear gene sequences with informative mutation rates (Benavides et al., unpublished.).

Multi-species Analysis of Patagonian Lizards: Comparative Phylogeography

To explore the congruence of co-distributed species, we generated phylogeographic hypotheses of *Liolaemus pictus*, *L. tenuis* and *L. lemniscatus* using cytb sequences of 92, 121, and 51 individuals from 10, 22 and 10 locations, respectively. Areas were delimited as 100-degree square latitude/longitude grids. Identical sequences from each area were removed from each data matrix, followed by phylogenetic analysis. Areas that were monophyletic (all sequences from a single area more closely related to each other than to a sequence from another area) were pruned to a single representative sequence, and then the individual haplotype names were replaced with their area name. A maximum agreement subtree (MAST) was generated for each pairwise combination of area trees, followed by randomization of trees with the same number of areas. For each pairwise comparison, the size of each MAST tree was then normalized by dividing the size of the mast tree by the number of areas common to both trees, and compared to the distribution frequency of the MAST scores of 1000 randomly generated trees with an equal number of shared areas as the original MAST tree. Congruence of area trees was inferred when the test was significant (i.e. larger than randomly generated MAST scores, 95%, for $\alpha = 0.05$). All three area trees were shown to be statistically congruent ($p = 0.001$), but one comparison (*L. pictus* x *L. lemniscatus*) was compromised by having too few areas in common (trees of three or fewer common areas will have a biased MAST score

due to the limited number of possible topological variants). The supertree of the area trees was generated using triplet fit MRP. Groups of areas on the tree that showed lineage breaks somewhat consistent with their geographic areas were identified (Fig. 2). Sufficient power to test whether these geographic regions differ from each other with respect to geophysical variables (MANOVA of regional climate data) or tectonic and glaciation events (correlation of divergence dates) will come from the addition of area trees for numerous other co-distributed Patagonian taxa.



In addition to the lizard described in the example above, we have made substantial progress demonstrating our ability to successfully carry out this work for each of the other taxa

to be included in this study. Preliminary work (e.g., sampling design, field collections, primer development, tissue and voucher archiving, morphometric analyses, etc.) is underway in some form for each of the groups to be evaluated in this study, including the fishes (Cussac et al 1998, Ruzzante et al 1998, 2003; Logan et al. 2000; Milano et al. 2002, 2005), frogs (Nuñez et al. 1999), crabs (Pérez-Losada et al. 2004) and plants (see L. Johnson Previous NSF Funding above). Using the methods outlined above, and illustrated with the lizard example, we are confident that we have selected a set of candidate taxa that are highly likely to reveal any patterns of shared historical effects among taxa in Patagonia.

EDUCATIONAL PROGRAM

Educational Objectives and Overview

This project will broaden the expertise of all the participating scientists and will train the next generation of students and young scientists in collaborative international research. Here we propose an educational program designed to accomplish three basic objectives: **1. Bring together key individuals from different scientific cultures to share ideas on how to best study and address biodiversity issues in temperate South America.** Our project targets biologists that operate in a wide range of social and cultural contexts. Workshops, field courses, and annual meetings will promote the exchange of different philosophies, research techniques, and technical expertise. This will promote new ways of looking at biodiversity issues in Patagonia. **2. Teach young scientists, graduate students, and undergraduate students how to flourish in broad international research partnerships.** By design, our project includes PIs and senior personnel with different levels of international experience—this is intended to create a mentoring environment for the next generation of young scientists. Largely through non-NSF funding, we plan to mentor over 50 individuals with little or no previous experience in international research (South American, Canadian, and US students); we will target undergraduate, graduate, and postdoctoral researchers representing the four host countries. **3. Establish a long-term program for scientific and educational interaction between biologists in the United States and Canada and their counterparts in temperate South America.** We are fundamentally committed to maintaining a long-term partnership between scientists and students in the U.S. and our counterparts in Patagonia. We will use some funding to develop a bi-lingual web-based communication network to promote ongoing correspondence among scientists and students. We will also establish an international internship program for students designed to function in perpetuity, with ongoing support coming directly from students and their host institutions. Finally, our project is designed to stimulate future research, include work in ecology, geology, and conservation science.

Cooperative Learning among PIs and Senior Personnel: Our project brings together a group of 17 senior level scientists. We have recruited individuals with different technical, taxonomic, and analytical skills, different levels of research experience in South America, and at different stages of their academic careers. Such variation creates an atmosphere where all participants are able to contribute and to learn from others. Our project promotes intellectual and cultural exchange among senior level scientists in several ways. First, it will help establish young scientists in South American research. For example, among the PIs, J. Johnson and L. Johnson are relatively young faculty members who will benefit greatly by working with Sites, Crandall, Ortí, Cussac, Ruzzante, and other biologists with extensive experience in Patagonia. Second, we will conduct field work together in Patagonia (with three research expeditions planned for each taxonomic group over the first three years of the grant) and laboratory work together in the United States and Canada (planned for years two through five). Third, we will hold annual meetings (described below; see timetable and locations of meetings in Budget Justification) where senior personnel can plan and update research and our educational objectives, report findings, and collaborate on publishing our results. Fourth, we will cooperate in training postdoctoral scientists, graduate students and undergraduate students. Fifth, we will

encourage sabbatical leaves for training in an international setting. Finally, we have invited Biff Birmingham and Chris Simon, two highly respected outside scientists, to meet annually with our senior personnel to provide feedback on our research and educational efforts.

International Training for Students: An essential element of this project is to train students and young scientists to be successful in an international education and research setting. We plan to develop individuals at the undergraduate, graduate, and postdoctoral levels using a combination of training opportunities. We will especially recruit native South Americans to these positions as possible to train and return the expertise and collaboration to their home countries. We have an outstanding track record of training South American visitors in our labs.

Study Abroad Internships – We will establish an international study abroad internship program for graduate and undergraduate students at the participating institutions. Each university and institute has agreed to receive a select number of students from other countries, with a commitment to provide those students with a research-based educational experience. In most cases, this internship will last 8 weeks (1 academic block) and will center on some aspect of the overall research project, but it need not be limited to this work alone. Support for these students will largely come from their host institutions, but with supplemental funding available through this grant. For example, BYU will match NSF funding up to \$20,000 per year for BYU undergraduate internships (BYU Mentoring Environment Grants to J. Johnson and L. Johnson and Sant Undergraduate Fund) and up to \$5,000 per year for BYU graduate internships (Integrative Biology Graduate Travel Fund). We anticipate recruiting a mix of undergraduate students from both science and non-science majors, and graduate students with strong promise to develop into future leaders in international biological research. The BYU Kennedy Center for International Studies has agreed to provide a cultural orientation seminar for U.S. students traveling to Chile and Argentina (see letter of support).

Regional Workshops and Field Course – We will also offer three 3-day workshops (at BYU, in Chile, and in Argentina) available to students, postdocs, and senior personnel at each of the participating institutions. These workshops will be taught by senior personnel, and will focus on Patagonian natural history, field techniques, sampling design, molecular methods, geometric morphometrics, and data analysis. Finally, we will design a four week field course (*InBio 549: Patagonian Natural History*) available for 8 graduate students to travel to Chile and Argentina to be introduced to the unique biological diversity of this region. This course will be team taught by a subset of the PIs and senior Chilean and Argentinean personnel in January 2008 and 2009.

Annual Meetings: Each year we will hold meetings to coordinate our research efforts and to participate in workshop and professional development activities. In November of year 1 we will hold a South American regional orientation meeting in Chile (in association with the regional Chilean workshop described above) designed to introduce individuals to one another and to lay out our sampling and field work plans. In years 2-4 we will hold annual meetings at BYU, at Centro Nacional Patagonico in Argentina, and at Snowbird Utah, respectively. The purpose of these meetings is to present progress updates, to plan future activities, and to engage in cross-training activities (see timetable in Budget Justification for specific activities). In our final year of funding we plan to hold two meetings. The first is to bring participants to the international scientific meetings sponsored by the *Society for the Study of Evolution* and *Society of Systematic Biologists*; we will propose to sponsor a symposium on comparative phylogeography. This meeting will also provide a venue for all participants to present their findings to the broader scientific community. Our second meeting will be a final symposium for the project to be held in Chile at the CIEP Conference Center (Patagonian Ecosystem Research Center; facility described below in Management Plan). We will invite all participants to present their findings and also invite representatives of from NGOs interested in Patagonian conservation (*The Nature Conservancy* and *The World Wildlife Fund*), government representatives from Chile and Argentina, and the 'next wave' of scientists and students that might be able to build upon work conducted in our project, including U.S. and South American

geologists, ecologists, and science educators. Our intent with both of these final meetings is to raise the profile of international collaborative research within the US research and education communities.

Communication: A project of this scope requires clear communication among participants, and a mechanism to communicate our findings to the broader scientific community and public. We will foster communication among participants using several formats. First, we will establish a bi-lingual web page (to be maintained at BYU) that will provide information on participants, progress updates, information on workshops, recruiting information for undergraduate and graduate students, and any other topics to keep participants up to date. Second, we will use the annual meetings and workshops described above as a way to allow face-to-face discussions about research, and to cross-train one another in research methods, taxonomy, natural history, etc. Finally, we anticipate several extended international exchanges (senior personnel on faculty leaves and students on internships) which will provide opportunities for individuals to become immersed in a new culture of learning and training. We will facilitate communication with the broader scientific community through our web page, by presenting our results at annual international meetings, and by publishing our work in high-quality, high-impact journals.

EVEN BROADER IMPACT OF PROJECT

Our project will have broad impact that goes beyond the current research and educational objectives listed above. **Conservation Partnerships:** Our project will provide key data and an educational framework for several conservation-related activities in Patagonia. We are working closely with *The Nature Conservancy* and with the *World Wildlife Fund* (see letters of support) to ensure that our research plan coincides with their efforts to define data-supported Freshwater Ecoregions of South America. In addition, the Chilean national government has called upon their scientists (see Table 2 for Chilean participants) to generate basic ecological and genetic data that can be used identify areas for government protection (currently 80% of the land of Chile is held by the government, including some of the most pristine temperate forests in the world). Our project will also help fulfill the objective of the Roger Sant Endowment at BYU: Sant is a board member of the *World Wildlife Fund* and has established an endowment of 1.1 million dollars in the BYU College of Biology –Agriculture, for the purpose of educating undergraduate students in conservation science, with an emphasis on field experience and the importance of worldwide conservation efforts. Our internship program will help accomplish this institutional objective (see D. Shiozawa letter of support). **Under-represented Groups:** Our work will promote the inclusion of several groups under-represented in evolutionary science, especially women, Native American, Black, and Hispanic populations. BYU is a large university attracts students from all U.S. states, from Native American reservations, and from countries all over the world. Consequently, we have several individuals from under-represented groups that can be recruited into our project, as we have demonstrated with our past research efforts (see Results from Prior NSF Support). **Creating a Foundation for New Research:** Our research will provide a model for conducting comparative phylogeography studies in areas of the world where few *a priori* data are available. We plan to share our insights via our web page (see above), through talks at scientific meetings, and at a proposed symposium of the *Society for the Study of Evolution* Annual Meetings in 2009. Our work will provide many of the first hypotheses for regional and local biogeographical patterns in Patagonia—this outcome will provide an independent framework against which geologists, community ecologists, and conservation biologists can test their own data. The project is also designed so that other phylogeographers can examine new taxa not included in the current project (e.g., mammals, birds, insects, etc.). In fact, we have identified several additional scientists with interests in the phylogeography of Patagonia that could be drawn into our comparative research effort beyond the duration of our funded project (e.g., Patagonian scarab beetle research group at Nebraska: [14](http://www-</p></div><div data-bbox=)

museum.unl.edu/research/entomology/SSSA.htm, or mammal phylogeography work in S. America by Duke Rogers (BYU) and Jim Patton (UCBerkeley).

Patagonian Partnerships: Although our primary focus will be on building partnerships between the U.S. scientists and our South American counterparts, the project will also provide an opportunity to strengthen scientific, NGO, and government ties between the two Patagonian countries: Argentina and Chile. We recognize that permanent solutions to conservation problems in Patagonia ultimately depend on the cooperation of scientists and governments in Patagonia; our project will promote this kind of cooperation. **Strengthening Taxonomic**

Expertise: We face a world-wide crisis of losing taxonomic expertise as senior scientists with strong taxonomic background retire without passing on their accumulated knowledge. By bringing together experts with such a wide-range of taxonomic skills (at workshops and annual meetings), our project will ensure promulgation and discovery of taxonomic knowledge in Patagonia. Further, our molecular and morphological based approaches will enhance this accumulated knowledge. Students and young scientists will particularly benefit by being trained in the taxonomy and natural history of Patagonia (see descriptions above of taxonomy-based internships and field courses available to students). **Strengthening International Studies at**

BYU: An important outcome of this project will be that it will further broaden the reach of the BYU Kennedy Center for International Studies (see letter of support). Currently, this program is exceptionally strong in international politics, social and cultural science, and government studies. Our project will add an important biological science element to this reputable center for international work. By integrating our efforts with the Kennedy Center, we will also ensure that our project will persist beyond the 5 year NSF funding period.

MANAGEMENT PLAN, INTERNATIONAL COORDINATION, & LOGISTICS

A project of this magnitude requires a well designed plan for managing resources, coordinating research efforts, promoting effective communication, and ensuring clear implementation of educational plans. Our project is designed to make optimal use of contributions from each participant, from their sponsoring institutions, and from several additional supporting entities. Here we outline how we intend to carry out this ambitious effort.

Management Responsibilities: J. Johnson, the PI, will assume responsibility for the coordination and execution of the overall project. This said, the PI and all four co-PIs will work closely together with our international partners to oversee the success of the project. Primary management stewardships for specific components of the project will be divided among PIs and senior personnel. The role of these individuals will be to *coordinate* efforts in these categories and to serve as a resource to all other participants on the project. These are as follows:

- (1) Research Design, Data Analysis, Publication – Crandall, Cussac, Avila, Ruzzante
- (2) Field Work & Travel Logistics – ARG: Sites, Avila & Cussac; CHL: Sites & Victoriano
- (3) Education - Graduate & Undergraduate Internships – J. Johnson & L. Johnson
- (4) Education - Faculty & Senior Personnel Development – Orti, Cussac, & Ruzzante
- (5) Meeting & Workshop Planning – J. Johnson, Walde, Habit, & Morando
- (6) Communications: Bilingual Web Resources, Language & Cultural Training – J. Johnson, Sites, Habit, and Morando.

In addition to these specific components, senior scientists grouped by taxonomic expertise will work together to design and carry out the field and laboratory components of field sampling and data collection for each taxonomic group. We expect close coordination among individuals working on aquatic taxa (fishes and crabs) and among individuals working on terrestrial taxa (lizards, plants, and frogs).

Project Execution: The timetable (see Budget Justification) outlines when different components of the project will be implemented. **Research Design and Analysis**—In our first year, we will hold a senior personnel orientation meeting in Chile (UConcepcion). At this time we will meet to discuss the sampling design and to outline the collecting schedules for each

species/species complex to be included in this project. During these meetings, a brief introduction to analysis techniques will also be discussed, preparatory to annual meetings in years 3-5 where discussion and training will focus on data analyses and publication. **Field Work & Travel Logistics**—Sites will oversee travel and field work; he has research experience in both Chile and Argentina and will consult with members of each taxonomic group to ensure optimal logistical arrangements. In each of the first three years, participants will conduct field work in Patagonia to collect tissues and voucher samples, and photograph specimens. Travel accommodations from the United States and Canada to Argentina and/or Chile will be arranged through the BYU Travel Center. Once in country, travel will be by four-wheel drive S.U.V. (three vehicles to be purchased by this grant; two for use in Argentina and one for use in Chile) or by vehicles made available through the partner institutions (see partner contributions below). Where necessary, we will also use vehicle rentals. Housing for field work will be at local hotels or at any of the sponsoring institutions when sampling efforts are close to these sites. All collecting sites are reasonably close to adequate medical facilities in case emergencies arise; J. Johnson has past certification as an emergency medical technician and several others are first aid certified. **Educational Internships**—Each participating organization has agreed to receive undergraduate or graduate students for 8-week internships, beginning in year 3 and continuing through year 5. J. Johnson and L. Johnson will oversee this program. It will be the responsibility of the sending institution to recruit intern candidates and to arrange financial support for these students to travel and subsist at the host location (some funding is available at host institutions (e.g. BYU has in the past partially funded visiting students/faculty from the Kennedy Center and Bean Museum, etc.). Housing accommodations will be arranged by the host institution and research support (through this grant) will be provided by the host institution. Current projections are that 10-12 students per year will participate in internships. **Faculty Development/Sabbaticals**—We will encourage faculty leaves and provide international professional development opportunities in years 2-5. Cussac, Orti, and Ruzzante will organize these efforts. Each of our annual meetings will include opportunities to learn new techniques, and to promote cross training in the natural history of Patagonia. To inform scientists of possible sabbatical opportunities, we will also compile and disseminate information about each of the respective senior scientists and work being conducted in their labs. **Meetings & Workshops**—J. Johnson, Orti, Habit, and Morando will oversee the organization of all meetings and workshops. In our first year The location and focus of these meetings is described above. BYU, Centro Nacional Patagonico, and the Universidad de Concepcion all have large meeting facilities that will accommodate up to 100 participants with sleeping and eating accommodations available near the meeting sites. Snowbird Resort in Utah and the Patagonian Ecosystem Research Center in southern Chile are meeting sites that occupy similar temperate alpine environments in opposite hemispheres. Each of these locations can also accommodate at least 100 individuals and both have on-site amenities. **Communications**—A bi-lingual web site for the project will be housed on the BYU BioAg web server and technical support will be provided by the BYU IT office. J. Johnson will oversee the maintenance of this site, including the flow and content of information posted. Translation will be supervised by Sites (N. America), Habit (CHL), and Morando (ARG), and will utilize the skills of the large bi-lingual undergraduate population at BYU.

Brigham Young University as the Flagship Institution: BYU has a remarkably strong track record of successful international research. In fact the BYU Kennedy Center for International Studies is one of the world's leading centers for coordinating collaborative international partnerships, especially between North and South America. Moreover, the Department of Integrative Biology and its faculty have an extensive history of collaborative research in Latin America. We will rely on this successful past to ensure success in the proposed work.

Unique Resources at BYU: BYU is particularly strong in its ability to recruit U.S. undergraduate and graduate students with multi-lingual capabilities, in part due to the

missionary language programs of the LDS church. This emphasis on language and cultural fluency also makes BYU an attractive location for foreign students and scientists to come and study. In fact, there are few universities as well-equipped to bridge the language barrier between Spanish and English. In addition, BYU has state-of-the-art DNA sequencing facilities, including a PhD-level full time director (Ed Wilcox) and technicians fully supported by the university. This substantially lowers our cost for conducting molecular work and provides a high level of on-site technical expertise. BYU also has secured internal funding commitments (\$20,000 per year) for undergraduate and graduate mentoring funds to send students abroad, and has a strong track record of securing funds through the Kennedy Center for supporting foreign students and scientists to come to study/conduct research at BYU.

Resources at University of Nebraska: The School of Biological Sciences at UNL offers competitive financial support for graduate student research and travel needs through several funding programs. A training grant from the DOEd to the Ecology and Evolution group provides fellowships and funds for educational needs, including travel and research.

International Partnerships and Institutional Contributions:

In each country, we have made arrangements with collaborators and their host institutions to provide field, research, and/or educational support (summarized in Table 4). These institutions and their planned contributions are detailed below.

Chile: *Universidad de Concepcion* (Victoriano,

Habit, Baeza, & Ruiz) is a large private university (17100 full-time students, 1100 graduate students, over 1,100 full-time faculty). This university has agreed to provide field support including collecting gear, student and technician time, and lab space. In addition, we will hold one of our first-year orientation meetings at U.Concepcion and our final symposium at the CIEP Conference Center (Patagonian Ecosystem Research Center), a facility managed in partnership with U.Concepcion. This center, located in the Andes of Chilean Patagonia, can house over 100 visiting researchers, and has a dining hall and auditorium. Victoriano and Habit have agreed to receive foreign students and visiting faculty in their laboratories, and have institutional support to send their students abroad to participate in the proposed international internship program (see letter from Dr. Oscar Parra).

Universidad Austral de Chile (Jara & Nunez) located in Valdivia Chile, a hotspot of freshwater crustacean endemism and biodiversity. They have agreed to provide field support including access to campus facilities in both Valdivia and Puerto Montt. These locations are ideal for staging collecting trips for frogs and crabs from coastal areas and islands. They will also strive to find complementary resources to support their investigators and students associated with this project (see letter from Dr. Oscar Balocchi, Director of Research).

Argentina: *Centro Nacional Patagonica* (CENPAT; Morando and Avila) is located in southern Argentina. They have agreed to provide field support, including sending students and researchers on field expeditions; they will also provide laboratory space and meeting space for

Table 4. International partnership institutions and their planned contributions to the overall project. Solid dots indicate that an institution can provide support.						
Country	Institution	Field Support	Lab Space	Study abroad site	Study abroad funding	Meeting Site
USA	Brigham Young University		•	•	•	•
	University of Nebraska		•	•	•	
Canada	Dalhousie University		•	•		
Argentina	Centro Nacional Patagonico	•	•	•		•
	Instituto de Botánica Darwinion	•	•	•		•
	Universidad de Comahue	•	•	•		
Chile	Universidad de Concepcion	•	•	•	•	•
	Universidad Austral de Chile	•	•	•		•

small meetings or conferences (see letter of support). They have offered their facilities to serve as a research center where samples can be stored and processed in Argentina. We will hold our third year annual meeting here at CENPAT (see letter of support from Andres Rivas).

Instituto de Botanico Darwinion (Poznar) is a major botanical research institution located in southeastern Argentina with significant floristic, systematic, and molecular systematic programs (www.darwin.edu.ar/) They have agreed to provide field support, including sending students and technicians on field expeditions; they will also provide laboratory space and meeting space for small or large conferences (see letter of support). They have offered their facilities to serve as a research center where samples can be stored and processed in Argentina. We will hold our first year educational workshop for Argentina at Darwinion (see support letter from F. Zuloaga).

Universidad Nacional del Comahue, Bariloche (Cussac) is located just east of the Andes in northern Patagonia. Cussac leads a group that has been working on the biology and ecology of native Patagonian fish over the past 15 years. They have extensive knowledge of the flora and fauna of the region, as well as experience sampling lakes from the Mendoza region to Tierra del Fuego. They will offer their expertise in the coordination of the fish sampling, as well as the use of equipment such as boats, motors, gillnets and electrofishing equipment. Undergraduate and graduate students working with Cussac will participate in the fieldwork.

Canada: Dalhousie University (Ruzzante, Walde) has an up-to-date multi-user molecular genetics laboratory (much of the equipment purchased or updated in the last 2 years). It has both experienced staff and a high through-put capacity (LI-COR, FMBIO, and CEQ). Over the past 10 years, it has been the site for the development of many microsatellite probes, for a wide variety of fish species. Substantial mtDNA sequencing has already been done for *Percichthys* in this lab. In addition Ruzzante has a digital imaging system (Zeiss) for obtaining and analyzing geometric morphometrics from digital photographs, as well as the necessary photography equipment. Both Ruzzante and Walde have worked in Patagonia since 1995 and speak Spanish (Ruzzante is a native speaker).

SYNOPSIS

One of the most pressing problems today in evolutionary biology is to uncover the relative contribution of past historical events in shaping region-wide patterns of biodiversity. A major limitation to this kind of work is that testing for historical effects on large geographic scales across multiple taxa typically requires collaboration among many scientists, each with unique taxonomic and technical expertise. In this study, we seek to understand how common historical events in temperate South America have affected a set of co-distributed plant and animal taxa in Patagonia. Our work relies heavily on international partnerships among individuals and institutions representing the U.S., Canada, Argentina, and Chile. Our project also reflects a firm commitment to educational training of young U.S. scientists and students, as well as their foreign counterparts, and will fully integrate educational opportunities into the fabric of the research program. In so doing, we expect to establish a long-term partnership in international research and education that will persist well beyond the 5 year funding period of this grant.