CORRELATIONS BETWEEN COMMUNITY DIVERSITY AND WITHIN-SPECIES GENETIC DIVERSITY IN AN AMPHIBIAN ASSEMBLAGE: POTENTIAL PROCESSES AND IMPLICATIONS FOR CONSERVATION MANAGEMENT

by

ANNA MIRIAM MCKEE

(Under the Direction of Travis C. Glenn and John C. Maerz)

ABSTRACT

When neutral processes (i.e., drift and dispersal) are responsible for distributions of species and genetic diversity, a positive correlation is expected between these scales of diversity. Under these circumstances, species diversity conservation efforts may also benefit the conservation of genetic diversity. However, habitat alteration can modify the strength of neutral processes, potentially leading to changes in the relationship between species and genetic diversity. The extent to which species are affected by habitat alterations may vary depending vagility and habitat restrictions. I investigated the associations of habitat features with species diversity and genetic diversity in two pond-breeding amphibian species with differing vagilities. In 2008 and 2009, I collected tissue samples from southern leopard frogs (*Rana sphenocephala*) and dwarf salamanders (*Eurycea quadridigitata*) from 10 and 9 isolated wetlands, respectively, in a longleaf pine reserve in southwest Georgia. I used microsatellite loci to estimate population genetic diversity and species data collected in 2006 from corresponding wetlands to estimate rarefied species richness. I used model selection to determine which local and landscape scale habitat features were most closely associated species and genetic diversity, as well as the spatial scale of greatest relevance. I also used model averaging to determine the directional association of each habitat feature with species and genetic diversity. Diversity was consistently lower in wetlands with more surrounding roads and generally greater in wetlands with more surrounding forest area. Dwarf salamanders showed greater allelic richness in less isolated wetlands and greater heterozygosity in larger wetlands. Southern leopard frog allelic richness was greatest in wetlands with

more surrounding agriculture and heterozygosity was greatest in less isolated wetlands. Species richness was greatest in wetlands with more surrounding forest area. To make this research relevant to a wider audience, I developed an activity for undergraduate students to help them understand the effects of neutral processes on species and genetic diversity. Prior to the activity, students were relatively familiar with the effects of neutral processes on genetic diversity; however they were less familiar with the effects on species diversity. The activity was effective in improving student knowledge of the effects of neutral processes on species diversity.

INDEX WORDS: Allelic richness, Amphibians, Biodiversity, Dispersal, *Eurycea*, Gene flow, Island biogeography, Landscape genetics, *Lithobates,* Longleaf pine, Microsatellite, PCR primers, *Rana*, SSR, STR, Species-area relationship, Species-genetic diversity correlation, Species richness

Correlations Between Community Diversity and Within-species Genetic Diversity in an Amphibian Assemblage: Potential Processes and Implications for Conservation Management

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DEDICATION

Half of this dissertation is dedicated to my mom and dad, Wendy and Art McKee, who have supported me [almost] without question, and my sister, Gwenny-Poo, whose philosophy on academics helped me make it as far as I have. A quarter is dedicated to my dogs, Easy Street and Jairo, the best personal trainers one could ask for. The final quarter is dedicated to Clark Jones, who was there for me at my worst.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL CONTEXT

1.1.1 PATTERNS AND PROCESSES THAT AFFECT BIODIVERSITY DISTRIBUTIONS

While explaining distributions of biodiversity has long been a central focus of ecology, continued global declines of biodiversity have also made understanding the patterns and processes that determine distributions of biodiversity critical for effective conservation management. In addition to its intrinsic value, biodiversity provides a number of important services as well, such as ecosystem function (Naeem et al. 1994, Tilman et al. 1996, Zavaleta et al. 2010), ecosystem regulation (McGrady-Steed et al. 1997), and ecosystem susceptibility to invasion (Kennedy et al. 2002, Stachowicz et al. 2002) and diseases (Knops et al. 1999, Schmidt and Ostfeld 2001, Altizer et al. 2003). Genetic diversity is an often overlooked component of biodiversity (Hooper et al. 2005), a term which is often used synonymously with species richness. Despite the importance of genetic diversity for populations' ability to adapt and population fitness (Frankham et al. 2002, Reed and Frankham 2003, Spielman et al. 2004) conservation efforts are primarily focused on protecting species diversity (Noss 1987, Myers et al. 2000, Possingham et al. 2001). The consequences to genetic diversity of managing for species diversity depend on the relationship between species and genetic diversity.

Theory suggests a positive relationship between species and genetic diversity when neutral forces (i.e., drift and dispersal) are the primary processes responsible for distributions of diversity at both the species and genetic level (Antonovics 1976, Vellend 2003). Management actions affecting the direction and magnitude of these forces are generally hypothesized to have similar effects on both species and genetic diversity (Redford and Richter 1999, Poiani et al. 2000). However, when deterministic forces (e.g., selection or species interactions) or interactions between species and genetic diversity have a greater influence on species

and (or) genetic diversity, the relationship between species and genetic is more difficult to predict and may be positive, negative, or neutral (Vellend 2005, Vellend and Geber 2005).

In many cases, diversity may be affected by a combination of neutral and deterministic forces (Leibold and McPeek 2006, Thompson and Townsend 2006). Distinguishing between these forces may be difficult in heterogeneous landscapes where different land cover types represent differing degrees of suitability and (or) resistance to dispersal (Thies and Tscharntke 1999, Haila 2002, Thompson and Townsend 2006, Goldberg and Waits 2010), and the effective area and isolation of a habitat may differ from the geometric measurements of area and Euclidean distance (Ricketts 2001, Lindenmayer and Fischer 2007). Processes responsible for distributions of species and genetic diversity may also act across both local and landscape scales (Levin 1992, With and Crist 1995, Ricketts 2001). Therefore, combining local and landscape-scale habitat features in studies of species and genetic diversity may be particularly important for understanding factors and processes that determine distributions of biodiversity (Levin 2000, Leibold et al. 2004, Laurance et al. 2007).

Studies on the species-genetic diversity correlations (SGDCs; Vellend 2003) have become more common within the last several years (Vellend 2004, Vellend 2005, Vellend and Geber 2005, Cleary et al. 2006, Evanno et al. 2009, Odat et al. 2010, Robinson et al. 2010, Finn and Poff 2011, Struebig et al. 2011, Blum et al. 2012, Wei and Jiang 2012). Results from these studies suggest that positive SGDCs are more likely when genetic diversity is measured in common versus rare species (Vellend 2005), species and diversity are measured during or following a disturbance event (Vellend 2004, Cleary et al. 2006, Evanno et al. 2009, but see Wei and Jiang 2012), or genetic diversity is measured in species with more habitat restrictions and limited dispersal (Struebig et al. 2011).

1.1.2 Pond-Breeding Amphibian Communities

In terms of vertebrate communities, pond-breeding amphibian species are suitable for studying the relationship between species and genetic diversity. Most amphibians have aquatic egg and larval development, which facilitates amphibian community surveys, followed by metamorphosis into terrestrial adults (Duellman

and Trueb 1994). Many of species that are terrestrial as adults are philopatric (Smith and Green 2005), suggesting that the breeding assemblages within wetlands should be somewhat consistent across years and therefore wetlands can serve as delineators for populations and communities.

A number of studies have been conducted on local and landscape scale habitat features associated with amphibians. At the local scale, features found to be associated with amphibian abundance and (or) diversity include pH, conductivity, water depth (Babbitt et al. 2006), wetland area (Burne and Griffin 2005), predatory fish (Hecnar and M'Closkey 1997, Babbitt et al. 2006, Murphy et al. 2010), hydroperiod (Snodgrass et al. 2000, Burne and Griffin 2005), isolation (Burne and Griffin 2005, Goldberg and Waits 2010, Murphy et al. 2010, Kirkman et al. 2012), emergent vegetation (Burne and Griffin 2005), and tree canopy cover (Burne and Griffin 2005). At the landscape scale, features associated with amphibian abundance and (or) diversity include surrounding forest area (Knutson et al. 1999, Houlahan et al. 2000, Guerry and Hunter 2002, Trenham and Shaffer 2005), roads (Fahrig et al. 1995, DeMaynadier and Hunter 2000, Carr and Fahrig 2001, Eigenbrod et al. 2008), agriculture (Knutson et al. 1999, Gray et al. 2004), and wetland density (Guerry and Hunter 2002, Trenham et al. 2003). The varying habitat features and spatial scales relevant to amphibian diversity indicate drift and dispersal are unlikely to be the only processes that dictate distributions of amphibian diversity among communities. However, if neutral processes are the primary ecological and evolutionary forces acting on amphibian communities and populations, a positive correlation between the two is still expected.

1.1.3 LONGLEAF PINE ECOSYSTEM

Within the southeastern US, the longleaf pine (*Pinus palustris*) ecosystem has a high number of endemic amphibian species (17 species; Means 2006), some of which are now threatened with extinction in large part due to the loss of habitat since European settlement. Historically, the longleaf pine ecosystem covered 37 million ha of the southeastern US (Frost 1993). However, now less than three percent of that habitat remains (Frost 2006). Isolated ephemeral wetlands associated with longleaf pine ecosystems provide breeding habitats that are naturally free of predatory fish. Prescribed burns are an essential component of

longleaf pine ecosystem management, and are important for maintaining suitable upland and wetland habitats for amphibians (Means 2006). In the uplands, fire helps prevent understory succession, helping to maintain a ground cover of grasses and forbs. Declines of the federally threatened flatwoods salamander (*Ambystoma cingulatum*) have been attributed in part to fire suppression in longleaf habitats, resulting in the loss of mature longleaf pine stands and ground cover of grasses and forbs (Palis 1997). Inundation and fire are the primary processes that help maintain vegetation of many isolated wetlands in longleaf pine ecosystems. During periods of drought, fires may enter isolated wetlands from the uplands, helping to maintain sparse overstories (mostly cypress) and abundant herbaceous vegetation (Kirkman 1995). Fire suppression enables succession to proceed, leading to shrub thickets and eventually hardwood encroachment and the loss of herbaceous vegetation (Russell et al. 1999).

1.1.4 Molecular Markers

The type of molecular marker that should be used to investigate genetic diversity within and among populations depends on the question of interest (Mariette et al. 2002, Morin et al. 2004). For population genetics questions, molecular markers that are selectively neutral and highly variable provide greater statistical power and information over a more recent time-scale than less variable markers (Avise 2004, Murphy and Evans 2011). Popular markers for landscape genetics include mitochondrial DNA sequences, allozymes, amplified fragment length polymorphisms, single nucleotide polymorphisms (SNPs), and microsatellites (Storfer et al. 2010, Murphy and Evans 2011). For a review of these markers and their advantages and disadvantages for various population genetic study objectives, see Murphy and Evans (2011). Of these markers, microsatellites, which are tandem repeats of short DNA sequences and occur across the nuclear genome of most taxa, have been the most popular markers used in animal landscape genetics studies (Storfer et al. 2010). However, this may change in the near future, as SNPs, which are loci that vary by a single base-pair and can provide broader genome coverage (Morin et al. 2004), become cheaper and easier to screen with high throughput sequencing (Seeb et al. 2011). In the meanwhile, microsatellites provide high information

content per locus and multiple loci can be combined in the genotyping process to provide results quickly and inexpensively (Selkoe and Toonen 2006).

1.1.5 Biodiversity and Human Dimensions

For the results of this study to be made relevant to biodiversity conservation for the general public, people must first understand what the term biodiversity means, and the fundamental ecological and evolutionary processes that affect distributions of species and genetic diversity. Unfortunately, the general public is generally unfamiliar with or uncomfortable explaining the meaning of the term "biodiversity" (Spash and Hanley 1995, Hunter and Brehm 2003, Christie et al. 2006, Fischer and Young 2007). Of the people that are familiar with the term biodiversity, their understanding tends to be the same as scientists and government agencies; limited to ideas of species diversity with little to no recognition of genetic diversity as a constituent of biodiversity (Spash and Hanley 1995). If biodiversity conservation efforts are to take genetic diversity into account, those making the decisions must first be aware of genetic diversity, its importance, and the processes that affect its distribution.

1.2 Purpose and Justification for Study

While SGDC studies have become more commonly recently, the number of studies is still quite limited. Most empirical studies thus far have focused on a single species for the genetic component (Vellend 2004, Cleary et al. 2006, Evanno et al. 2009, Odat et al. 2010, Finn and Poff 2011, Blum et al. 2012, but see Robinson et al. 2010, Struebig et al. 2011), limiting the inferences that can be made regarding how differing characteristics within community-types affect SGDCs. Additionally, few empirical studies have investigated SGDCs in vertebrate communities (Vellend 2004, Cleary et al. 2006, Evanno et al. 2009, Odat et al. 2010, Finn and Poff 2011, but see Blum et al. 2012, Struebig et al. 2011). The purpose of this dissertation was to investigate the relationship between, and habitat predictors of, species diversity and genetic diversity in species with differing habitat and dispersal characteristics, within a community of vertebrate species of general conservation concern located in an ecosystem of conservation concern. Additionally, I was interested

in making this research more relevant to a wider audience, by helping undergraduate students understand the constituents of biodiversity as well as the fundamental ecological and evolutionary theories behind SGDCs, and their relevance to conservation.

1.3 STUDY LOCATION

One large (11,800 ha) remaining tract of longleaf pine that is managed with prescribed fire to maintain a longleaf and wiregrass (*Aristida stricta*) dominant landscape is the Jones Ecological Research Center at Ichauway, located in Baker County, GA (Figure 1.1). While the majority of Ichauway is managed for longleaf pine, approximately 1200 ha of Ichauway are used for agriculture and an additional 120 ha for food plots. The area immediately surrounding Ichauway is composed primarily of center-pivot agriculture (Michener et al. 1998). This longleaf pine research site has numerous (approximately 30) ephemeral wetlands and high amphibian diversity (31 species recorded at between 1990 and 2004; Smith et al. 2006). An additional benefit of conducting this research at Ichauway was the pre-existing amphibian community data for 29 wetlands, which had been collected during the winter and spring breeding seasons in 2006. Modeling species and genetic diversity as functions of features representing neutral forces (i.e., wetland area and isolation) as well as landscape scale features that may modify these forces or represent deterministic processes (i.e., surrounding agriculture, forest, wetland, and roads) across varying scales previously suggested as relevant to amphibians (i.e., 0.5, 1.0, and 2.5 km; Houlahan and Findlay 2003, Piha et al. 2007, Veysey et al. 2011) will facilitate a better understanding of which processes are responsible for distributions of species and genetic diversity, as well predict how various management strategies are likely to affect species and genetic diversity.

1.4 FOCAL SPECIES

The ideal candidate species for the genetic component of a study should be widely distributed but not necessarily ubiquitous, have a larval and adult stage and be easy to capture in both stages, and at least moderately philopatric. To better understand how differing habitat restrictions and vagilities affect the relationship between and habitat predictors of species and genetic diversity, the selected species should also

have differing degrees of habitat restrictions and vagilities. I chose to focus on the dwarf salamander (*Eurycea quadridigitata*; Figure 1.2a) and the southern leopard frog (*Rana sphenocephala*, also referred to as *Lithobates sphenocephalus*; Figure 1.2b), which are widespread in the southeastern US and relatively abundant in our study area (Cash 2008, Means 2008). Dwarf salamanders are presumably less vagile and have stricter habitat restrictions than southern leopard frogs because of the salamanders' small size (22-26 mm snout-vent length; SVL) and lack of lungs compared to southern frogs, which are medium sized anurans (50-130 mm SVL) that have lungs.

1.5 Biodiversity Activity

The biodiversity activity was developed for an upper level undergraduate ecology course in the Warnell School of Forestry and Natural Resources at the University of Georgia, Athens, GA. Warnell is a professional school for which the mission statement includes "to prepare leaders in the conservation and sustainable management of forests and other natural resources." As a number of these students are interested in or may end up working as land managers, providing them with a fundamental understanding of the processes regulating biodiversity may help future land managers predict how various management strategies are likely to affect species and genetic diversity. The activity was based on an approach of using a concept (species diversity) that was more tangible to students to help explain a concept (genetic diversity) with which students may be less comfortable.

1.6 Objectives

The studies described in this dissertation were designed to address the following objectives:

- Develop microsatellite loci for both dwarf salamanders and southern leopard frog to study population genetic structure, genetic diversity, and gene flow across varying habitat types and scales (Chapter 2 and 3).
- Determine the extent to which genetic diversity varies among populations of dwarf salamanders and southern leopard frogs (Chapter 4).

- Determine which habitat features in a longleaf pine ecosystem best predict genetic diversity in dwarf salamanders and southern leopard frogs (Chapter 4) and species diversity in pond-breeding amphibian communities (Chapter 5).
- Determine the directional association between longleaf pine ecosystem habitat features and genetic diversity in a species with greater habitat restrictions, genetic diversity in a habitat generalist species (Chapter 4), and species diversity in pond-breeding amphibian communities (Chapter 5).
- Determine the spatial scale at which habitat features are most strongly associated with genetic diversity in populations of a species with limited vagility and more habitat restrictions, genetic diversity in populations of a highly vagile habitat generalist species (Chapter 4), and species diversity in pond-breeding amphibian communities (Chapter 5).
- Determine the relationship between species diversity in pond-breeding amphibian communities and genetic diversity in populations of a species with limited vagility and more habitat restrictions versus genetic diversity in populations of a highly vagile habitat generalist species (Chapter 5), and
- Develop and assess the effectiveness of an activity designed to help undergraduate students (Chapter 6):
    - Understand how neutral processes affect species and genetic diversity on islands and/or habitat patches.
    - Apply the concepts of island biogeography to alternative systems.
    - Understand how habitat size and isolation play into decisions regarding habitat reserves for conserving biodiversity.

1.7 LITERATURE CITED

Altizer, S., D. Harvell, and E. Friedle. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution **18**:589-596.

Antonovics, J. 1976. The Input from Population Genetics: "The New Ecological Genetics". Systematic Botany **1**:233-245.

Avise, J. C. 2004. Molecular markers, natural history, and evolution. Sinauer, Sunderland, MA.

Babbitt, K. J., M. J. Baber, and L. A. Brandt. 2006. The effect of woodland proximity and wetland characteristics on larval anuran assemblages in an agricultural landscapeThis is contribution No. 82 of the MacArthur Agro-Ecology Research Center. Canadian Journal of Zoology **84**:510-519.

Blum, M., M. Bagley, D. Walters, S. Jackson, F. Daniel, D. Chaloud, and B. Cade. 2012. Genetic diversity and species diversity of stream fishes covary across a land-use gradient. Oecologia **168**:83-95.

Burne, M. R. and C. R. Griffin. 2005. Habitat associations of pool-breeding amphibians in eastern Massachusetts, USA. Wetlands Ecology and Management **13**:247-259.

Carr, L. W. and L. Fahrig. 2001. Effect of Road Traffic on Two Amphibian Species of Differing Vagility. Conservation Biology **15**:1071-1078.

Cash, W. B. 2008. Southern Leopard Frog (*Rana sphenocephala*).*in* J. B. Jensen, C. D. Camp, and W. Gibbons, editors. Amphibians and reptiles of Georgia. University of Georgia Press, Athens, Georgia.

Christie, M., N. Hanley, J. Warren, K. Murphy, R. Wright, and T. Hyde. 2006. Valuing the diversity of biodiversity. Ecological Economics **58**:304-317.

Cleary, D. F. R., C. Fauvelot, M. J. Genner, S. B. J. Menken, and A. Ø. Mooers. 2006. Parallel responses of species and genetic diversity to El Niño Southern Oscillation-induced environmental destruction. Ecology Letters **9**:304-310.

DeMaynadier, P. G. and M. L. J. R. Hunter. 2000. Road effects on amphibian movements in a forested landscape. Natural Areas Journal **20**:56-65.

Duellman, W. E. and L. Trueb. 1994. The Biology of Amphibians. Johns Hopkins University Press, Baltimore, Maryland.

Eigenbrod, F., S. J. Hecnar, and L. Fahrig. 2008. The relative effects of road traffic and forest cover on anuran populations. Biological Conservation **141**:35-46.

Evanno, G., E. Castella, C. Antoine, G. Paillat, and J. Goudet. 2009. Parallel changes in genetic diversity and species diversity following a natural disturbance. Molecular Ecology **18**:1137.

Fahrig, L., J. H. Pedlar, S. E. Pope, P. D. Taylor, and J. F. Wegner. 1995. Effect of road traffic on amphibian density. Biological Conservation **73**:177-182.

Finn, D. S. and N. L. R. Poff. 2011. Examining spatial concordance of genetic and species diversity patterns to evaluate the role of dispersal limitation in structuring headwater metacommunities. JNABS Journal **30**:273-283.

Fischer, A. and J. C. Young. 2007. Understanding mental constructs of biodiversity: Implications for biodiversity management and conservation. Biological Conservation **136**:271-282.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Frost, C. 2006. History and Future of the Longleaf Pine Ecosystem. Pages 9-42 *in* S. Jose, E. J. Jokela, and D. L. Miller, editors. The Longleaf Pine Ecosystem: Ecology, Siviculture, and Restoration. Springer, New York, NY.

Frost, C. C. 1993. Four Centuries of Changing Landscape Patterns in the Longleaf Pine Ecosystem. Pages 17-43 *in* The Longleaf Pine Ecosystem: Ecology, Restoration, and Management. Proceedings of the Tall Timbers Fire Ecology Conference, Tall Timbers Research Station.

Goldberg, C. and L. Waits. 2010. Comparative landscape genetics of two pond breeding amphibian species in a highly modified agricultural landscape. Molecular Ecology **19**:3650-3663.

Gray, M. J., L. M. Smith, and R. I. Leyva. 2004. Influence of agricultural landscape structure on a Southern High Plains, USA, amphibian assemblage. Landscape Ecology **19**:719-729.

Guerry, A. D. and M. L. Hunter. 2002. Amphibian Distributions in a Landscape of Forests and Agriculture: an Examination of Landscape Composition and Conf iguration. Conservation Biology **16**:745-754.

Haila, Y. 2002. A conceptual genealogy of fragmentation research: From island biogeography to landscape ecology. Ecological Applications **12**:321-334.

Hecnar, S. J. and R. T. M'Closkey. 1997. The effects of predatory fish on amphibian species richness and distribution. Biological Conservation **79**:123-131.

Hooper, D., F. Chapin Iii, J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. Lawton, D. Lodge, M. Loreau, and S. Naeem. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs **75**:3-35.

Houlahan, J. E. and C. S. Findlay. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences **60**:1078-1094.

Houlahan, J. E., C. S. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature **404**:752-755.

Hunter, L. M. and J. Brehm. 2003. Brief Comment: Qualitative Insight into Public Knowledge of, and Concern with, Biodiversity. Human Ecology **31**:309-320.

Kennedy, T. A., S. Naeem, K. M. Howe, J. M. H. Knops, D. Tilman, and P. Reich. 2002. Biodiversity as a barrier to ecological invasion. Nature **417**:636-638.

Kirkman, L. K. 1995. Impacts of fire and hydrological regimes on vegetation in depression wetlands of southeastern USA. Pages 10-20 *in* Tall Timbers Fire Ecology Conference, Tall Timbers Research Station, Tallahassee, FL.

Kirkman, L. K., L. L. Smith, P. F. Quintana-Ascencio, M. J. Kaeser, S. W. Golladay, and A. L. Farmer. 2012. Is species richness congruent among taxa? Surrogacy, complementarity, and environmental correlates among three disparate taxa in geographically isolated wetlands. Ecological Indicators **18**:131-139.

Knops, J. M. H., D. Tilman, N. M. Haddad, S. Naeem, C. Mitchell, J. Haarstad, M. Ritchie, K. Howe, P. Reich, and E. Siemann. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters **2**:286-293.

Knutson, M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, and M. J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology **13**:1437-1446.

Laurance, W. F., H. E. M. Nascimento, S. G. Laurance, A. Andrade, R. M. Ewers, K. E. Harms, R. C. C. Luizao, and J. E. Ribeiro. 2007. Habitat fragmentation, variable edge effects, and the landscape-divergence hypothesis. PLoS One **2**:e1017.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, and D. Tilman. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters **7**:601-613.

Leibold, M. A. and M. A. McPeek. 2006. Coexistence of the niche and neutral perspectives in community ecology. Ecology **87**:1399-1410.

Levin, S. A. 1992. The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. Ecology **73**:1943-1967.

Levin, S. A. 2000. Multiple scales and the maintenance of biodiversity. Ecosystems **3**:498-506.

Lindenmayer, D. B. and J. Fischer. 2007. Tackling the habitat fragmentation panchreston. Trends in Ecology & Evolution **22**:127-132.

Mariette, S., V. Le Corre, F. Austerlitz, and A. Kremer. 2002. Sampling within the genome for measuring within-population diversity: trade-offs between markers. Molecular Ecology **11**:1145-1156.

McGrady-Steed, J., P. M. Harris, and P. J. Morin. 1997. Biodiversity regulates ecosystem predictability. Nature **390**:162-165.

Means, B. 2006. Vertebrate Faunal Diversity. Pages 157-213 *in* S. Jose, E. J. Jokela, and D. L. Miller, editors. The Longleaf Pine Ecosystem: Ecology, Siviculture, and Restoration. Springer, New York, NY.

Means, B. 2008. Dwarf Salamander Complex (*Eurycea quadridigitata*).*in* J. B. Jensen, C. D. Camp, and W. Gibbons, editors. Amphibians and reptiles of Georgia. University of Georgia Press, Athens, Georgia.

Michener, W. K., E. R. Blood, J. B. Box, C. A. Couch, S. W. Golladay, D. J. Hippe, R. J. Mitchell, and B. J. Palik. 1998. Tropical storm flooding of a coastal plain landscape. Bioscience **48**:696-705.

Morin, P. A., G. Luikart, R. K. Wayne, and S. N. P. w. group. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution **19**:208-216.

Murphy, M., R. Dezzani, D. Pilliod, and A. Storfer. 2010. Landscape genetics of high mountain frog metapopulations. Molecular Ecology **19**:3634-3649.

Murphy, M. A. and J. S. Evans. 2011. Genetic Patterns as a Function of Landscape Process: Applications of Neutral Genetic Markers for Predictive Modeling in Landscape Ecology. Pages 161-188 *in* C. A. Drew, Y. F. Wiersma, and F. Huettmann, editors. Predictive Species and Habitat Modeling in Landscape Ecology. Springer New York.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. Da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature **368**:734-737.

Noss, R. F. 1987. From plant communities to landscapes in conservation inventories: a look at The Nature Conservancy (USA). Biological Conservation **41**:11-37.

Odat, N., F. H. Hellwig, G. Jetschke, and M. Fischer. 2010. On the relationship between plant species diversity and genetic diversity of Plantago lanceolata (Plantaginaceae) within and between grassland communities. Journal of Plant Ecology **3**:41-48.

Palis, J. G. 1997. Breeding Migration of Ambystoma cingulatum in Florida. Journal of Herpetology **31**:71-78.

Piha, H., M. Luoto, M. Piha, and J. Merilä. 2007. Anuran abundance and persistence in agricultural landscapes during a climatic extreme. Global Change Biology **13**:300-311.

Poiani, K. A., B. D. Richter, M. G. Anderson, and H. E. Richter. 2000. Biodiversity conservation at multiple scales: Functional sites, landscapes, and networks. Bioscience **50**:133-146.

Possingham, H., S. Andelman, B. Noon, S. Trombulak, and H. Pulliam. 2001. Making smart conservation decisions. Pages 225-244 *in* M. E. Soule and G. H. Orians, editors. Conservation biology: research priorities for the next decade. Island Press, Washington, D.C.

Redford, K. H. and B. D. Richter. 1999. Conservation of Biodiversity in a World of Use. Conservation Biology **13**:1246-1256.

Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology **17**:230-237.

Ricketts, T. H. 2001. The matrix matters: effective isolation in fragmented landscapes. The American Naturalist **158**:87-99.

Robinson, J. D., E. Diaz-Ferguson, M. F. Poelchau, S. Pennings, T. D. Bishop, and J. Wares. 2010. Multiscale diversity in the marshes of the Georgia Coastal Ecosystems LTER. Estuaries and Coasts **33**:865-877.

Russell, K. R., D. H. Van Lear, and D. C. Guynn. 1999. Prescribed fire effects on herpetofauna: review and management implications. Wildlife Society Bulletin **27**:374-384.

Schmidt, K. A. and R. S. Ostfeld. 2001. Biodiversity and the dilution effect in disease ecology. Ecology **82**:609-619.

Seeb, J., G. Carvalho, L. Hauser, K. Naish, S. Roberts, and L. Seeb. 2011. Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. Molecular Ecology Resources **11**:1-8.

Selkoe, K. A. and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters **9**:615-629.

Smith, L. L., D. A. Steen, J. M. Stober, M. C. Freeman, S. W. Golladay, L. M. Conner, and J. Cochrane. 2006. The Vertebrate Fauna of Ichauway, Baker County, GA. Southeastern Naturalist **5**:599-620.

Smith, M. A. and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography **28**:110-128.

Snodgrass, J. W., M. J. Komoroski, A. L. Bryan, and J. Burger. 2000. Relationships among Isolated Wetland Size, Hydroperiod, and Amphibian Species Richness: Implications for Wetland Regulations. Conservation Biology **14**:414-419.

Spash, C. L. and N. Hanley. 1995. Preferences, information and biodiversity preservation. Ecological Economics **12**:191-208.

Spielman, D., B. W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences of the United States of America **101**:15261.

Stachowicz, J. J., H. Fried, R. W. Osman, and R. B. Whitlatch. 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology **83**:2575-2590.

Storfer, A., M. A. Murphy, S. F. Spear, R. Holderegger, and L. P. Waits. 2010. Landscape genetics: where are we now? Molecular Ecology **19**:3496-3514.

Struebig, M. J., T. Kingston, E. J. Petit, S. C. Le Comber, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2011. Parallel declines in species and genetic diversity in tropical forest fragments. Ecology Letters **14**:582-590.

Thies, C. and T. Tscharntke. 1999. Landscape structure and biological control in agroecosystems. Science **285**:893.

Thompson, R. and C. Townsend. 2006. A truce with neutral theory: local deterministic factors, species traits and dispersal limitation together determine patterns of diversity in stream invertebrates. Journal of Animal Ecology **75**:476-484.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature **379**:718-720.

Trenham, P. C., W. D. Koenig, M. J. Mossman, S. L. Stark, and L. A. Jagger. 2003. Regional dynamics of wetland-breeding frogs and toads: turnover and synchrony. Ecological Applications **13**:1522-1532.

Trenham, P. C. and H. B. Shaffer. 2005. Amphibian upland habitat use and its consequences for population viability. Ecological Applications **15**:1158-1168.

Vellend, M. 2003. Island biogeography of genes and species. The American Naturalist **162**:358-365.

Vellend, M. 2004. Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. Ecology **85**:3043-3055.

Vellend, M. 2005. Species diversity and genetic diversity: parallel processes and correlated patterns. The American Naturalist **166**:199-215.

Vellend, M. and M. A. Geber. 2005. Connections between species diversity and genetic diversity. Ecology Letters **8**:767-781.

Veysey, J., S. Mattfeldt, and K. Babbitt. 2011. Comparative influence of isolation, landscape, and wetland characteristics on egg-mass abundance of two pool-breeding amphibian species. Landscape Ecology **26**:661-672.

Wei, X. and M. Jiang. 2012. Contrasting relationships between species diversity and genetic diversity in natural and disturbed forest tree communities. New Phytologist **193**:779-786.

With, K. A. and T. O. Crist. 1995. Critical thresholds in species' responses to landscape structure. Ecology:2446-2459.

Zavaleta, E. S., J. R. Pasari, K. B. Hulvey, and G. D. Tilman. 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. Proceedings of the National Academy of Sciences **107**:1443.

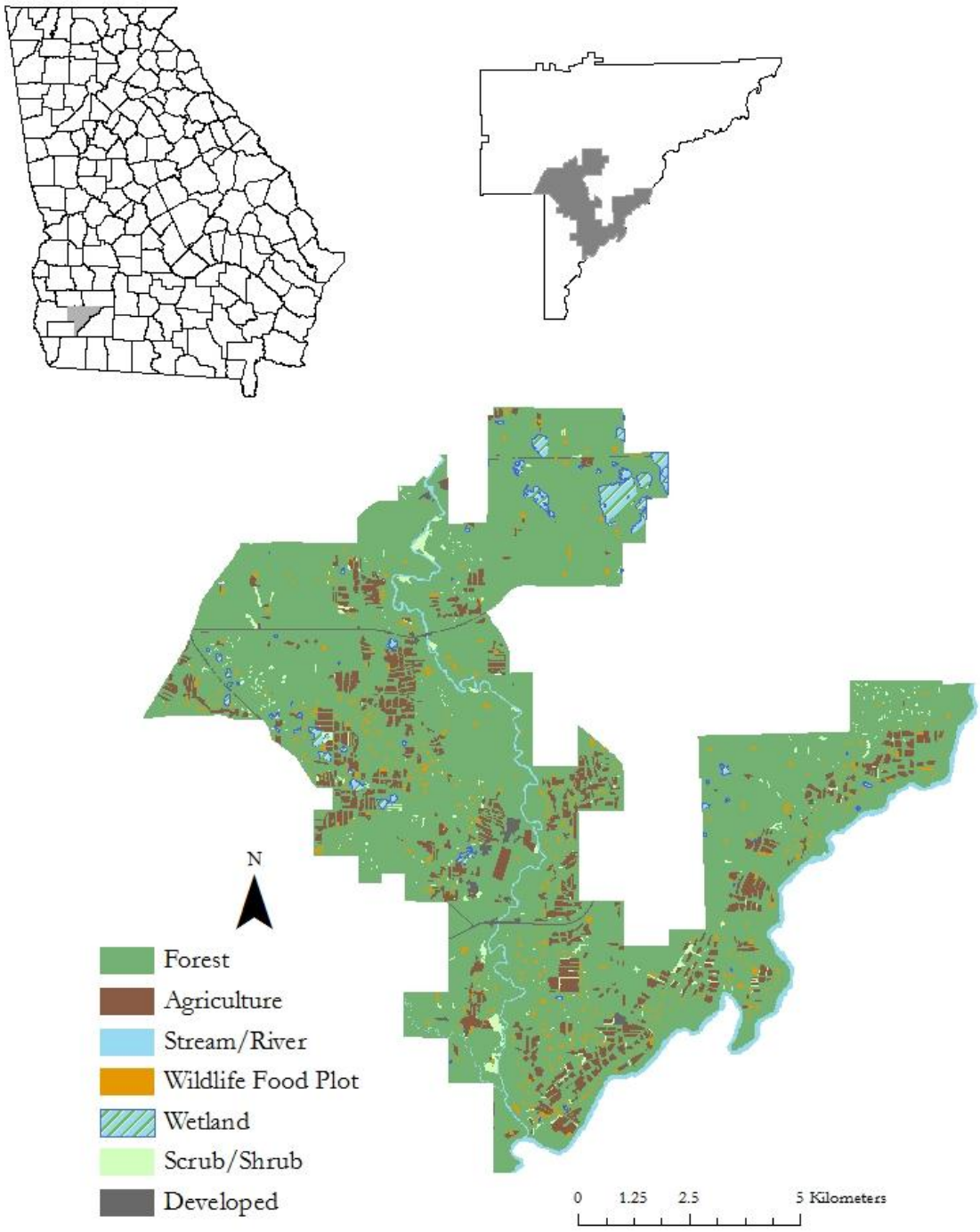

Figure 1.1. Location and land cover types of Ichauway in Baker County, GA.

a)

b)

Figure 1.2. Focal amphibian species for the genetic component of this dissertation. a) Dwarf salamander (*Eurycea quadridigitata*). b) Southern leopard frog (*Rana sphenocephala*). Printed with the permission from Todd Pierson.

CHAPTER 2

DEVELOPMENT AND CHARACTERIZATION OF 18 MICROSATELLITE LOCI FOR THE SOUTHERN LEOPARD FROG, *RANA SPHENOCEPHALA*[1]

[1] A.M. McKee and T.C. Glenn. *Conservation Genetics Resources.* 3:267–269. Reprinted here with permission of the publisher.

2.1 ABSTRACT

We isolated and characterized 18 microsatellite loci for the Southern Leopard Frog, *Rana sphenocephala*. Loci were screened in 30 individuals of *R. sphenocephala*. The number of alleles per locus ranged from 5 to 21, observed heterozygosity ranged from 0.200 to 0.933, and the probability of identity values ranged from 0.008 to 0.299. These new loci are tools that can be used to study population genetic structure, genetic diversity, and gene flow across varying habitat types and scales.

2.2 PRIMER DEVELOPMENT AND CHARACTERIZATION

The Southern Leopard Frog (*Rana sphenocephala,* also commonly referred to as *Lithobates sphenocephalus*) is widely distributed throughout the southeastern US. Its range extends as far north as New York, and as far west as Texas, Oklahoma, and Nebraska. *Rana sphenocephala* is considered common throughout much of its range (Lannoo 2005) and thus serves as a model frog species in many conservation programs. Despite their wide distribution, relatively little is known about phylogeography in *R. sphenocephala*. We therefore sought to obtain microsatellite markers for this species.

We extracted genomic DNA from *R. sphenocephala* (leg skin and muscle) tissue (preserved in 95% EtOH) using standard phenol-chloroform procedures (Sambrook et al. 1989). Genomic DNA was then serially enriched twice for microsatellites using three probe mixes following Glenn and Schable (2005), with the changes described in Lance et al. (2010) and used the SimpleX-2 linker (Henningsen et al. 2010). All methods for sequencing, microsatellite identification, primer design, and primer screening are as described in Erickson et al. (2010).

Ninety-six primer pairs were screened for amplification and polymorphism on DNA from eight *R. sphenocephala* specimens. Genomic DNA was extracted from individuals using the DNeasy tissue protocol (Qiagen, Valencia, CA). PCR amplification was performed in 12.5 μL volume reactions with 10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.36μM unlabeled primer, 0.04μM tag labeled primer, 0.36μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units JumpStart Taq DNA Polymerase (Sigma), and ~20 ng DNA template using an Applied Biosystems GeneAmp 9700.

Most loci were amplified using one of two touchdown PCR protocols (Don et al. 1991), TD65 or TD58 (Table 1). Each touchdown protocol included an 8°C span of annealing temperatures (65-57°C and 58-50°C respectively). Touchdown cycling parameters consisted of 16 cycles of 95°C for 30s, highest annealing temperature of 65°C or 58°C (decreased by 0.5°C per cycle) for 30s, and 72°C for 45s; and 24 cycles of 95°C for 30s, 57°C or 50°C for 30s, and 72°C for 45s. Amplified products were run on an ABI-3730xl sequencer and compared with Naurox size standard prepared as described in DeWoody et al. (2004) except that unlabeled primers started with GTTT. Results were analyzed using GENEMAPPER version 4.0 (Applied Biosystems). Eighteen of the tested primer pairs amplified high quality PCR product and displayed polymorphisms.

Thirty specimens of *R. sphenocephala*, collected from a single wetland at the Joseph W. Jones Ecological Research Center (JERC) at Ichauway in Baker County, GA, were assessed for genetic variability at these loci. Conditions and characteristics of the 18 loci are given in Table 2.1. We estimated the number of alleles per locus (*k*), observed and expected heterozygosity (H_o and H_e), and tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium using GENEPOP v4.0 (Raymond and Rousset 1995, Rousset 2008). Probability of identity (PI) was estimated in GenAlEx v6.0 (Peakall and Smouse 2006). After Bonferroni corrections for multiple comparisons, linkage disequilibrium was not detected at any of the 153 locus-pairwise comparisons; however 7 loci deviated from expectations under HWE. To test for possible sex linkage, all loci were screened against seven male and seven female *R. sphenocephala* samples from the Savannah River Site in Aiken County, SC. One locus, Rasp55, exhibited characteristics of a sex-linked marker in that only known females were blindly scored as heterozygotes.

2.3 ACKNOWLEDGEMENTS

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2.4 LITERATURE CITED

DeWoody, J. A., J. Schupp, L. Kenefic, J. Busch, L. Murfitt, and P. Keim. 2004. Universal method for producing ROX-labeled size standards suitable for automated genotyping. Biotechniques **37**:348-353.

Don, R. H., P. T. Cox, B. J. Wainwright, K. Baker, and J. S. Mattick. 1991. 'Touchdown'PCR to circumvent spurious priming during gene amplification. Nucleic Acids Research **19**:4008.

Erickson, M., D. Scott, K. Jones, C. Hagen, and S. Lance. 2010. Development and characterization of ten microsatellite loci for the eastern spadefoot toad, *Scaphiopus holbrookii*. Conservation Genetics Resources **2**:143-145.

Glenn, T. C. and N. A. Schable. 2005. Isolating Microsatelline DNA Loci. Methods in Enzymology **395**:202-222.

Henningsen, J., S. Lance, K. Jones, C. Hagen, J. Laurila, R. Cole, and K. Perez. 2010. Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae). Conservation Genetics Resources **2**:247-250.

Lance, S. L., J. E. Light, K. L. Jones, C. Hagen, and J. C. Hafner. 2010. Isolation and characterization of 17 polymorphic microsatellite loci in the kangaroo mouse, genus Microdipodops (Rodentia: Heteromyidae). Conservation Genetics Resources **2**:139-141.

Lannoo, M. J. 2005. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley, CA.

Peakall, R. O. D. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248.

Rousset, F. C. O. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Table 2.1. Details for 18 polymorphic microsatellite loci developed for the southern leopard frog, *Rana sphenocephala*. The annealing temperature (T_A °C) where TD65 and TD58 indicates touchdown protocols with a highest annealing temperature of 65°C and 58°C, respectively; size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; the number of individuals genotyped is N; *k* is the number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus.

Locus	Primer Sequence 5' → 3'	Repeat motif	T_A	Size (bp)	N	*k*	H_o	H_e	PI
Rasp01	F: ACCTAGGGATTGCTGCATAA	(ATCT)	TD65	284 - 323	30	8	0.200†	0.742	0.110
	R: *AGCGAAAGGCAGACTCGATA								
Rasp03	F: TATGCCGTCAGTGCCACATC	(AGT)	TD65	283 - 324	30	13	0.767	0.913	0.019
	R: *GGTGCTAAGAGGACCACACA								
Rasp07	F: *TGGAGTTGTGCCACTTGTGA	(GAT)	TD58	290 - 305	29	6	0.690	0.621	0.185
	R: CAGACGCCAATATTTGTGCAAG								
Rasp09	F: GGTGAAACCCTGGAGACGTA	(ATCT)	TD58	316 - 364	30	13	0.933	0.902	0.023
	R: *CATGGCCAACAGAGTGGAAA								
Rasp10	F: *CTTCTGGGATGCGGATACATT	(ATCT)	TD58	152 - 216	30	13	0.933	0.901	0.023
	R: TGGTTGCAGAGATATTACGC								
Rasp13	F: *TCCCTAGTTCACTGTCGTTTATC	(GTTT)	TD65	190 - 215	30	6	0.433†	0.714	0.128
	R: AGCCAAAGCAGTCAAAGCAG								
Rasp16	F: *GCAGCAGTTTCGGTGTGATA	(ATGT)	TD65	266 - 286	28	5	0.214†	0.612	0.196
	R: GGATGATGCAGTTTCTCGGC								
Rasp17	F: TGCACTTCTTCCCTGGCTAA	(ATCT)	TD65	233 - 290	30	12	0.800	0.885	0.030
	R: *CAGGTCACCAGGCTCTTACA								
Rasp20	F: TGATGGTCAGGTCCACAAACT	(ATCT)	TD65	140 - 283	29	19	0.517†	0.949	0.009
	R: *CCTTATCCTGTTGGCAGCAAT								
Rasp28	F: *AAACTGGAGCCCTTCAACCT	(ACT)	TD65	234 - 274	28	11	0.536†	0.873	0.036
	R: CAGATGCCCTGTCTAACTTTGT								
Rasp37	F: *AGTCGAGGCGCAGAGAAC	(ATGT)(ATCT)	TD58	212 - 300	29	21	0.793	0.942	0.010
	R: GCCTAGTGCGTACAAGACTAT								

Rasp42	F: *GCTTGGGAAGGTTTCTGGTG	(AGAT)	TD65	366 - 431	30	12	0.867	0.902	0.023
	R: AGTGCACAGGTGGAGACATT								
Rasp45	F: TACTACTGTTCGGAGGCCCA	(CTTT)	TD65	159 - 221	29	17	0.551†	0.907	0.021
	R: *GGGAGAGAGAATAAATAAGGAGGC								
Rasp50	F: AAATGTGTGATTCCTCCTGC	(AGAT)	58.4	421 - 477	27	11	0.778	0.859	0.042
	R: *TTGTTCGATTGTCAGGGCTC								
Rasp51	F: ACACAGTGCAGTATCGCAA	(AGAT)	63.1	387 - 449	26	16	0.846	0.932	0.014
	R: *CCATGTGACCAGCTATGTGGA								
Rasp53	F: ACGATGTGGCATCCTTCTGT	(AGAT)	TD65	266 - 319	28	13	0.679†	0.881	0.031
	R: *TGGGTTGTTACCGTACGTGG								
Rasp55§	F: AGTCACTGTGGCGGATCTTT	(ATCT)	58.4	152 - 237	28	16	0.714	0.851	0.043
	R: *TGGTCTTTGTGTCTGGGAGC								
Rasp67	F: *GAGCAGCACAGTGGAGGTAA	(CATT)	63.1	315 - 330	30	5	0.333	0.489	0.299
	R: ACACAATCATTTGCAGGGTG								

* Indicates CAG tag (5'- CAGTCGGGCGTCATCA-3') label. † Indicates significant deviations from Hardy-Weinberg expectations after Bonferroni corrections. § Indicates evidence of sex linkage.

CHAPTER 3

DEVELOPMENT AND CHARACTERIZATION OF 12 MICROSATELLITE LOCI FOR THE DWARF SALAMANDER, *EURYCEA QUADRIDIGITATA*[2]

[2] A.M. McKee and T.C. Glenn. *Conservation Genetics Resources.* 3:633–635. Reprinted here with permission of the publisher.

3.1 ABSTRACT

We isolated and characterized 12 microsatellite loci for the Dwarf Salamander, *Eurycea quadridigitata*. Loci were screened in 30 individuals of *E. quadridigitata*. The number of alleles per locus ranged from 2 to 9, observed heterozygosity ranged from 0.2 to 0.9, and the probability of identity values ranged from 0.034 to 0.689. These new loci can be used to study population genetic structure and potentially help determine cryptic species divergence.

3.2 PRIMER DEVELOPMENT AND CHARACTERIZATION

The dwarf salamander, *Eurycea quadridigitata*, occurs throughout the southeastern United States (Bonett and Chippindale 2011). Its range extends from North Carolina, south to Florida, and as far west as Texas. Despite the current official recognition of *E. quadridigitata* as a single species, mitochondrial work has noted deep divergences within the species (Chippindale et al. 2000), and expert opinion suggests that *E. quadridigitata* consists of at least two (Chippindale et al. 2000) and possibly as many as four cryptic species (Bonett and Chippindale 2011). We sought to obtain microsatellite markers for *E. quadridigitata* to investigate fine scale population structure and potential cryptic species divergence among individuals collected from specific localities (i.e., putative populations).

We extracted genomic DNA from *E. quadridigitata* tail tissue preserved in 95% EtOH using standard phenol-chloroform procedures (Sambrook et al. 1989). Genomic DNA was then serially enriched twice for microsatellites using three probe mixes following Glenn and Schable (2005), with the changes described in Henningsen et al. (2010) and used the SimpleX-2 linker (Henningsen et al. 2010). All methods for sequencing, microsatellite identification, primer design, and primer screening are as described in Henningsen et al. (2010).

Forty-eight primer pairs were screened for amplification and polymorphism on DNA from eight *E. quadridigitata* specimens. Genomic DNA was extracted as described above. PCR amplification was performed in 12.5 μL volume reactions with 10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.36μM unlabeled primer, 0.04μM tag labeled primer, 0.36μM universal dye-labeled primer, 2.1 mM $MgCl_2$, 0.8 mM dNTPs, 0.5

units JumpStart Taq DNA Polymerase (Sigma), and ~40 ng DNA template using an Applied Biosystems GeneAmp 9700. Euqu09 was amplified with Amplitaq Gold buffer and polymerase (Applied Biosystems).

Most loci were amplified using one of three touchdown PCR protocols (Don et al. 1991), TD65, TD60, or TD58 (Table 1). Each touchdown protocol included an 8°C span of annealing temperatures (65-57°C, 60-52°C, and 58-50°C respectively). Touchdown cycling parameters consisted of 16 cycles of 95°C for 30s, highest annealing temperature of 65°C, 60°C, or 58°C (decreased by 0.5°C per cycle) for 30s, and 72°C for 45s; and 24 cycles of 95°C for 30s, 57°C or 50°C for 30s, and 72°C for 45s. Loci that did not amplify with a touchdown PCR protocol were screened with the same PCR protocol but using a single annealing temperature (Table 1) for all 40 cycles. PCR products were run on an ABI-3730xl sequencer and compared with Naurox size standard prepared as described in DeWoody et al. (2004) except that unlabeled primers started with GTTT. Results were analyzed using GENEMAPPER version 4.0 (Applied Biosystems). Twelve of the tested primer pairs amplified high quality PCR product and displayed polymorphisms.

Thirty specimens of *E. quadridigitata*, collected from a single wetland at the Joseph W. Jones Ecological Research Center (JERC) at Ichauway in Baker County, GA, were assessed for genetic variability at these loci. Conditions and characteristics of the 12 loci are given in Table 3.1. We estimated the number of alleles per locus (*k*), observed and expected heterozygosity (H_o and H_e), and tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium using GENEPOP v4.0 (Raymond and Rousset 1995, Rousset 2008). Probability of identity (PI) was estimated in GENALEX v6.0 (Peakall and Smouse 2006). After sequential Bonferroni corrections for multiple comparisons, linkage disequilibrium was not detected at any of the 66 locus-pairwise comparisons; however 3 loci (Euqu04, Euqu31, and Euqu45) deviated from expectations under HWE.

3.3 ACKNOWLEDGEMENTS

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3.4 LITERATURE CITED

Bonett, R. M. and P. T. Chippindale. 2011. Eurycea quadridigitata. AmphibiaWeb: Information on amphibian biology and conservation. Berkeley, California: AmphibiaWeb, Berkeley, California.

Chippindale, P., A. Price, J. Wiens, and D. Hillis. 2000. Phylogenetic relationships and systematic revision of central Texas hemidactyliine plethodontid salamanders. Herpetological Monographs **14**:1-80.

DeWoody, J. A., J. Schupp, L. Kenefic, J. Busch, L. Murfitt, and P. Keim. 2004. Universal method for producing ROX-labeled size standards suitable for automated genotyping. Biotechniques **37**:348-353.

Don, R. H., P. T. Cox, B. J. Wainwright, K. Baker, and J. S. Mattick. 1991. 'Touchdown'PCR to circumvent spurious priming during gene amplification. Nucleic Acids Research **19**:4008.

Glenn, T. C. and N. A. Schable. 2005. Isolating Microsatelline DNA Loci. Methods in Enzymology **395**:202-222.

Henningsen, J., S. Lance, K. Jones, C. Hagen, J. Laurila, R. Cole, and K. Perez. 2010. Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae). Conservation Genetics Resources **2**:247-250.

Peakall, R. O. D. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248.

Rousset, F. C. O. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Table 3.1. Details for 12 polymorphic microsatellite loci developed for the dwarf salamander, *Eurycea quadridigitata*. The annealing temperature (T_A °C) where TD65, TD60, and TD58 indicates touchdown protocols with a highest annealing temperature of 65°C, 60°C and 58°C, respectively; size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; the number of individuals genotyped is N; *k* is the number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus.

Locus	Primer Sequence 5' → 3'	Repeat motif	T_A	Size (bp)	N	*k*	H_o	H_e	PI
Euqu01	F: GTTTAATGACAGGTAAGACGGGCA	(CTGT)	TD65	283 - 317	28	7	0.821	0.810	0.071
	R: *AATCACACCACCCAAGTCGT								
Euqu04	F: GTTTACTCGTGAATTTGAGGTAGG	(AAGG)	57	291 - 319	29	5	0.448†	0.735	0.122
	R: *GGCATAGCTGTGTAACTGGG								
Euqu09‡	F: *AAGTGAAACGCATCCACCAG	(CATT)	TD65	154 - 158	30	2	0.200	0.183	0.689
	R: GTTTCTGTGAGCGGTTGCAAGAAT								
Euqu16	F: *GCACAGGAACCAATTGACCC	(CTGT)	TD58	143 - 207	30	8	0.700	0.832	0.057
	R: GTTTGAGGGCCACCAATGATTTA								
Euqu17	F: *AGCTAGCATTGGTGTTCTGGA	(AATG)	TD58	184 - 229	30	9	0.867	0.880	0.034
	R: GTTTAATGCTGCCATCAGGTAGGG								
Euqu20	F: *CAGGGACCAAGAGAATTGCC	(AATG)	TD65	253 - 269	30	4	0.500	0.656	0.195
	R: GTTTAAGCTCCTGCAACTACCCAA								
Euqu24	F: *AGTGTTTCTTGCTTAGTGCC	(CATT)	TD60	113 - 141	30	5	0.633	0.646	0.201
	R: GTTTAACAGAGCCCACCTTGA								
Euqu25	F: *TCCAGGTGCATTCTCAACCA	(AAAC)	TD65	301 - 304	29	2	0.241	0.216	0.643
	R: GTTTGTGGCAATATGCCTCACAAGT								
Euqu31	F: *TTTGTCCACAGCAGCCTGAA	(GATT)	TD58	323 - 349	30	6	0.400†	0.625	0.181
	R: GTTTGCCTTGAATTGGGTGCAAA								
Euqu36	F: *CGGTTCGGGAAATCACAGTT	(AGAT)	55.9	297 - 352	30	7	0.800	0.813	0.069
	R: GTTTACAGCAGTTTGCTACTAGTCTG								
Euqu45	F: GTTTGGCTTGAGTGCTCTAGAAAGG	(ACAT)	55.9	148 - 157	28	3	0.321†	0.657	0.202

	R: *CCAGTATTGAACTGGGACCAT								
Euqu46	F: GTTTAATGGCACGTTGTTTGCCG	(CATT)	TD65	246 - 270	30	7	0.900	0.782	0.085
	R: *AGCTAGGCTTGGAAGGTGTT								

* Indicates CAG tag (5'- CAGTCGGGCGTCATCA-3') label; † Indicates significant deviations from Hardy-Weinberg expectations after sequential Bonferroni corrections. ‡ Indicates Amplitaq Gold DNA polymerase was used in place of Jumpstart Taq.

CHAPTER 4

LOCAL AND LANDSCAPE PREDICTORS OF GENETIC DIVERSITY IN POPULATIONS OF TWO POND-BREEDING AMPHIBIAN SPECIES WITH DIFFERING VAGILITIES[3]

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4.1 ABSTRACT

Habitat alteration often modifies population dynamics and connectivity of pond-breeding amphibian species; however, the strength and direction of effects of these alterations may vary among species with differing vagilities and habitat preferences. We investigated the associations of habitat features and spatial scales with genetic diversity in two pond-breeding amphibian species with differing vagilities. In 2008 and 2009 we sampled larva and adults of these two species at 30 isolated wetlands in a longleaf pine reserve and surrounding area in southwest Georgia. We genotyped 12 microsatellite loci in dwarf salamanders (*Eurycea quadridigitata*) from 9 wetlands. We also genotyped 11 microsatellite loci for southern leopard frogs (*Lithobates sphenocephalus*) from 10 isolated wetlands. We used model selection to determine which local (wetland area, isolation, and hydroperiod) and landscape scale land cover features (wetlands, forest, agriculture, and roads), as well as the spatial scale (0.5, 1.0, and 2.5-km) of landscape features, were most closely associated with allelic richness and observed heterozygosity for both species. We used model averaging to determine the directional association of each local and landscape feature with genetic diversity in both species. Dwarf salamanders had more significant directional associations with local and landscape scale features than the southern leopard frog. Dwarf salamanders allelic richness was greater in less isolated wetlands and greater heterozygosity in larger wetlands with more wetland area with 0.5-km. Southern leopard frog allelic richness was greatest in wetlands with more agricultural area within 2.5-km, and observed heterozygosity was greatest in less isolated wetlands. Genetic diversity of both species was generally greater in wetlands with fewer surrounding roads and surrounding forest area. Our results indicate the important effect upland habitat and roads may have on amphibian genetic diversity. Conservation plans should focus on minimizing roads and maintaining forest cover for amphibian dispersal and non-breeding habitat.

4.2 INTRODUCTION

The maintenance of genetic diversity is widely accepted as important to the conservation and management of wildlife. Genetic diversity is the grist of evolution, and facilitates the maintenance of a diversity of phenotypes that provide for local adaptation. Further, genetic diversity is important for

population persistence because it allows for adaptive responses to environmental change (Lande 1988, Semlitsch 2000). Finally, evidence suggests that populations with higher genetic diversity have greater fitness than less genetically diverse populations (Guyer and Bailey 1993, Rowe et al. 1999, Reed and Frankham 2003, Luquet et al. 2011); therefore, population management may be less predictable or effective without the management and maintenance of high genetic diversity. While the value of genetic diversity may be widely accepted, the challenges of managing for genetic diversity can be daunting. The ability to measure and monitor genetic diversity will be limited by time, expertise and cost; and the general discomfort with genetics and evolutionary processes among the general public (Vellend 2003, McKee et al. 2012) and many land managers is likely to lead to limited incorporation of genetic goals in management plans. In contrast, most managers are comfortable with the management of habitats or keystone species, which are easier and more tangible to assess; therefore, establishing relationships between habitat variables and genetic diversity will facilitate the management of genetic diversity via more traditional habitat management.

Effective maintenance of genetically diverse populations requires an understanding of the evolutionary processes responsible for determining the gain or loss of genetic diversity. Over an ecological time frame, genetic diversity in populations is gained by gene flow from other populations and lost through genetic drift (Jaenike 1973, Vellend 2005, Cleary et al. 2006). Although natural selection is also a mechanism of evolution, the effect of selection may be difficult to predict (Vellend and Geber 2005). Therefore, management efforts to maintain or increase genetic diversity in populations should focus on maximizing gene flow (by maximizing dispersal) and minimizing genetic drift (by maximizing effective population sizes). Habitat alteration and fragmentation have led to increased population isolation for many terrestrial species. The effect of habitat isolation on dispersal can be largely dependent on the intervening matrix (Shields 1982, Joly et al. 2001, Cushman 2006, Franklin and Lindenmayer 2009, but see Marsh 2004) especially in species with limited vagility, such as pond-breeding amphibians (Prugh et al. 2008).

Many pond-breeding amphibian species are believed to occur as metapopulations because of their limited vagility (Sinsch 1990, Gibbs 1998, Bowne and Bowers 2004), high philopatry and fidelity to breeding sites (see Smith and Green 2005 for review), and because wetlands are relatively small discrete entities

embedded in large matrices of terrestrial habitats (Marsh and Trenham 2001, Smith and Green 2005). Metapopulations depend on occasional dispersal among subpopulations for long-term persistence through rescue events (Semlitsch and Bodie 1998, Hanski 1999, Semlitsch 2002) and gene flow (Lande 1988, Rowe et al. 1999, Cushman 2006), which are important for counteracting the effects of genetic drift and inbreeding (Lande 1988, Rowe et al. 1999, Semlitsch 2000, Andersen et al. 2004, Luquet et al. 2011). In instances where pond-breeding amphibian population dynamics resemble source-sink dynamics as opposed to the classic metapopulation model, maintaining dispersal among source populations is still essential for the long term persistence of the source populations (Harrison 1991).

A number of studies have tested the relationships between habitat characteristics and amphibian species presence and abundance. For example, local wetland characteristics, such as hydroperiod (Pechmann et al. 1989, Semlitsch et al. 1996, Snodgrass et al. 2000) and predatory fish (Piha et al. 2007, Murphy et al. 2010), as well as landscape scale features, such as land use (*i.e.,* forests and agriculture) (Gagné and Fahrig 2007, Piha et al. 2007, Eigenbrod et al. 2008), can affect species distributions (Simon et al. 2009). These studies are helpful for understanding the habitat features associated with amphibian distributions, but the results do not necessarily translate to information about which habitat features are closely associated with evolutionary potential or inbreeding. Amphibian landscape genetics studies have become more common in the literature, however few studies have focused on more than one species simultaneously (Scribner et al. 2001, Andersen et al. 2004, Spear et al. 2005, Greenwald et al. 2009, Richter et al. 2009, but see Goldberg and Waits 2010, Murphy et al. 2010), despite a recognized need to consider genetic data from multiple species for a more comprehensive approach to conservation (Whiteley et al. 2006). For conservation efforts to be effective across an amphibian community, differences in characteristics, such as vagility and habitat requirements, among species must be taken into consideration (Levin 1992). Species with differing habitat requirements and vagilities are often associated with different habitat features (Liner et al. 2008, Simon et al. 2009) across different scales (Cushman 2006, but see Piha et al. 2007, Eigenbrod et al. 2008, Veysey et al. 2011). The effects of habitat alteration may therefore differ in strength and operate at different scales among species (Hanski 1998, Ficetola and De Bernardi 2004, Swihart and Verboom 2004, Cushman 2006).

The southeastern United States has undergone major habitat alteration, most notably the conversion of longleaf pine forest first to other species of intensive planted pine and more recently in southwestern Georgia, to extensive center-pivot agriculture. Historically, the longleaf pine, wiregrass ecosystem covered between 22-37 million ha of the southeastern coastal plain (Frost 1993, Ware et al. 1993). The longleaf pine ecosystem is now reduced to less than three percent of its original range, with the rest now mostly comprised of pine plantations and agricultural land (Frost 2006). Concurrent with the loss of longleaf pine ecosystem has been the loss of associated seasonally ponded, isolated wetlands, which contribute significantly to the biodiversity of the region (Kirkman et al. 1999, Kirkman et al. 2012). These wetlands are generally ephemeral, preventing the establishment of predatory fish populations and providing important habitat for a number of endemic invertebrate and amphibian species (Guyer and Bailey 1993), including the threatened flatwoods salamander (*Ambystoma cingulatum*), gopher frog (*Rana capito*), and striped newt (*Notophthalmus perstriatus*). The longleaf forest has been listed as a threatened ecosystem (Noss et al. 1995), which has drawn attention and resources towards habitat restoration (e.g., The Nature Conservancy's longleaf pine restoration projects, federally funded conservation easements, incentive programs in the federal Farm Bill, and the Natural Resources Conservation Service incentive programs). To inform and optimize longleaf pine habitat restoration for amphibian conservation, we must first understand the local wetland and landscape scale habitat features associated with amphibian populations.

We examined populations of two pond-breeding amphibian species with differing vagilities and habitat requirements in native longleaf pine ecosystems to determine 1) the extent to which genetic diversity varies among populations, 2) which habitat features best predict genetic diversity, 3) the directional association between habitat features and genetic diversity, and 4) the spatial scale at which habitat features are most strongly associated with genetic diversity. We focused on dwarf salamanders (*Eurycea quadridigitata*) and southern leopard frogs (*Rana sphenocephala*, also commonly known as *Lithobates sphenocephalus*) in a longleaf pine reserve is southwestern Georgia (GA), USA. As adults, both species are strongly associated with wetlands and wetland edges during breeding and non-breeding seasons. Although they both utilize aquatic habitats for

mating and larval development, and (semi-) terrestrial habitats as adults they differ in vagility and microhabitat requirements and may require different approaches at different spatial scales to optimize conservation efforts.

We used an information theoretic approach to compare how well local wetland characteristics versus anthropogenic and natural landscape scale land cover features were able to predict population genetic diversity of both species. We predicted genetic diversity of the southern leopard frog, which has higher vagility (Smith and Green 2005) and is able to breed in a variety of wetland types (Liner 2006), would be best predicted by habitat features at greater landscape scales compared to the dwarf salamander, which we predicted would be more closely associated with local habitat and small landscape scale features because of its restricted dispersal (Pechmann et al. 2001) and microhabitat conditions (Mount 1975, Petranka 1998). Based on these differences in habitat restrictions, we hypothesized that genetic diversity in the dwarf salamander would be more closely tied (more significant associations) to habitat features than the southern leopard frog. In terms of the directional associations with habitat features, based on the literature, we had multiple biological and associated statistical hypotheses for the relationships between each species and many of the local and landscape variables (Table 4.1).

4.3 METHODS

4.3.1 FOCAL SPECIES

The dwarf salamander and the southern leopard frog are widespread in the southeastern US and they are relatively abundant in our study area (Cash 2008, Means 2008). The morphology and physiology of the dwarf salamander restrict when and where this species can disperse. Adults are generally 22-26 mm snout-vent length (SVL; Means 2008), making them one of the smallest vertebrates in the US. This species is a member of the lungless family of salamanders (Plethodontidae). Their lack of lungs requires them to respire through their skin and the tissues lining their mouths, all of which must remain moist for gas exchange to occur, making them sensitive to desiccation. This species most commonly breeds in wetlands with longer hydroperiods, however it may be susceptible to fish predation (Liner 2006), and therefore still require ephemeral breeding sites. Outside of breeding season, post-metamorphic dwarf salamanders are commonly

found in moist habitats, such as beneath cover objects around pond edges and swamps (Mount 1975, Petranka 1998).

Southern leopard frogs are medium sized anurans (adults are generally 50-130 mm SVL). Their tadpoles are unpalatable to native/local fish species, and leopard frogs are therefore are not restricted in breeding sites by the presence of predatory fish (Baber 2001, Babbitt et al. 2006). Adults have lungs and powerful legs, which facilitate mobility. The lower surface-area to volume ratio of a larger-bodied species reduces the rate at which gas exchange and water loss occurs and may make them less susceptible to environmental stress (Lindstedt and Boyce 1985). Some movement data are available, the maximum recorded movement distance for the dwarf salamander was 0.6-km (Pechmann et al. 2001), whereas the northern leopard frog (*L. pipiens*; a close relative to the southern leopard frog) has been recorded to move distances of 8.0-km (Seburn et al. 1997, Lehtinen and Galatowitsch 2001, Smith and Green 2005).

4.3.2 STUDY AREA

Study wetlands (amphibian breeding sites; the unit of study) were located at the Jones Ecological Research Center at Ichauway (31°13'16.88"N and 84°28'37.81"W; Figure 4.1) located in Baker County, Georgia. Ichauway is an 11,800 ha longleaf pine (*Pinus palustris*) reserve, spotted with numerous isolated wetlands, bounded by the Flint River on the eastern border, and 23-km of Ichauwaynochaway Creek runs through the property. Study wetlands (also referred to as staff gauge wetlands) at Ichauway vary in size (0.2 – 76.4 ha), hydroperiod (number days per year the wetland is at least 25% full; 11 – 225 days), and vegetation type (grass-sedge marshes, cypress savannas, and cypress-gum swamps). Marshes tend to be the largest of the wetland types and have moderate hydroperiods. Cypress savannas are generally the smallest wetlands and have the shortest hydroperiods. Cypress-gum swamps tend to be of intermediate size and have the longest hydroperiods (Kirkman et al. 2000). Intervening habitat among the study wetlands included forest, agriculture, open water, wildlife food plots, scrub and shrubs, and paved and dirt roads. The properties surrounding Ichauway are composed almost entirely of center-pivot agricultural fields.

4.3.3 FIELD SAMPLING

Of the 90 possible isolated wetlands on Ichauway that could be sampled for amphibians, we restricted our surveys to 30, 29 of which are included in an on-going, long-term monitoring study of relatively undisturbed isolated wetlands on Ichauway. The condition of these 29 wetlands suggests they can be used as reference sites for comparison with more degraded isolated wetlands in the region(Brinson and Rheinhardt 1996). The wetland not included in the long-term monitoring study (DS2; referred to as Psk; Kirkman et al. 2012) is a hardwood depression that maintains water by runoff from adjacent agricultural fields. We collected larval and adult dwarf salamanders and larval southern leopard frogs from nine and ten wetlands, respectively, during the breeding season in 2008 and 2009 (Table 4.2; University of Georgia IACUC permit #A2009-10030-0). For each species, attempts were made to collect a minimum of 30 samples per wetland from at least nine wetlands; however this was not possible for either species in 2008. We assumed that the genetic composition of breeding assemblages from the incompletely surveyed wetlands would be similar in 2008 and 2009 based on the philopatric tendencies of both species (for review see Blaustein et al. 1994, Smith and Green 2005). Therefore, sites where the collection goals were not met in 2008 were revisited in 2009 to collect additional samples. For the southern leopard frog, one wetland was sampled in 2008 only, two were sampled in 2009 only, and seven were sampled in 2008 and 2009 (Table 4.2). In addition to the hardwood depression site (Psk) three of all three wetland types were sampled for leopard frogs. For the dwarf salamander, one wetland was sampled both years, while five and four wetlands were sampled in 2008 and 2009, respectively (Table 4.2). Dwarf salamander populations were sampled from eight cypress-gum swamps and Psk. Larval amphibians were collected with dipnets and funnel traps.

To obtain genetic samples representative of each wetland, dipnet sweeps were distributed equally around the perimeter and shallow microhabitats (<0.5 m) of each surveyed wetland. To avoid collecting full siblings, we collected a maximum of one individual per sweep when wetlands were large enough and larvae were sufficiently abundant. However, in several instances larvae were sparse and collected opportunistically. Additionally, total rainfall in Baker County in 2008 was approximately 7.6 cm below normal (www.georgiaweather.net) and wetlands did not fill to capacity and dried quickly (unpublished data). At these

sites, dipnetting was still distributed equally around the perimeter of the standing water, however the likely collection of siblings was difficult to avoid. Funnel traps were used for supplemental sampling at sites where attaining target sample sizes proved difficult from dipnetting alone. Traps were distributed around the perimeter of the wetland and in shallow microhabitats and checked daily. In cases where tadpole species identification was questionable, individuals were collected and reared in the lab to metamorphosis when identification was possible. We were unable to collect a sufficient number of larval dwarf salamanders at any of the sites and we therefore supplemented our larval salamander samples with adult samples. Adult dwarf salamanders were collected opportunistically from under cover objects around the edges of the surveyed wetlands. All individuals caught in the field were brought back to the lab where they were euthanized in MS-222 and stored in 95% EtOH for genetic analysis.

4.3.4 MICROSATELLITE AMPLIFICATION

Genomic DNA was isolated from southern leopard frogs using silica-binding techniques whereas dwarf salamander DNA was isolated using phenol chloroform. Leopard frog DNA samples were screened at 16 microsatellite loci (Rasp01, Rasp03, Rasp07, Rasp09, Rasp10, Rasp13, Rasp16, Rasp17, Rasp20, Rasp28, Rasp37, Rasp42, Rasp45, Rasp50, Rasp53, and Rasp55(McKee et al. 2011b)and dwarf salamander DNA samples were screened at 12 microsatellite loci (Mckee et al. 2011a) using a 3730xl Genetic Analyzer and GeneMapper software (Applied Biosystems, Inc.). Negative controls were run with samples to ensure systematic contamination was not an issue. We used GENEMAPPER v4.0 (Applied Biosystems) to manually create allele bins and inspect allele calls. Approximately ten percent of the samples were rescreened at each locus to estimate genotyping error rates for each locus. Error rates per reaction were calculated following Hoffman and Amos (2005), where the rate represents the number of inconsistent genotypes divided by the total number of reactions compared (Table 4.3).

4.3.5 GENETIC ANALYSIS

We screened genotypes in COLONY 2 (Mac version) to detect full siblings in samples. The inclusion of full siblings in population genetic analyses can lead to inaccurate estimates of population genetic parameters (Struebig et al. 2011). When two samples had a probability of full sibship greater than 90%, the individual with the more complete genotype set was retained for analysis while the other sibling was removed from further analyses. Pairwise-loci tests for linkage disequilibrium were performed in GENEPOP 4.0 (web version, default settings; Raymond and Rousset 1995, Rousset 2008) with the Markov chain method and default parameter settings. Sequential Bonferroni corrections were applied to account for multiple comparisons (Weir 1990).

Null alleles are a statistical problem when trying to estimate allele frequencies because presence of null alleles results in higher rates of apparent observed homozygosity, leading to biased allele frequency estimates. We estimated null allele frequencies for each locus in MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004), with 10,000 iterations. We used the Chakraborty estimator (Chakraborty et al. 1992), because this estimator ignores non-amplified samples and we were uncertain whether non-amplification in our study was a result of defective PCRs or null alleles. MICROCHECKER results suggested high rates of null alleles within populations at a number of loci for each species (Table 4.3), but especially for the leopard frog. In most of these instances, null allele estimates were unrealistically high given the successful rate of amplification for those loci (e.g., Lisph.41 at Rasp20 had a null allele estimate of 1.0, despite the amplification of 17 out of 19 samples). We believed the exaggerated null allele estimates were due to multiple generations of relatives breeding at the same wetland, which would cause higher rates of inbreeding and therefore a greater frequency of homozygotes than expected. Even after accounting for the effect of inbreeding on homozygosity, five leopard frog loci (Rasp01, Rasp16, Rasp20, Rasp28, and Rasp42) were still highly suspect of not following Mendelian inheritance. These loci were statistically out of Hardy-Weinberg (HW) equilibrium in five or more leopard frog populations even after Bonferroni Corrections (Table 4.3). Additionally, these loci had evidence of significant null allele frequencies in eight to ten leopard frog populations (Table 4.3). We therefore removed these loci from analysis.

Probability of identity, private alleles within populations, expected heterozygosity under HW (H_e), and observed heterozygosity (H_o) for each locus were calculated in GENALEX v6.41 (Peakall and Smouse 2006). We tested for deviations from HW in GENEPOP web version 4.0.10 (Raymond and Rousset 1995, Rousset 2008) using exact tests at alpha = 0.05. The fixation index (F), which measures how far a population deviates from HW, was calculated for each population across loci, based on the equation (1- H_o/ H_e).

4.3.6 GENETIC DIVERSITY

We used allelic richness (r_g) and H_o as our metrics of genetic diversity. Allelic richness is indicative of a population's ability to adapt to changing environmental conditions whereas heterozygosity is often associated with short-term fitness in populations (James 1971, Petit et al. 1998). We calculated r_g in FSTAT v2.9.3.2 (Goudet 1995), which uses rarefaction to standardize allelic richness (r) estimates based on the smallest sample size of gene copies (g) (Hurlbert 1971, Mousadik and Petit 1996). For both species, one of the sites (Psk) had significantly lower r_g compared to the other sites. We performed analyses both with and without Psk to understand how inclusion of these outlier populations would affect our interpretation of local and landscape associations with genetic diversity in dwarf salamanders and southern leopard frogs. A number of results from the model selection and model averaging analyses for both species differed between analyses with and without Psk. We present genetic results for analyses with and without Psk. However, because of the heavy bias these outlier values caused in model selection and model averaging, we present only results without Psk for the subsequent local and landscape modeling analyses. Results from analyses with Psk are available as supplementary material (Appendix A).

4.3.7 LOCAL AND LANDSCAPE HABITAT CHARACTERIZATION

We used staff gauges, located in the deepest point of our sample sites, to designate the centers of the wetlands. In ArcMap 9 (ESRI), we created circle buffers around the center of each wetland (Piha et al. 2007) with radii of 0.5, 1.0, and 2.5-km (Houlahan and Findlay 2003, Piha et al. 2007, Veysey et al. 2011). We used 2006 National Land cover Data (NLCD; www.mrlc.gov/nlcd_2006.php) and Hawth's Tools

(www.spatialecology.com) Thematic Raster Summary by Polygon to calculate the percent area of each land cover feature (http://www.mrlc.gov/nlcd_definitions.php) within the buffers. Because our sample sizes were relatively small compared to the number of land cover classifications, we reduced our land cover types of interest to development (DEVEL; sum of all development land cover types), forest (FOREST; sum of all forest land cover types), agriculture (AG; sum of pasture and row crop), and wetland (WTLND; sum of wooded wetlands and emergent herbaceous wetlands). Ichauway is located in a rural area so development within and adjacent to our study area primarily consisted of roads. Thus, DEVEL was generally an indicator of road density rather than houses or urbanization. These percent variables were arcsine square root transformed and tested for normality with the Shapiro-Wilk test. Additional variables we believed might be of biological relevance to both species (Table 4.2) were wetland area (AREA), isolation (ISO), and hydroperiod (HYDRO). AREA was estimated from survey contours (all wetlands except Psk; see Kirkman et al. 2012) and hand-digitizing aerial photography (Psk; see Kirkman et al. 2012).These data were natural logarithmically transformed for subsequent analysis. Isolation was calculated with Hanski's isolation index (S_i; Hanski and Thomas 1994) using relative distances from all 90 wetlands on Ichauway as well as 34 wetlands within a 0.25-km buffer around Ichauway (Kirkman et al. 2012). A positive relationship between genetic diversity and ISO would indicate that genetic diversity was greater in more isolated sites. Hydroperiod was calculated as the average number of days over a calendar year that a wetland was at least 25% full based on staff gauge data collected from 2000 – 2011 (Kirkman et al. 2012). We chose to use this metric of hydroperiod, as opposed to the average number of days per year a wetland was 100% full, because amphibians bred in the wetlands even when they were not completely full (A. McKee personal observation).

4.3.8 MODEL SELECTION AND MODEL AVERAGING

Model selection was performed in SAM v4.0 (Rangel et al. 2010). We separated predictor variables into two categories: local variables (AREA, HYRO, and ISO) and landscape (DEVEL, FOREST, AG, and WTLND). We performed four rounds of model selection for both genetic diversity parameters of each species both with and without Psk (a total of 32 rounds of model selection). Top models were selected based

on the lowest Akaike's Information Criteria value, corrected for small sample size (AICc) (Burnham and Anderson 2002). We tested for correlations between predictor variables with Spearman rank-order correlation tests and we used a condition number (CN) to determine how much multicollinearity was an issue within models (Lazaridis 2007). A CN< 2 indicates that multicollinearity is not an issue, whereas a CN> 5 indicates multicollinearity is likely to greatly affect estimates. We were unable to cross-validate our models to determine their predictive ability because our sample sizes were too small to withhold data for validation purposes. We therefore assessed the predictive ability of our top models based on relative AICc and R^2 values. We calculated model averaged estimates and 95% confidence intervals for each predictor variable in SAM v4.0 (Rangel et al. 2010). When the 95% CI of the estimates did not cross zero, we considered this variable statistically significant and noted the direction of effect.

The lack of independence of spatially autocorrelated variables can lead to inaccurate relationships with dependent variables because of biased estimates of statistical error and inflated R^2 estimates (Anselin and Griffith 1988, Smith et al. 2006). When predictor variables are spatially autocorrelated the risk of a Type I error may be greater because of the lack of independence among values causes underestimates of standard errors (Legendre 1993, Lennon 2000, but see Diniz et al. 2003). We used Moran's I to examine the spatial autocorrelation of predictor variables.

4.4 RESULTS

We collected 30-31 dwarf salamanders from 9 wetlands and 30-40 leopard frogs from 10 wetlands (Table 4.2). After removing full siblings from the analysis, the number of dwarf salamanders per wetland ranged from 27-31 and the number of southern leopard frogs ranged from 15-30 (Table 4.2). After removing full siblings, estimates of F ranged from 0.05 to 0.13 for the dwarf salamander and -0.08 to 0.16 for the southern leopard frog.

4.4.1 LOCUS CHARACTERISTICS

The genotyping error rate per locus for both dwarf salamander and southern leopard frog markers ranged from 0 (no disagreements between scored genotypes) to 0.11, with the average for both species at 003 errors per reaction at a given locus (Table 4.3). Five of 12 dwarf salamander loci deviated from HWE after Bonferroni corrections (Table 4.3), and nine southern leopard frog loci (all except Rasp07 and Rasp09) deviated from HWE after Bonferroni corrections (Table 4.3). We did not find evidence of linkage disequilibrium for the dwarf salamander. For the leopard frog we found that every locus had evidence of linkage disequilibrium ($p \leq 0.05$) in at least one population; however no pair of loci was suspect of linkage disequilibrium in more than four populations.

4.4.2 GENETIC DIVERSITY PARAMETERS

After Bonferroni corrections, three dwarf salamander populations did not differ significantly from HW equilibrium expectations (Euqua.04, Euqua.11, and Euqua.sk; Table 4.2). All other populations of dwarf salamanders and southern leopard frogs differed significantly from HW equilibrium even after Bonferroni corrections, as expected given the philopatric tendencies of our focal species. Results from the half sibling analysis in COLONY suggested that we sampled a number of half siblings, some of which were collected during the same year and some of which were collected over separate years.

Southern leopard frog populations had, on average, greater r_g and H_o than dwarf salamander populations. Dwarf salamander r_g within populations ranged from 4.43 to 7.15 and H_o ranged from 0.55 to 0.64when Psk was included (Table 4.4). Southern leopard frog r_g within populations ranged from 3.57 to 9.85 and H_o ranged from 0.67 to 0.78when Psk was included (Table 4.4). Multicollinearity was not an issue for any of the top models of genetic diversity for either species (CN<2 for all models). Neither genetic diversity parameter for either species exhibited significant spatial autocorrelation when Psk was removed from the analysis (Appendix C).

4.4.3 SUMMARY OF LOCAL AND LANDSCAPE CHARACTERISTICS

For both species, on average, FOREST was the most dominant and DEVEL was the least dominant landscape type surrounding study wetlands. The percent AG within buffers generally increased with increasing buffer size. There was no statistically significant difference in leopard frog allelic richness or heterozygosity among wetland types (Appendix D). The cypress savanna wetlands from which leopard frogs were collected were on average smaller and had shorter hydroperiods than marshes or cypress-gum swamps (0.44 ha versus 1.99 and 2.94 ha respectively, Appendix E; 123 days versus 141 and 182 days respectively). Dwarf salamander study wetlands were on average larger and more isolated than southern leopard frog study sites. Dwarf salamander study wetlands ranged from 1.56 – 7.28 ha with an average size of 3.71 ha, southern leopard frog sites ranged in size from 0.09 – 5.88 ha with an average of 1.66 ha. Pairwise Euclidean distances between wetlands ranged from 0.754 – 12.148-km for the southern leopard frog and 0.755 – 12.035-km for the dwarf salamander. The isolation indices for the dwarf salamander study sites ranged from -8.86 to -2.63. The isolation indices for the southern leopard frog study sites ranged from -13.49 to 2.63.

Land cover variables FOREST and AG were highly correlated at all spatial scales across both species' sites; R ranged from -0.92 to -0.95for southern leopard frog sites and -0.83 to -0.93 for dwarf salamander sites. Local variables, AREA and HYDRO, were highly correlated (R = 0.75) at southern leopard frog sites. Isolation was highly correlated with landscape variables at the largest spatial scale at both species' sites. At southern leopard frog sites, ISO was correlated with $FOREST_{2.5km}$ (R = 0.77), whereas at dwarf salamander sites, ISO was correlated with $AG_{2.5km}$ (R= -0.74) and AREA (-0.88). All local and landscape variables, except for AREA and HYDRO, exhibited some evidence of spatial autocorrelation for the southern leopard frog (Appendix C). Most instances of spatial autocorrelation for the southern leopard frog were negative, indicating that values were more different than expected by random chance. The only evidence of spatial autocorrelation of landscape variables for the dwarf salamander was for $WTLND_{1.0km}$. The local variables AREA and ISO both had evidence of spatial autocorrelation for the dwarf salamander (Appendix C).

4.4.4 TOP MODELS

Top models of dwarf salamander genetic diversity suggested stronger associations (greater R^2 values) with local and landscape variables than the top models of southern leopard frog genetic diversity (Table 4.5). Dwarf salamander genetic diversity was generally best predicted by the area of wetlands within surrounding buffers, indicating that sites in greater proximity to other wetlands (*i.e.,* greater connectivity) tended to have greater genetic diversity. However the most relevant scale of connectivity differed between r_g and H_o. Allelic richness was most strongly associated with $WTLND_{2.5km}$ (AICc =18.141, R^2 = 0.623; Table 4.5), which suggests that r_g at a wetland is related to allelic richness harbored within the metapopulation. In contrast, H_o was best modeled $WTLND_{0.5km}$ (AICc =-35.457, R^2 = 0.74; Table 4.5). On average, the top models of dwarf salamander r_g explained slightly more variance than the top models of dwarf salamander H_o (mean R^2 = 0.46versus 0.40, respectively; Table 4.5). Neither $WTLND_{2.5km}$ nor $WTLND_{0.5km}$ exhibited evidence of spatial autocorrelation, therefore the relationships between these variables and r_g and H_o, respectively, are unlikely to be a result of a statistical artifact.

No single variable stood out as a consistent predictor of southern leopard frog r_g as each landscape variable was present in one landscape scale top model, and the average fit of the top models was relatively poor (mean R^2= 0.14, Table 4.5). The top model of southern leopard frog r_g was $AG_{2.5km}$ (AICc = 17.631, Table 4.5), which showed that r_g was greater in wetlands with more surrounding agriculture; however, the fit of this model was relatively poor (R^2 = 0.314, Table 4.5). Although DEVEL was the top model of southern leopard frog H_o across all landscape scales, the overall top model of southern leopard frog H_o was ISO (AICc = -33.906, R^2 = 0.333; Table 4.5), which showed that H_o was greater in more isolated wetlands. The predictor variables in the southern leopard frog overall top models of r_g and H_o both were significantly spatially autocorrelated, indicating the possibility that these relationships may have resulted from statistical biases as opposed to a true relationship between southern leopard frog genetic diversity and habitat. Both ISO and $AG_{2.5km}$ were highly correlated with $FOREST_{2.5km}$, though they were not correlated with each other, suggesting an association between forest cover and leopard frog genetic diversity as well.

4.4.5 MODEL AVERAGED ESTIMATES

We found significant associations between habitat features across all spatial scales and genetic diversity for both species (Table 4.6). In dwarf salamanders, less isolated wetlands with shorter hydroperiods supported greater r_g whereas larger wetlands supported greater H_o (Table 4.6). Larger and more isolated wetlands had greater H_o in the southern leopard frog whereas leopard frog r_g did not have any directional associations with any local variables (Table 4.6).

For both species, DEVEL was negatively associated with genetic diversity and AREA was positively associated with H_o (Table 4.6). Forest cover was generally positively associated with genetic diversity in both species, except for $FOREST_{1.0km}$, which had a significant negative association with southern leopard frog r_g (Table 4.6). Dwarf salamanders and leopard frog generally had opposite directional associations with AG and WTLND; dwarf salamanders were positively associated with WTLND and negatively associated with AG whereas southern leopard frogs generally had negative and positive associations with WTLND and AG, respectively (Table 4.6).

4.5 DISCUSSION

Genetic diversity provides variation that enables populations to adapt to changing environmental conditions; however it is an often-overlooked component in biodiversity conservation management. The incorporation of genetic diversity considerations into conservation management plans may be facilitated by determining relationships between genetic diversity parameters and habitat variables, which compared to population genetic diversity estimates, are more tangible and easier to assess for management purposes. We sought to determine the level of genetic diversity in two pond-breeding amphibian species with differing vagilities, and to use these genetic diversity parameter estimates to determine which landscape features and scales were most closely associated with amphibian population genetic diversity in a longleaf pine ecosystem.

4.5.1 GENETIC DIVERSITY WITHIN AND AMONG POPULATIONS

Southern leopard frogs had greater allelic richness than dwarf salamanders overall. Allelic richness values among populations had more variance for both species than observed heterozygosity, which did not vary greatly among populations. Several leopard frog loci were suspect of having null alleles, although this may have been a partial cause of population deviations from HW equilibrium, we believe a violation of the random mating assumption of HW likely had a stronger role. Some degree of inbreeding is to be expected in pond-breeding amphibian populations because of their philopatric tendencies (Shields 1982). Within populations, we collected a number of full siblings, which we removed from analysis. We also collected a number of half siblings within populations, most of which were collected within the same year. These half-siblings could have come from the same clutch, indicating multiple paternity, or from separate clutches, indicating promiscuous males (if females only lay one clutch per year). Several half siblings were collected within the same population across years suggesting philopatry at these sites and supporting previous findings of the philopatric tendencies of these species (Smith and Green 2005). We detected some evidence of linkage disequilibrium at all leopard frog loci, however, all of the alleged linked loci were suspect in fewer than half the populations, and that populations that exhibited linkage disequilibrium differed among loci. We therefore believed the linkage disequilibrium was an artifact of inbreeding as opposed to other possible causes (e.g. linked loci, segregation distortion, cryptic species, etc.), which suggests that our estimates of observed heterozygosity and allelic richness were representative of the observed diversity within the sampled populations.

4.5.2 LOCAL VARIABLES

Below average rainfall in southwest Georgia from 2006-2008 (total rainfall deviated from average -10.2, -43.2, and -7.6 cm respectively, in Baker County, Georgia) likely influenced the relationship between our focal species and the local and landscape variables during at least the first year of our sample collection. The positive association between area, and southern leopard frog heterozygosity was possibly a function of the relationships among wetland area, hydroperiod, and type. At Ichauway marshes and cypress-gum swamps are

alligator habitats because of their larger areas and longer hydroperiods (Kirkman et al. 2000, Liner 2006, Subalusky et al. 2009). Southern leopard frogs were collected from all three wetlands types present at Ichauway, but H_o was greater in larger wetlands (marshes and cypress-gum swamps). Alligator wallows and burrows in these wetlands may provide refugia for leopard frogs during the non-breeding season or during periods of drought (Kushlan 1974, Kushlan and Kushlan 1980, Finlayson and Moser 1991), which in turn could help maintain population sizes and reduce the risk of subsequent inbreeding (Frankham et al. 2002) and loss of allelic richness (Nei et al. 1975, Leberg 1992, Spencer et al. 2000). Heterozygosity in the southern leopard frog was greater at more isolated wetlands. Although this relationship may have been exaggerated due to spatial autocorrelation, the positive association between heterozygosity and the most isolated wetlands agreed with our hypothesis, which was based on results from a study on spotted salamanders (*Ambystoma maculatum*) and wood frogs (*L. sylvaticus*) (Veysey et al. 2011), that individuals would gather at more isolated sites due to limited habitat options.

Dwarf salamander allelic richness was greatest in less isolated wetlands with shorter hydroperiods. We were only able to collect dwarf salamanders from cypress-gum swamps, which on average have the longest hydroperiods of the wetlands at Ichauway, so a negative relationship between allelic richness and hydroperiod suggests that allelic richness was greatest in wetlands with longer hydroperiods that were still short enough to prevent predatory fish from persisting (Liner 2006). We believe the relationship between dwarf salamander allelic richness and isolation is valid, despite spatial autocorrelation confounding the relationship. Isolation was highly correlated with the best predictor, wetland area within 2.5 km, of dwarf salamander allelic richness, which is theoretically also a measure of isolation. Unlike the positive relationship between leopard frog heterozygosity and isolation, this indicates the overall importance of connectivity to alternative breeding habitats for maintaining allelic richness within dwarf salamander populations, which is consistent with the results of other studies that have found a positive association between amphibian species richness and/or amphibian species distributions and proximity to other wetlands (Sjögren 1991, Gulve 1994, Skelly et al. 1999).

4.5.3 LANDSCAPE-SCALE VARIABLES

Other studies on pond-breeding amphibians have suggested that dispersal is common enough when wetlands are separated by several hundred meters, without intervening barriers, that they function more as a single population as opposed to separate populations (Scribner et al. 2001, Petranka et al. 2004, Gamble et al. 2007, Zamudio and Wieczorek 2007, Veysey et al. 2011). Our results for the dwarf salamander also support this. Observed heterozygosity, which is often indicative of effective population size, was positively associated with wetland area within 0.5-km. The maximum recorded distance for dwarf salamander movement is only 0.6-km (Pechmann et al. 2001, Smith and Green 2005), which was the maximum measure of the study, suggesting inferences about dispersal distances may have been limited by the spatial scale of the study. A number of studies have suggested that amphibian dispersal measurements are often limited because of study scale constraints (Smith and Green 2005) and, in fact, long distance dispersal events may be more common in pond-breeding amphibian species than previously thought (Smith and Green 2005). The positive relationship between salamander allelic richness and wetland area within 2.5-km suggests that some degree of gene flow occurs among populations at this scale, enabling the introduction or reintroduction of alleles lost through drift. Houlahan and Findlay (2003) found a strong positive relationship between wood frog presence and proportion of wetlands within 0.75 and 3-km of study wetland edges, and a positive but weaker relationship between the same landscape predictors and presence of northern leopard frogs and spotted salamanders.

Between species, road area (DEVEL) had one of the most consistent directional relationships with genetic diversity. A number of previous studies have found that roads can be barriers to dispersal (Vos and Chardon 1998, Carr and Fahrig 2001) and decrease amphibian abundance and diversity (Houlahan and Findlay 2003). In some cases, the negative effect of roads on amphibian abundance/species richness can outweigh the benefit of proximal woodlands (Eigenbrod et al. 2008, but see Simon et al. 2009 for species that were not strongly associated with road density) of the landscape variables included in our study, development was one of the only variables associated with both genetic diversity parameters for the leopard frog. More vagile species may be more sensitive to effects of habitat fragmentation in the short term because they are more likely to encounter the altered habitat (Gibbs 1998, Homan et al. 2004, Cushman 2006). Eigenbrod et

al. (2008) found that more vagile amphibian species were more sensitive to the detrimental effects of road traffic in the short term because their greater vagility increased the rate of road encounters. However, we also found evidence that our less vagile species was sensitive to the presence of roads. The majority of roads at Ichauway are dirt roads, which may be greater barriers to dispersal to small amphibians than pavement because of the potential for additional water-loss.

Based on the model-averaged estimates, forests generally had a positive directional relationship with genetic diversity in both species. This was in agreement with our hypothesis for the dwarf salamander; forest cover is necessary for upland habitat and dispersal in many pond-breeding amphibians (Knutson et al. 1999, Guerry and Hunter 2002, Trenham and Shaffer 2005). In addition to providing upland and dispersal habitat for the southern leopard frog (Graeter et al. 2008), the positive relationship may also indicate the importance of drought refugia. We hypothesized that both species would be negatively associated with agriculture because of its potential as a barrier to dispersal (Table 4.1; Rothermel and Semlitsch 2002, Rothermel 2004), and our dwarf salamander results supported this hypothesis. For the southern leopard frog, even though agriculture within 2.5-km was the top model of allelic richness, the low R^2 value and lack of significance dispute a true relationship between agriculture and genetic diversity. A study on the short-term movement patterns of southern leopard frogs in clear-cut versus forested habitat showed that within a 24-hour period, the frogs initially preferred to move through the altered habitat versus forested habitat, however they ultimately preferred forested habitat (Graeter et al. 2008). This suggests that although southern leopard frogs may prefer forested habitat, altered habitats may be suitable temporary environments.

4.5.4 THE IMPORTANCE OF SCALE

The relative strength of the top landscape scale models versus the top local models of dwarf salamander genetic diversity suggest that habitat features at the landscape scale, 0.5 to 2.5-km in our study, are more relevant than local scale features to genetic diversity in our less vagile species. A similar result was found for spotted salamander abundance (Veysey et al. 2011), suggesting that populations of less vagile species may be more influenced by features of the upland and dispersal habitat compared to breeding habitat.

Alternatively, Piha et al. (2007) found that landscape scale variables were better predictors of common frog (*Rana temporaria*) egg abundance after a periods of drought, compared to the stronger relationship between local variables and egg mass abundance during normal weather conditions. This warrants the possibility that the spatial scale of greatest relevance between amphibian genetic diversity and habitat features may also shift depending on weather conditions. However, the scale of greatest relevance to the southern leopard frog is still debatable. The poor goodness-of-fit for our southern leopard frog top models lead us to question if our study was conducted at the correct scale for capturing the relationship between landscape and genetic diversity in our more vagile species (see Smith and Green 2005 for review of study scale and inference limitations in amphibian studies). The disagreements in directional effect of forest and agriculture on allelic richness versus heterozygosity in the southern leopard frog also support that we may have measured diversity and/or landscape variables at biologically inappropriate scales (Comps et al. 2001).

4.5.5 Recommendations for amphibian conservation in longleaf pine restoration projects

Genetic diversity is essential for populations to be able to adapt to changing environmental conditions. Current conservation strategies for pond-breeding amphibians generally focus on protecting breeding wetlands, without much consideration for protecting upland or dispersal habitat. Our results suggest that long term population persistence of pond-breeding amphibians in the southeastern US will require maintaining habitat that facilitates gene flow within metapopulations.

Area was positively associated with heterozygosity in the southern leopard frog. We believed this was due to wetland characteristics preferred by alligators, which may create and maintain drought refugia. For southern leopard frogs, focusing on restoring marsh and cypress-gum swamps and maintaining connectivity to alligator habitat would likely benefit leopard frog metapopulation persistence, especially over periods of drought. The consistent positive relationships between wetland area in surrounding buffers and genetic diversity in the dwarf salamander indicates that maintaining, restoring, and/or creating wetlands should be of high priority for management of dwarf salamander genetic diversity. We agree with Semlitsch (2002) recommendations to maintain high densities of wetlands and to restore or create stepping-stone wetlands.

Correlations between predictor variables confounded our ability to distinguish the effects of forest versus agriculture. Based on studies that have shown associations between amphibian occurrence and both forests and agriculture (Knutson et al. 1999, Guerry and Hunter 2002, Rothermel and Semlitsch 2002, Rothermel 2004, Trenham and Shaffer 2005), we believe dwarf salamander genetic diversity is likely influenced by both of these landscape features. For the southern leopard frog, the lack of consistent associations with agriculture, and the positive relationships between heterozygosity and forest cover suggest that forest was the more strongly associated feature, possibly because of the importance of drought refuge during our study. We recommend maintaining and/or restoring forest cover for the benefit of both species, and minimizing surrounding agriculture for dwarf salamanders and other forest-dwelling salamanders. Previous recommendations call for maintaining a critical minimum ~0.16 – 0.2-km forest buffer around wetlands (Semlitsch 1998, Semlitsch 2000, Semlitsch 2002) for amphibians. A number of other studies have suggested 1-2-km is the critical distance beyond which gene flow is unlikely to occur (Berven and Grudzien 1990, Sjögren 1991, Vos and Chardon 1998, Hranitz and Diehl 2000, Newman and Squire 2001, Scribner et al. 2001, Conroy and Brook 2003). Our results suggest that distances up to 2.5-km are likely still relevant to the maintenance of genetic diversity.

Similar to a number of previous studies that have shown roads to be detrimental to amphibian populations (see Fahrig and Rytwinski 2009 for review), we found that roads were negatively associated with genetic diversity in both species. Given that roads can be more detrimental to amphibian populations than deforestation (Fahrig et al. 1995, Vos and Chardon 1998, Houlahan and Findlay 2003), limiting road density within and among wetlands should be a primary goal, as it will benefit species with a range of vagilities. Our results suggest that landscape features at distances up to 2.5-km away from wetlands may affect genetic diversity in species, even those with limited vagility. This emphasizes the importance of considering landscape scale and non-breeding habitat features when developing conservation plans for amphibians. Given that 2.5-km was the largest buffer included in our study, we acknowledge the possibility that features at distances greater than 2.5-km may influence gene flow (Smith and Green 2005). Despite this limitation, the information gained from our study can be interpreted from a habitat management perspective in that it provides evidence

for targeting specific local and landscape features for conserving genetic diversity in southern leopard frogs and dwarf salamanders. The translation of genetic jargon into a language understood by land managers and the general public enables the field of landscape genetics to bridge the proverbial gap between academia and the general public, a necessity for timely and effective conservation management of genetic diversity in populations.

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4.7 LITERATURE CITED

Andersen, L. W., K. Fog, and C. Damgaard. 2004. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (Hyla arborea). Proceedings of the Royal Society B: Biological Sciences **271**:1293.

Anselin, L. and D. A. Griffith. 1988. Do spatial effecfs really matter in regression analysis? Papers in Regional Science **65**:11-34.

Babbitt, K. J., M. J. Baber, and L. A. Brandt. 2006. The effect of woodland proximity and wetland characteristics on larval anuran assemblages in an agricultural landscapeThis is contribution No. 82 of the MacArthur Agro-Ecology Research Center. Canadian Journal of Zoology **84**:510-519.

Baber, M. J. 2001. Understanding anuran community structure in temporary wetlands: the interaction and importance of landscape and biotic processes. Florida International University, Miami, FL.

Berven, K. A. and T. A. Grudzien. 1990. Dispersal in the Wood Frog (Rana sylvatica): Implications for Genetic Population Structure. Evolution **44**:2047-2056.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology **8**:60-71.

Bowne, D. R. and M. A. Bowers. 2004. Interpatch movements in spatially structured populations: a literature review. Landscape Ecology **19**:1-20.

Brinson, M. M. and R. Rheinhardt. 1996. The role of reference wetlands in functional assessment and mitigation. Ecological Applications **6**:69-76.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, NY.

Cabe, P. R., R. B. Page, T. J. Hanlon, M. E. Aldrich, L. Connors, and D. M. Marsh. 2007. Fine-scale population differentiation and gene flow in a terrestrial salamander (Plethodon cinereus) living in continuous habitat. Heredity **98**:53-60.

Carr, L. W. and L. Fahrig. 2001. Effect of Road Traffic on Two Amphibian Species of Differing Vagility. Conservation Biology **15**:1071-1078.

Cash, W. B. 2008. Southern Leopard Frog (*Rana sphenocephala*).*in* J. B. Jensen, C. D. Camp, and W. Gibbons, editors. Amphibians and reptiles of Georgia. University of Georgia Press, Athens, Georgia.

Chakraborty, R., M. Andrade, S. Daiger, and B. Budowle. 1992. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Annals of Human Genetics **56**:45-57.

Cleary, D. F. R., C. Fauvelot, M. J. Genner, S. B. J. Menken, and A. Ø. Mooers. 2006. Parallel responses of species and genetic diversity to El Niño Southern Oscillation-induced environmental destruction. Ecology Letters **9**:304-310.

Comps, B., D. Gömöry, J. Letouzey, B. Thiébaut, and R. Petit. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. Genetics **157**:389.

Conroy, S. D. S. and B. W. Brook. 2003. Demographic sensitivity and persistence of the threatened white-and orange-bellied frogs of Western Australia. Population Ecology **45**:105-114.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation **128**:231-240.

Diniz, J. A. F., L. M. Bini, and B. A. Hawkins. 2003. Spatial autocorrelation and red herrings in geographical ecology. Global Ecology and Biogeography **12**:53-64.

Eigenbrod, F., S. J. Hecnar, and L. Fahrig. 2008. The relative effects of road traffic and forest cover on anuran populations. Biological Conservation **141**:35-46.

Fahrig, L., J. H. Pedlar, S. E. Pope, P. D. Taylor, and J. F. Wegner. 1995. Effect of road traffic on amphibian density. Biological Conservation **73**:177-182.

Fahrig, L. and T. Rytwinski. 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecology and Society **14**:21.

Ficetola, G. F. and F. De Bernardi. 2004. Amphibians in a human-dominated landscape: the community structure is related to habitat features and isolation. Biological Conservation **119**:219-230.

Finlayson, M. and M. Moser, editors. 1991. Wetlands. International Waterfowl and Wetlands Research Bureau, Oxford.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Franklin, J. F. and D. B. Lindenmayer. 2009. Importance of matrix habitats in maintaining biological diversity. Proceedings of the National Academy of Sciences **106**:349.

Frost, C. 2006. History and Future of the Longleaf Pine Ecosystem. Pages 9-42 *in* S. Jose, E. J. Jokela, and D. L. Miller, editors. The Longleaf Pine Ecosystem: Ecology, Siviculture, and Restoration. Springer, New York, NY.

Frost, C. C. 1993. Four Centuries of Changing Landscape Patterns in the Longleaf Pine Ecosystem. Pages 17-43 *in* The Longleaf Pine Ecosystem: Ecology, Restoration, and Management. Proceedings of the Tall Timbers Fire Ecology Conference, Tall Timbers Research Station.

Gagné, S. A. and L. Fahrig. 2007. Effect of landscape context on anuran communities in breeding ponds in the National Capital Region, Canada. Landscape Ecology **22**:205-215.

Gamble, L. R., K. McGarigal, and B. W. Compton. 2007. Fidelity and dispersal in the pond-breeding amphibian, Ambystoma opacum: implications for spatio-temporal population dynamics and conservation. Biological Conservation **139**:247-257.

Gibbs, J. P. 1998. Amphibian movements in response to forest edges, roads, and streambeds in southern New England. The Journal of Wildlife Management **62**:584-589.

Goldberg, C. and L. Waits. 2010. Comparative landscape genetics of two pond breeding amphibian species in a highly modified agricultural landscape. Molecular Ecology **19**:3650-3663.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity **86**:485.

Graeter, G. J., B. B. Rothermel, and J. W. Gibbons. 2008. Habitat Selection and Movement of Pond Breeding Amphibians in Experimentally Fragmented Pine Forests. The Journal of Wildlife Management **72**:473-482.

Greenwald, K. R., H. L. GIBBS, and T. A. Waite. 2009. Efficacy of Land Cover Models in Predicting Isolation of Marbled Salamander Populations in a Fragmented Landscape. Conservation Biology **23**:1232-1241.

Guerry, A. D. and M. L. Hunter. 2002. Amphibian Distributions in a Landscape of Forests and Agriculture: an Examination of Landscape Composition and Conf iguration. Conservation Biology **16**:745-754.

Gulve, P. S. 1994. Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. Ecology **75**:1357-1367.

Guyer, C. and M. A. Bailey. 1993. Amphibians and reptiles of longleaf pine communities. Pages 139-158 *in* The Longleaf Pine Ecosystem: Ecology, Restoration, and Management Proceedings of the Tall Timbers Fire Ecology Conference. Tall Timbers Research Station, Tallahassee, Florida.

Hanski, I. 1998. Metapopulation dynamics. Nature **396**:41-49.

Hanski, I. 1999. Metapopulation Ecology. Oxford University Press, USA.

Hanski, I. and C. D. Thomas. 1994. Metapopulation dynamics and conservation: a spatially explicit model applied to butterflies. Biological Conservation **68**:167-180.

Harrison, S. 1991. Local extinction in a metapopulation context: an empirical evaluation. Biological Journal of the Linnean society **42**:73-88.

Hoffman, J. and W. Amos. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology **14**:599-612.

Homan, R. N., B. S. Windmiller, and J. M. Reed. 2004. Critical thresholds associated with habitat loss for two vernal pool-breeding amphibians. Ecological Applications **14**:1547-1553.

Houlahan, J. E. and C. S. Findlay. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences **60**:1078-1094.

Hranitz, J. M. and W. J. Diehl. 2000. Allozyme variation and population genetic structure during the life history of Bufo woodhousii fowleri (Amphibia: Anura). Biochemical Systematics and Ecology **28**:15-27.

Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology **52**:577-586.

Jaenike, J. R. 1973. A steady state model of genetic polymorphism on islands. The American Naturalist **107**:793-795.

James, J. 1971. The founder effect and response to artificial selection. Genet. Res **16**:241-250.

Joly, P., C. Miaud, A. Lehmann, and O. Grolet. 2001. Habitat matrix effects on pond occupancy in newts. Conservation Biology **15**:239-248.

Kirkman, L., S. Golladay, L. Laclaire, and R. Sutter. 1999. Biodiversity in southeastern, seasonally ponded, isolated wetlands: management and policy perspectives for research and conservation. Journal of the North American Benthological Society **18**:553-562.

Kirkman, L. K., P. C. Goebel, L. West, M. B. Drew, and B. J. Palik. 2000. Depressional wetland vegetation types: a question of plant community development. Wetlands **20**:373-385.

Kirkman, L. K., L. L. Smith, P. F. Quintana-Ascencio, M. J. Kaeser, S. W. Golladay, and A. L. Farmer. 2012. Is species richness congruent among taxa? Surrogacy, complementarity, and environmental correlates among three disparate taxa in geographically isolated wetlands. Ecological Indicators **18**:131-139.

Knutson, M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, and M. J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology **13**:1437-1446.

Kushlan, J. A. 1974. Observations on the role of the American alligator (Alligator mississippiensis) in the southern Florida wetlands. Copeia **1974**:993-996.

Kushlan, J. A. and M. S. Kushlan. 1980. Everglades alligator nests: nesting sites for marsh reptiles. Copeia **1980**:930-932.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455.

Lazaridis, A. 2007. A note regarding the condition number: the case of spurious and latent multicollinearity. Quality and Quantity **41**:123-135.

Leberg, P. L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution **46**:477-494.

Legendre, P. 1993. Spatial Autocorrelation: Trouble or New Paradigm? Ecology **74**:1659-1673.

Lehtinen, R. M. and S. M. Galatowitsch. 2001. Colonization of restored wetlands by amphibians in Minnesota. The American Midland Naturalist **145**:388-396.

Lennon, J. J. 2000. Red shifts and red herrings in geographical ecology. Ecography **23**:101-113.

Levin, S. A. 1992. The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. Ecology **73**:1943-1967.

Lindstedt, S. L. and M. S. Boyce. 1985. Seasonality, fasting endurance, and body size in mammals. The American Naturalist **125**:873-878.

Liner, A. E. 2006. Wetland predictors of amphibian distributions and diversity within the Southeastern U.S. Coastal Plain. University of Georgia, Athens, GA.

Liner, A. E., L. L. Smith, S. W. Golladay, S. B. Castleberry, and J. W. Gibbons. 2008. Amphibian Distributions within Three Types of Isolated Wetlands in Southwest Georgia. The American Midland Naturalist **160**:69-81.

Luquet, E., P. David, J. P. Lena, P. Joly, L. Konecny, C. Dufresnes, N. Perrin, and S. Plenet. 2011. Heterozygosity–fitness correlations among wild populations of European tree frog (Hyla arborea) detect fixation load. Molecular Ecology **20**:1877-1887.

Marsh, D. M. and P. C. Trenham. 2001. Metapopulation Dynamics and Amphibian Conservation. Conservation Biology **15**:40-49.

Mckee, A. M., S. L. Lance, K. L. Jones, C. Hagen, and T. C. Glenn. 2011a. Development and characterization of 12 microsatellite loci for the Dwarf Salamander, *Eurycea quadridigitata*. Conservation Genetics Resources **3**:1-3.

McKee, A. M., S. L. Lance, K. L. Jones, C. Hagen, and T. C. Glenn. 2011b. Development and characterization of 18 microsatellite loci for the Southern Leopard Frog, *Rana sphenocephala*. Conservation Genetics Resources **3**:267-269.

McKee, A. M., J. C. Maerz, L. L. Smith, and T. C. Glenn. 2012. Chapter 4: Local and Landscape Predictors of Genetic Diversity in Populations of Two Pond-breeding Amphibian Species with Differing Vagilities. University of Georgia, Athens, GA.

Means, B. 2008. Dwarf Salamander Complex (*Eurycea quadridigitata*).*in* J. B. Jensen, C. D. Camp, and W. Gibbons, editors. Amphibians and reptiles of Georgia. University of Georgia Press, Athens, Georgia.

Mount, R. H. 1975. The Reptiles and Amphibians of Alabama. University Alabama Press, Tuscaloosa, Alabama.

Mousadik, A. and R. Petit. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theoretical and Applied Genetics **92**:832-839.

Murphy, M., R. Dezzani, D. Pilliod, and A. Storfer. 2010. Landscape genetics of high mountain frog metapopulations. Molecular Ecology **19**:3634-3649.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution **29**:1-10.

Newman, R. A. and T. Squire. 2001. Microsatellite variation and fine scale population structure in the wood frog (Rana sylvatica). Molecular Ecology **10**:1087-1100.

Noss, R. F., E. T. LaRoe, and J. M. Scott. 1995. Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. US Dept. of the Interior, National Biological Service.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Pechmann, J. H. K., R. A. Estes, D. E. Scott, and J. W. Gibbons. 2001. Amphibian colonization and use of ponds created for trial mitigation of wetland loss. Wetlands **21**:93-111.

Pechmann, J. H. K., D. E. Scott, J. Whitfield Gibbons, and R. D. Semlitsch. 1989. Influence of wetland hydroperiod on diversity and abundance of metamorphosing juvenile amphibians. Wetlands Ecology and Management **1**:3-11.

Petit, R. J., A. El Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology **12**:844-855.

Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press Washington, DC.

Petranka, J. W., C. K. Smith, and A. Floyd Scott. 2004. Identifying the minimal demographic unit for monitoring pond-breeding amphibians. Ecological Applications **14**:1065-1078.

Piha, H., M. Luoto, M. Piha, and J. Merilä. 2007. Anuran abundance and persistence in agricultural landscapes during a climatic extreme. Global Change Biology **13**:300-311.

Prugh, L. R., K. E. Hodges, A. R. E. Sinclair, and J. S. Brashares. 2008. Effect of habitat area and isolation on fragmented animal populations. Proceedings of the National Academy of Sciences **105**:20770.

Rangel, T. F., J. A. F. Diniz Filho, and L. M. Bini. 2010. SAM: a comprehensive application for Spatial Analysis in Macroecology. Ecography **33**:46-50.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248.

Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology **17**:230-237.

Richter, S. C., B. I. Crother, and R. E. Broughton. 2009. Genetic consequences of population reduction and geographic isolation in the critically endangered frog, *Rana sevosa*. Copeia **2009**:799-806.

Rothermel, B. B. 2004. Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. Ecological Applications **14**:1535-1546.

Rothermel, B. B. and R. D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology **16**:1324-1332.

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

Rowe, G., T. Beebee, and T. Burke. 1999. Microsatellite heterozygosity, fitness and demography in natterjack toads Bufo calamita. Animal Conservation **2**:85-92.

Scribner, K. T., J. Arntzen, N. Cruddace, R. Oldham, and T. Burke. 2001. Environmental correlates of toad abundance and population genetic diversity. Biological Conservation **98**:201-210.

Seburn, C., D. Seburn, and C. Paszkowski. 1997. Northern leopard frog (Rana pipiens) dispersal in relation to habitat. Pages 64-72 *in* D. M. Green, editor. Herpetological Conservation: Canadian studies of a global problem. Society for the study of amphibians and reptiles.

Semlitsch, R., D. Scott, J. Pechmann, and J. Gibbons. 1996. Structure and dynamics of an amphibian community: evidence from a 16-year study of a natural pond. Long-term studies of vertebrate communities. Academic Press, San Diego, California, USA:217–248.

Semlitsch, R. D. 1998. Biological Delineation of Terrestrial Buffer Zones for Pond Breeding Salamanders. Conservation Biology **12**:1113-1119.

Semlitsch, R. D. 2000. Principles for Management of Aquatic-Breeding Amphibians. The Journal of Wildlife Management **64**:615-631.

Semlitsch, R. D. 2002. Critical Elements for Biologically Based Recovery Plans of Aquatic Breeding Amphibians. Conservation Biology **16**:619-629.

Semlitsch, R. D. and J. R. Bodie. 1998. Are small, isolated wetlands expendable? Conservation Biology **12**:1129-1133.

Shields, W. M. 1982. Philopatry, inbreeding, and the evolution of sex. State University of New York Press, Albany, NY.

Simon, J. A., J. W. Snodgrass, R. E. Casey, and D. W. Sparling. 2009. Spatial correlates of amphibian use of constructed wetlands in an urban landscape. Landscape Ecology **24**:361-373.

Sinsch, U. 1990. Migration and orientation in anuran amphibians. Ethology Ecology & Evolution **2**:65-79.

Sjögren, P. 1991. Extinction and isolation gradients in metapopulations: the case of the pool frog (Rana lessonae). Biological Journal of the Linnean society **42**:135-147.

Skelly, D. K., E. E. Werner, and S. A. Cortwright. 1999. Long-term distributional dynamics of a Michigan amphibian assemblage. Ecology **80**:2326-2337.

Smith, L. L., W. J. Barichivich, J. S. Staiger, K. G. Smith, and C. K. Dodd Jr. 2006. Detection probabilities and site occupancy estimates for amphibians at Okefenokee National Wildlife Refuge. The American Midland Naturalist **155**:149-161.

Smith, M. A. and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography **28**:110-128.

Snodgrass, J. W., M. J. Komoroski, A. L. Bryan, and J. Burger. 2000. Relationships among Isolated Wetland Size, Hydroperiod, and Amphibian Species Richness: Implications for Wetland Regulations. Conservation Biology **14**:414-419.

Spear, S. F., C. R. Peterson, M. D. Matocq, and A. Storfer. 2005. Landscape genetics of the blotched tiger salamander (Ambystoma tigrinum melanostictum). Molecular Ecology **14**:2553-2564.

Spencer, C., J. Neigel, and P. Leberg. 2000. Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. Molecular Ecology **9**:1517-1528.

Struebig, M. J., T. Kingston, E. J. Petit, S. C. Le Comber, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2011. Parallel declines in species and genetic diversity in tropical forest fragments. Ecology Letters **14**:582-590.

Subalusky, A. L., L. L. Smith, and L. A. Fitzgerald. 2009. Detection of American alligators in isolated, seasonal wetlands. Applied Herpetology **6**:199-210.

Swihart, R. and J. Verboom. 2004. Assessing biodiversity consequences of land-use decisions: a role for ecologically scaled landscape indices. Conserving biodiversity in agricultural landscapes: model-based planning tools. Purdue University Press, West Lafayette, Indiana:81–101.

Trenham, P. C. and H. B. Shaffer. 2005. Amphibian upland habitat use and its consequences for population viability. Ecological Applications **15**:1158-1168.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**:535-538.

Vellend, M. 2003. Island biogeography of genes and species. The American Naturalist **162**:358-365.

Vellend, M. 2005. Species diversity and genetic diversity: parallel processes and correlated patterns. The American Naturalist **166**:199-215.

Vellend, M. and M. A. Geber. 2005. Connections between species diversity and genetic diversity. Ecology Letters **8**:767-781.

Veysey, J., S. Mattfeldt, and K. Babbitt. 2011. Comparative influence of isolation, landscape, and wetland characteristics on egg-mass abundance of two pool-breeding amphibian species. Landscape Ecology **26**:661-672.

Vos, C. C. and J. Chardon. 1998. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. Journal of Applied Ecology **35**:44-56.

Ware, S., C. Frost, and P. D. Doerr. 1993. Southern mixed hardwood forest: the former longleaf pine forest. Pages 447-493 *in* W. H. Martin, S. G. Boyce, and A. C. Echternacht, editors. Biodiversity of the southeastern United States: Lowland terrestrial communities. John Wiley & Sons, New York, NY.

Weir, B. S. 1990. Genetic data analysis. Methods for discrete population genetic data. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Whiteley, A. R., P. Spruell, and F. W. Allendorf. 2006. Can common species provide valuable information for conservation? Molecular Ecology **15**:2767-2786.

Zamudio, K. R. and A. M. Wieczorek. 2007. Fine-scale spatial genetic structure and dispersal among spotted salamander (Ambystoma maculatum) breeding populations. Molecular Ecology **16**:257-274.

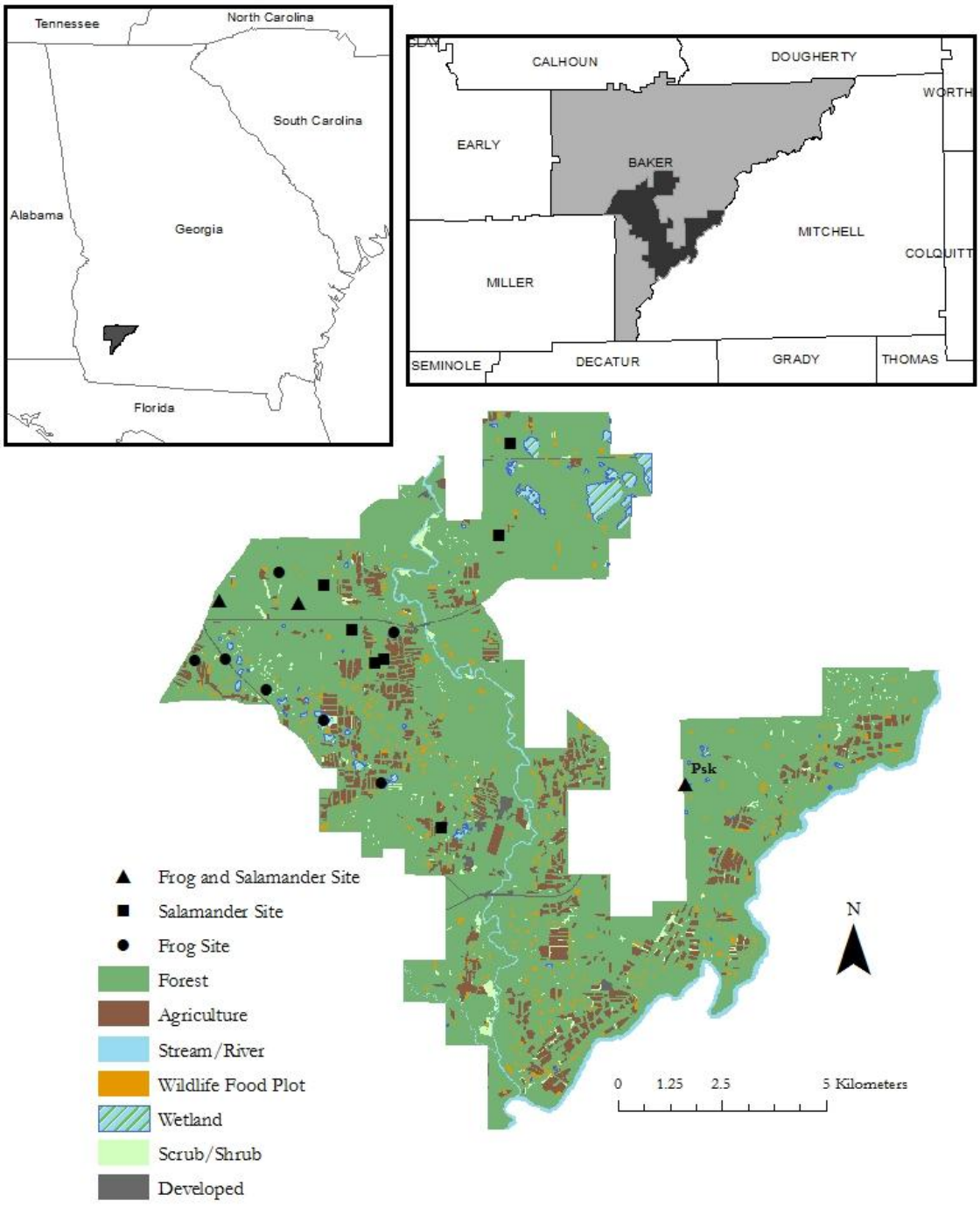

Figure 4.1. Map of Ichauway and the seasonal wetlands within the Ichauway boundary.

Table 4.1. Biological and statistical hypotheses for the relationships between habitat features and dwarf salamander (DS; *Eurycea quadridigitata*) and southern leopard frog (SLF; *Lithobates sphenocephalus*) genetic diversity. Local habitat features refer to variables associated with the breeding wetland (AREA, wetland area; ISO, wetland isolation; HYDRO, wetland hydroperiod) and landscape habitat features (AG, agriculture; DEVEL, development but primarily roads; FOREST, forest; WTLND, wetlands) refer to variables measured in circular buffers with radii 0.5-2.5-km around the wetland.↑ represents an expected increase in genetic diversity; ↓ represents an expected decrease in genetic diversity; NS indicates that no significant relationship expected.

Predictor	Effect	Biological Justification and References
Local		
AREA		
DS	↑/ NS	• (↑) More area indicates higher carrying capacity, neutral genetic theory; (Antonovics 1976, Antonovics 2003, Vellend 2004). • (NS) No significant relationship between wetland area and dwarf salamander presence (Snodgrass et al. 2000).
SLF	↑/NS	• (↑) More area indicates higher carrying capacity, neutral genetic theory; (Antonovics 1976, Antonovics 2003, Vellend 2004). • (NS) No significant relationship between wetland area and southern leopard frog presence (Snodgrass et al. 2000).
ISO		
DS	↑/↓	• (↑) Individuals may congregate at more isolated sites because of reduced habitat options (Veysey et al. 2011b). • (↓) Isolation may reduce immigration (Sjögren 1991, Marsh and Fegraus 1999).
SLF	↑/↓	• (↑) Individuals may congregate at more isolated sites because of reduced habitat options (Veysey et al. 2011b). • (↓) Isolation may reduce immigration (Sjögren 1991, Marsh and Fegraus 1999).
HYDRO		
DS	NS	• (NS) Dwarf salamanders are associated with aquatic habitats year-round (Means 2008, Bonett and Chippindale 2011), however fish may be predators of dwarf salamander larvae (Liner 2006). Dwarf salamander presence was not significantly related to wetland hydroperiod (Snodgrass et al. 2000).
SLF	↑/NS	• (↑) Southern leopard frog larvae are unpalatable to local fish species and therefore may thrive in wetlands with longer hydroperiods(Baber 2001, Babbitt et al. 2006). • (NS) Southern leopard frog presence was not significantly related to wetland hydroperiod (Snodgrass et al. 2000).
Landscape		
DEVEL		
DS	↓	• (↓) Development and roads may be partial barriers to dispersal and/or a source of mortality(Gibbs 1998, Vos and Chardon 1998, Carr and Fahrig 2001b).
SLF	↓	• (↓) Development and roads may be partial barriers to dispersal and/or a source of mortality (Gibbs 1998, Vos and Chardon 1998, Carr and Fahrig 2001b). Species with higher vagility may be affected by roads in the short-term than species with lower vagility(Cushman 2006).
FOREST		

DS	↑	• (↑) Forest cover is necessary for upland habitat and dispersal in many other pond-breeding amphibian species (Knutson et al. 1999, Guerry and Hunter 2002, Trenham and Shaffer 2005). Adult dwarf salamanders are often found under woody debris around pond margins (Means 2008).
SLF	↑/NS	• (↑/NS) In the short-term, leopard frogs may display an initial preference for movement through clear-cut areas, but eventually prefer forested habitat (Graeter et al. 2008)
AG		
DS	↓	• (↓) Agricultural landscapes may be partial barriers to amphibian dispersal because of the potential for water loss (Rothermel and Semlitsch 2002, Rothermel 2004).
SLF	↓	• (↓) Agricultural landscapes may be partial barriers to amphibian dispersal because of the potential for water loss (Rothermel and Semlitsch 2002, Rothermel 2004).
WTLND		
DS	↑	• (↑) Dwarf salamanders are associated with aquatic habitat year-round (Means 2008, Bonett and Chippindale 2011).
SLF	↑	• (↑) Southern leopard frogs may breed year-round in Georgia and are generally associated with aquatic habitats year-round (Cash 2008).

Table 4.2. Summary of population parameters in 9 populations of dwarf salamanders (*Euqua quadridigitata*; Euqua) and 10 populations of southern leopard frogs (*Lithobates sphenocephalus,* Lisph). Genetic diversity parameters based on estimates from 12 microsatellite loci in the dwarf salamanders and 11 microsatellite loci in the southern leopard frog. * Indicates population is in Hardy-Weinberg equilibrium after Bonferroni corrections, Latitude/Longitude of sites are in UTM, *N* is the sample size by year before (and after) removing full siblings, P is the number of private alleles, r_g is the mean number of alleles rarefied to 24 individuals (min sample size, dwarf salamander) and 13 individuals (leopard frog) ± the interlocus standard error, H_e is the expected heterozygosity (calculated as Nei's unbiased gene diversity; Nei (1987)) ± the inter-locus standard error, H_o is the observed heterozygosity ± the interlocus standard error, and *F* is the fixation index. P-value is the p-value from HWE exact tests in GENEPOP, § Indicates outlier values based on μ±1.96*standard error.

Population	Latitude/Longitude	N_{2008}	N_{2009}	P	r_g	H_e	H_o	*F*	P-value
Euqua.00	16 R 741137.17 m E 3466868.2 m N	10 (10)	21 (20)	1	6.68 (±0.87)	0.65(±0.09)	0.58(±0.08)	0.08(±0.04)	<0.0001
Euqua.01	16 R 734138.75 m E 3463106.12 m N	-	31 (31)	0	6.32 (±0.61)	0.63(±0.08)	0.57(±0.08)	0.11(±0.06)	<0.0001
Euqua.03	16 R 736048.12 m E 3463054.93 m N	31 (30)	-	2	6.89 (±0.71)	0.67(±0.08)	0.59 (±0.09)	0.13(±0.06)	0.0001
Euqua.04*	16 R 736666.06 m E 3463489 m N	31 (30)	-	1	7.15 (±0.83)	0.69(±0.06)	0.64 (±0.07)	0.07 (±0.05)	0.0305
Euqua.11*	16 R740856.45 m E 3464681.54 m N	-	31 (29)	4	6.11 (±0.63)	0.61(±0.08)	0.55 (±0.08)	0.05 (±0.06)	0.0853
Euqua.52	16 R 737998.405 m E 3461651.875 m N	31 (29)	-	2	6.81 (±0.69)	0.67(±0.07)	0.58 (±0.06)	0.11(±0.05)	<0.0001
Euqua.58	16 R 739472.5 m E 3457629.75 m N	-	30 (27)	1	5.21 (±0.45)	0.66(±0.06)	0.57 (±0.07)	0.10(±0.06)	0.0007
Euqua.68	16 R737330.65 m E 3462395.76 m N	31 (31)	-	0	6.85 (±0.64)	0.69(±0.06)	0.60(±0.05)	0.11 (±0.05)	0.001
Euqua.sk*	16 R 745332.5 m E 3458686 m N	31 (28)	-	0	4.43 (±0.38)§	0.62(±0.05)	0.59(±0.07)	0.08(±0.08)	0.0244
Lisph.01	16 R 734138.75 m E 3463106.12 m N	1 (1)	30 (30)	4	9.85 (±0.82)	0.85 (± 0.03)	0.74 (±0.04)	0.12 (±0.05)	<0.0001
Lisph.02	16 R 735598.04 m E 3463772.73 m N	2 (1)	29 (23)	1	9.38 (± 0.87)	0.85 (±0.03)	0.76 (±0.05)	0.09 (±0.04)	<0.0001
Lisph.03	16 R 736048.12 m E 3463054.93 m N	18 (6)	17 (14)	2	9.19 (±0.84)	0.83 (±0.04)	0.71 (±0.06)	0.13 (±0.05)	<0.0001
Lisph.27	16 R 733556.49 m E 3461669.34 m N	16 (13)	15 (15)	2	9.38 (±0.76)	0.84 (±0.03)	0.70 (±0.05)	0.16 (±0.05)	<0.0001
Lisph.41	16 R735278.02 m E 3460955.73 m N	-	30 (19)	2	9.24 (±0.89)	0.82 (±0.04)	0.72 (±0.05)	0.09 (±0.05)	<0.0001
Lisph.46	16 R736650.59 m E 3460216.03 m N	6 (4)	26 (25)	4	9.72 (±1.00)	0.84 (±0.04)	0.72 (±0.05)	0.13 (±0.03)	<0.0001
Lisph.53	16 R738359.54 m E 3462353.85 m N	19 (15)	12 (12)	2	8.33 (±0.76)	0.83 (±0.03)	0.72 (±0.06)	0.10 (±0.08)	<0.0001
Lisph.55	16 R738047.28 m E 3458732.26 m N	14 (4)	26 (21)	5	9.77 (±0.84)	0.87 (±0.02)	0.78 (±0.02)	0.08 (±0.03)	<0.0001
Lisph.96	16 R734309.96 m E 3461709.4 m N	-	31 (25)	2	9.34 (±0.83)	0.83 (±0.04)	0.71 (±0.04)	0.13 (±0.03)	<0.0001
Lisph.sk	16 R745332.5 m E 3458686 m N	40 (18)	-	1	3.57 (±0.31) §	0.63 (±0.03) §	0.67 (±0.08)	-0.08 (±0.12) §	<0.0001

Table 4.3. Characteristics of microsatellite loci used to estimate inbreeding, heterozygosity, and allelic richness in dwarf salamander (*Eurycea quadridigitata*; Euqu) and southern leopard frog (*Lithobates sphenocephalus*; Rasp) populations in southwestern Georgia (U.S.A.). PI represents the probability of identity averaged across loci. P-value is from Hardy-Weinberg Equilibrium (HWE) exact tests in GENEPOP. ᵞ Indicates a dwarf salamander locus. ‡Indicates loci were out of HWE after Bonferroni Corrections. § Indicates loci that were removed from analysis because of strong deviations from HWE (>4 populations out of HWE after Bonferroni corrections for multiple comparisons). Chakraborty null allele frequency estimates averaged across populations with suspected null alleles, number of populations contributing to the estimate in parentheses.

Locus	# of alleles	r_g	Allele size range (bp)	H_e	H_o	Genotyping error rate estimate	F_{ST}	PI	P-value	Null allele frequency
Euqu01ᵞ	11	9.34	279-317	0.86	0.83	0.00	0.09	0.08	1.00	-
Euqu04‡ᵞ	12	9.02	268-319	0.83	0.59	0.11	0.08	0.09	0.00	0.18 (6)
Euqu09ᵞ	3	2.91	154-170	0.30	0.31	0.00	0.04	0.55	1.00	-
Euqu16ᵞ	13	9.72	143-211	0.81	0.74	0.06	0.04	0.08	0.11	-
Euqu17ᵞ	15	11.74	181-237	0.90	0.88	0.02	0.05	0.04	1.00	-
Euqu20‡ᵞ	4	3.53	253-269	0.34	0.18	0.00	0.13	0.56	0.00	0.47 (4)
Euqu24ᵞ	10	6.52	113-150	0.77	0.67	0.00	0.06	0.12	0.01	0.16 (1)
Euqu25ᵞ	4	3.40	301-324	0.31	0.30	0.00	0.06	0.55	1.00	-
Euqu31‡ᵞ	7	6.45	323-349	0.81	0.60	0.05	0.09	0.12	0.00	0.20 (3)
Euqu36‡ᵞ	27	16.65	297-441	0.92	0.72	0.02	0.05	0.03	0.00	0.16 (5)
Euqu45‡ᵞ	6	4.42	136-157	0.63	0.44	0.11	0.06	0.23	0.00	0.23 (5)
Euqu46ᵞ	11	8.65	241-287	0.80	0.76	0.04	0.07	0.11	0.08	-
Rasp01‡§	9	8.00	272-328	0.81	0.20	0.03	0.09	0.12	0.00	0.63 (9)
Rasp03‡	14	10.98	283-339	0.91	0.79	0.00	0.05	0.04	0.00	0.10 (5)
Rasp07	3	4.63	287-308	0.59	0.62	0.00	0.05	0.25	0.25	-
Rasp09	11	10.57	196-372	0.90	0.90	0.00	0.04	0.04	0.85	-
Rasp10‡	14	10.80	152-224	0.90	0.79	0.00	0.06	0.06	0.01	0.09 (2)
Rasp13‡	5	5.20	190-215	0.71	0.50	0.00	0.05	0.16	0.00	0.22 (7)
Rasp16‡§	5	4.64	262-286	0.57	0.21	0.00	0.03	0.25	0.00	0.48 (10)
Rasp17‡	13	9.00	230-290	0.87	0.80	0.00	0.03	0.05	0.00	0.09 (1)
Rasp20‡§	14	14.63	140-302	0.94	0.30	0.02	0.06	0.03	0.00	0.60 (9)
Rasp28‡§	10	9.10	234-280	0.88	0.60	0.10	0.04	0.05	0.00	0.22 (9)
Rasp37‡	20	14.63	212-303	0.94	0.79	0.10	0.05	0.03	0.00	0.10 (6)
Rasp42‡§	12	10.91	362-435	0.91	0.67	0.04	0.04	0.04	0.00	0.18 (8)
Rasp45‡	16	11.72	159-228	0.92	0.68	0.02	0.06	0.04	0.00	0.20 (6)
Rasp50‡	11	8.98	421-493	0.85	0.64	0.02	0.05	0.08	0.00	0.26 (5)
Rasp53‡	15	11.37	266-335	0.91	0.79	0.02	0.05	0.04	0.00	0.11 (3)
Rasp55‡	11	9.38	152-242	0.81	0.67	0.10	0.05	0.09	0.00	0.19 (4)

Table 4.4. Means and ranges of allelic richness (r_g) and observed heterozygosity (H_o) across all populations including Psk and excluding Psk for both the dwarf salamander (*Eurycea quadridigitata*) and the southern leopard frog (*Lithobates sphenocephalus*).

Parameter	# Pops	Avg. (± SD)	Range
Dwarf Salamander			
r_g			
	9	6.27(±0.90)	4.43 – 7.15
	8	6.50(±0.62)	5.21 - 7.15
H_o			
	9	0.59 (±0.02)	0.55 - 0.64
	8	0.59 (±0.03)	0.55 - 0.64
Southern Leopard Frog			
r_g			
	10	8.78(±1.88)	3.57 – 9.85
	9	9.35 (±0.45)	8.33 – 9.85
H_o			
	10	0.72(±0.03)	0.59 - 0.68
	9	0.73(±0.03)	0.59 - 0.65

Table 4.5. Top models of allelic richness (r_g) and observed heterozygosity (H_o) for the dwarf salamander (*Eurycea quadridigitata*) and the southern leopard frog (*Lithobates sphenocephalus*). Condition number (CN) is the degree of multicollinearity in the model, when CN < 2, multicollinearity is not an issue in the model. AICc Wi is the model weight relative to all other models tested for the same species, genetic diversity parameter, number of populations, and at the same spatial scale. * Indicates the top model for a given parameter and number of populations. ‡ Indicates the 95% confidence interval of the variable does not cross 0.

Parameter/ Scale	Variable	Coeff.	SE	t	95% CI	r^2	CN	AICc	AICc Wi
Dwarf Salamander									
r_g									
local	Constant‡	4.927	0.783	6.295	3.393 - 6.462	0.416	1	21.645	0.793
	ISO‡	-0.236	0.114	-2.067	-0.461 - -0.012				
0.5-km	Constant ‡	4.300	1.66	2.59	1.046 - 7.554	0.229	1	23.861	0.329
	WTLND	5.843	4.372	1.336	-2.726 - 14.413				
1.0-km	Constant ‡	4.095	0.855	4.79	2.419 - 5.77	0.577	1	19.059	0.831
	WTLND‡	7.539	2.635	2.862	2.376 - 12.703				
2.5-km*	Constant ‡	2.954	1.136	2.601	0.728 - 5.18	0.623	1	18.141	0.875
	WTLND‡	12.074	3.834	3.149	4.559 - 19.589				
H_o									
local	Constant‡	0.562	0.029	19.328	0.505 - 0.619	0.106	1	-25.586	0.384
	AREA	0.018	0.021	0.844	-0.023 - 0.058				
0.5-km*	Constant ‡	0.418	0.041	10.265	0.338 - 0.498	0.74	1	-35.457	0.948
	WTLND‡	0.443	0.107	4.13	0.233 - 0.653				
1.0-km	Constant ‡	0.506	0.045	11.308	0.418 - 0.594	0.35	1	-28.139	0.47
	WTLND	0.248	0.138	1.799	-0.022 - 0.518				
2.5-km	Constant ‡	0.466	0.061	7.657	0.347 - 0.586	0.392	1	-28.671	0.621
	WTLND‡	0.405	0.206	1.968	0.002 - 0.808				
Southern Leopard Frog									
r_g									
local	Constant‡	9.349	0.158	59.319	9.04 - 9.658	0.048	1	20.576	0.344
	AREA	0.079	0.133	0.593	-0.182 - 0.341				
0.5-km	Constant‡	9.69	0.40	24.16	8.903 - 10.475	0.105	1	20.021	0.316
	DEVEL	-1.44	1.59	-0.91	-4.546 - 1.674				
1.0 -km	Constant‡	10.43	1.19	8.75	8.093 - 12.766	0.106	1	20.01	0.293
	FOREST	-1.20	1.32	-0.91	-3.784 - 1.384				
2.5km*	Constant‡	8.17	0.68	12.09	6.844 - 9.493	0.314	1	17.631	0.328
	AG	2.14	1.20	1.79	-0.206 - 4.489				
H_o									
local*	Constant‡	0.774	0.025	30.959	0.725 - 0.823	0.333	1	-33.906	0.67
	ISO‡	0.005	0.003	1.87	<.001 - 0.01				
0.5-km	Constant‡	0.76	0.02	37.61	0.724 - 0.803	0.316	1	-33.681	0.517
	DEVEL	-0.15	0.08	-1.80	-0.302 - 0.013				

1.0-km	Constant‡	0.78	0.03	28.39	0.722 - 0.829	0.303	1	-33.509	0.412
	DEVEL	-0.20	0.12	-1.75	-0.425 - 0.025				
2.5-km	Constant‡	0.96	0.14	6.88	0.684 - 1.23	0.277	1	-33.174	0.324
	DEVEL	-0.98	0.60	-1.64	-2.156 - 0.194				

Table 4.6. Model averaged estimate directional effects of local and landscape scale predictor variables of allelic richness (r_g) and observed heterozygosity (H_o) in the dwarf salamander (*Eurycea quadridigitata*) and the southern leopard frog (*Lithobates sphenocephalus*).

Parameter/ Scale	AREA[4]	HYDRO[5]	ISO[6]	DEVEL[7]	FOREST[7]	AG[7]	WTLND[7]
E. quadridigitata							
r_g							
local		-	-				
0.5-km				-	+	-	+
1.0-km				-	+	-	+
2.5-km				-	+	-	+
H_o							
local	+						
0.5-km				-	+	-	+
1.0-km					+	-	+
2.5-km					+		+
L. sphenocephalus							
r_g							
local							
0.5-km				-			
1.0-km					-	+	-
2.5-km							-
H_o							
local	+		+				
0.5-km				-	+		-
1.0-km				-	+	-	-
2.5-km				-	+	+	-

[4] Estimated from survey contours (all wetlands except Psk; see (Kirkman et al. 2012)) and hand-digitizing aerial photography (Psk; see (Kirkman et al. 2012))

[5] Represents hydroperiod; calculated as the average number of days over a calendar year that a wetland was at least 25% full based on staff gauge data collected from 2000 - 2011(Kirkman et al. 2012)

[6] Represents isolation; calculated with Hanski's isolation index (S_i; Hanski and Thomas 1994) using relative distances from all 90 wetlands on Ichauway as well as 34 wetlands within a 250 m buffer around Ichauway (Kirkman et al. 2012)

[7] DEVEL represents development (primarily roads); FOREST represents evergreen, mixed, and deciduous forest cover; AG represents center pivot agriculture and pastures; WTLND represents herbaceous and wooded wetlands. Calculated based on 2006 National Land cover Data (NLCD; www.mrlc.gov/nlcd_2006.php) as the percent area of each land cover feature within circular buffers with given radii.

CHAPTER 5

CORRELATIONS BETWEEN AND LANDSCAPE PREDICTORS OF SPECIES AND ALLELIC RICHNESS IN POND-BREEDING AMPHIBIAN COMMUNITIES[8]

[8] A.M. McKee, L.L. Smith, J.C. Maerz, and T.C. Glenn. To be submitted to: *Conservation Biology*.

5.1 ABSTRACT

When neutral processes (drift and dispersal) are responsible for distributions of species and genetic diversity, a positive correlation is expected between species and genetic diversity. Under these circumstances, species diversity conservation efforts may also benefit the conservation of genetic diversity. To investigate the relationship between and habitat predictors of species and genetic diversity, we performed amphibian surveys at 15 wetlands at Ichauway, a longleaf pine reserve in Baker County, Georgia. We collected 265 dwarf salamander (*Eurycea quadridigitata*) and 246 leopard frog (*Lithobates sphenocephalus*) tissue samples from 8 and 9 of these wetlands, respectively. Dwarf salamander and southern leopard frog DNA samples were screened at 12 and 11 microsatellite loci, respectively. We used Pearson correlations to determine the relationship between species and allelic richness. To determine which variables were most closely associated with species and allelic richness, we modeled species richness (SR) and allelic richness (AR) as functions of local (wetland area, hydroperiod, and isolation) and landscape scale land cover variables (forest, agriculture, wetlands, and development) across three spatial scales (0.5, 1.0, and 2.5 km). Dwarf salamander AR was not correlated with SR (r^2=0.103, P=0.439), whereas southern leopard frog AR was negatively correlated with SR (r^2=0.5, P=0.033), suggesting that species and (or) genetic diversity were not determined by neutral processes. However, single outlier sites for both focal genetic species were driving these linear trends, suggesting the statistical significance of the relationships may differ from the ecological significance. Allelic richness of dwarf salamanders was best predicted by the area of wetlands within 2.5km (β=12.07; 95% CI: 4.56-19.59; r^2=0.62), and the top model of southern leopard frog AR was the area of agriculture within 2.5km (β=2.14; 95% CI: -0.21-4.49; r^2=0.31). Species richness was best predicted by the area of forests within 2.5km (β=14.74; 95% CI: 4.73-24.74; r^2=0.39). These results suggest that a management strategy that protects wetlands and forested habitats would benefit amphibian species and genetic diversity at Ichauway.

5.2 INTRODUCTION

Resources for biodiversity conservation are limited (Dalton 2000, Wilson et al. 2006). While maintaining genetic diversity is important for populations' abilities to adapt to changing conditions and

therefore long term population persistence, resources for conserving biodiversity are often allocated towards maintaining species diversity. Fortunately, under certain conditions, protecting species diversity in communities may help protect genetic diversity in populations as well. The theory of island biogeography (MacArthur and Wilson 1967) posits that extinction, as a function of habitat area, and colonization, as a function of habitat isolation, are the primary processes that determine the distribution of species among communities. When parallel processes at the genetic level (i.e., genetic drift and gene flow) are the primary forces that dictate distributions of genetic diversity in populations, a positive relationship is expected between species and genetic diversity (Vellend 2003), and communities with greater species diversity are also expected to have greater genetic diversity within the populations. When this is the case, management actions meant to optimize species diversity are also likely to help maintain genetic diversity (Cleary et al. 2006). While positive correlations between species and genetic diversity are still possible when selection or co-existence processes have a greater influence on distributions of species and/or genetic diversity, the outcome of the relationship is much more difficult to predict (Vellend and Geber 2005). Understanding the relationship between biodiversity and habitat features, including area and isolation, may help to elucidate what processes affect distributions of biodiversity and provide information that may help managers best utilize resources for conservation of species and genetic diversity.

The longleaf pine ecosystem in the US, of which less than three percent of the original extent remains (Frost 1993), is an area of great conservation concern, and the increasing focus of restoration projects. This ecosystem is a home to 17 endemic amphibian species (Means 2006), several of which are now listed as threatened at the state or federal level. The general life-cycle is very similar for many of these amphibian species, in that breeding and larval development occur in aquatic habitats, however the majority of their adult life is spent in upland habitats surrounding the wetlands. Despite these similarities, pond-breeding amphibians within the longleaf pine ecosystem display a range of vagilities (Smith and Green 2005) and habitat restrictions. Therefore, understanding the processes and habitat features associated with species and genetic diversity across amphibians with differing vagilities and habitat restrictions may help determine where to focus conservation efforts in remaining longleaf pine habitat, direct restoration efforts to help better protect a

range of amphibian species, or even possibly inform decisions regarding locations for amphibian species reintroductions.

The objectives of our study were to use previously published species and genetic diversity data (Kirkman et al. 2012, McKee et al. 2012) from amphibian breeding communities in a longleaf pine habitat reserve to determine the relationship between species and genetic diversity, and compare the habitat features associated with species versus genetic diversity in pond-breeding amphibian communities. Additionally, we sought to determine how these relationships differed when genetic diversity was measured in a highly vagile, habitat generalist species, versus when genetic diversity was measured in a less vagile species with more habitat restrictions. Results from McKee et al. (2012) found a strong positive relationship between the area of wetlands surrounding breeding sites and allelic richness in dwarf salamanders, a specialist to the longleaf pine ecosystem (Means 2006) and a species with limited vagility (previously recorded maximum distance of movement was 600m; Pechmann et al. 2001). The same study found that genetic diversity in the southern leopard frog, a species that is considered more of a habitat generalist (Butterfield et al. 2011), with a wider habitat distribution (Means 2006), and greater vagility (Smith and Green 2005) was not strongly associated the landscape. Based on these results we developed the hypothesis that species and genetic diversity would not be positively correlated for both the dwarf salamander and the southern leopard frog, because of the differences in habitat associations between dwarf salamander and southern leopard frog genetic diversity. We also hypothesized that if we did find a positive correlation between species diversity and genetic diversity for one of the species, the correlation would be with the dwarf salamander genetic diversity because of its strength of association with the landscape.

5.3 METHODS

5.3.1 STUDY AREA

Study wetlands (amphibian breeding sites; the unit of study) were located at the Jones Ecological Research Center at Ichauway (31°13'16.88"N and 84°28'37.81"W) located in Baker County, Georgia (Figure 5.1). Ichauway is an 11,800 ha longleaf pine (*Pinus palustris*) reserve, with numerous isolated lime-sink wetlands

varying in size, hydroperiod, and type. Marshes tend to be the largest of the wetland types and have moderate hydroperiods. Cypress savannas are generally the smallest wetlands and have the shortest hydroperiods. Cypress-gum swamps tend to be of intermediate size and have the longest hydroperiods (Kirkman et al. 2000). Intervening habitat among the study wetlands included forest, agriculture, open water, wildlife food plots, scrub/shrub habitat, and paved and dirt roads.

5.3.2 AMPHIBIAN SURVEYS AND COLLECTION

Amphibian species surveys were conducted as described in Liner et al. (2006) and Kirkman et al. (2012) from January – April 2006. Briefly, survey effort was standardized across wetlands and methods included dipnetting (300 sweeps per wetland per season; Dodd 2003), automated frog call recorders (two nights per season), and crawfish and minnow traps (five of each type per wetland for four nights per season).

Southern leopard frog and dwarf salamander tissue samples for genetic analysis were collected as described in McKee et al. (2012). Briefly, we collected larval and adult dwarf salamanders and larval southern leopard frogs from 9 and 10 wetlands, respectively, during the breeding seasons in 2008 and 2009 (Table 5.1; University of Georgia IACUC permit #A2009-10030-0). One site from which both dwarf salamander and southern leopard frog samples were collected was not included in the 2005-2006 amphibian surveys. We therefore did not include data from this site in our subsequent analyses. Attempts were made to collect a minimum of 30 samples of each species per wetland from at least 9 wetlands; however this was not possible for either species in 2008. Because of the philopatric nature of these species, we assumed that the genetic composition of breeding assemblages from the incompletely surveyed wetlands would be similar in 2008 and 2009 (for review see Blaustein et al. 1994, Smith and Green 2005). Therefore, sites where the collection goals were not met in 2008 were revisited in 2009 to collect additional samples.

Southern leopard frogs were collected from nine wetlands, three of each type. Two wetlands were sampled in 2009 only and seven were sampled in 2008 and 2009 (Table 5.1). Dwarf salamanders were collected from eight wetlands, all of which were cypress-gum swamps. One wetland was sampled in both 2008 and 2009, while four wetlands were sampled in 2008 only and four more in 2009 only (Table 5.1). There

were only two wetlands from which we collected both southern leopard frogs and dwarf salamanders, for a total of 15 wetlands sampled. Larval amphibians were collected with dipnets and funnel traps. To obtain genetic samples representative of each wetland, dipnet sweeps were distributed equally around the perimeter and shallow microhabitats (<0.5 m) of each surveyed wetland. To avoid collecting full siblings, we collected a maximum of one individual per sweep when wetlands were large enough and larvae were sufficiently abundant. However, in several instances larvae were sparse and collected opportunistically.

Total rainfall in 2008 was approximately 7.6 cm below normal for the area and wetlands remained small relative to their standard capacity and dried quickly (unpublished data). At these sites, dipnetting was still distributed equally around the perimeter of the wetland, however the likely collection of siblings was difficult to avoid. Funnel traps were used for supplemental sampling at sites where attaining target sample sizes proved difficult from dipnetting alone. Traps were distributed around the perimeter of the wetland and in shallow microhabitats and checked daily. In cases where tadpole species identification was questionable, individuals were collected and reared in the lab to metamorphosis when identification was possible. We were unable to collect a sufficient number of larval dwarf salamanders at any of the sites and we therefore supplemented our larval salamander samples with adult samples. Adult dwarf salamanders were collected opportunistically from under cover objects around the edges of the surveyed wetlands. All individuals caught in the field were brought back to the lab where they were euthanized in MS-222 and stored in 95% EtOH for genetic analysis.

5.3.3 MICROSATELLITE AMPLIFICATION

Microsatellite amplification was performed as described in McKee et al. (2012). Briefly, whole genomic southern leopard frog DNA was isolated using silica-binding techniques whereas dwarf salamander DNA was isolated using phenol chloroform. Southern leopard frog DNA samples were screened at 16 microsatellite loci (all except Rasp51 and Rasp67; McKee et al. 2011b) and dwarf salamander DNA samples were screened at 12 microsatellite loci (Mckee et al. 2011a). All loci were genotyped using a 3730xl Genetic Analyzer and GENEMAPPER software v4.0 (Applied Biosystems, Inc.) with manually created allele bins and

visually inspected allele calls. Negative controls were run with samples to ensure systematic contamination was not an issue. To estimate genotyping error rates, we rescreened approximately 10% of the samples for each locus. Error rates per reaction were calculated following Hoffman and Amos (2005), where the rate represents the number of inconsistent genotypes divided by the total number of reactions compared (see McKee et al. 2012 for detailed results).

5.3.4 STATISTICAL ANALYSES

Genetic analysis was performed as described in McKee et al. (2012) with minor modifications. To test for full siblings within wetlands, we screened genotypes in COLONY 2 (Mac version). When the probability of sibship between two samples was greater than 90%, we retained the individual with the more complete genotype set for analysis while the other sibling was removed from further analyses. We tested for linkage disequilibrium in GENEPOP 4.0 (web version, default settings); (Raymond and Rousset 1995, Rousset 2008) with the Markov chain method and default parameter settings. Sequential Bonferroni corrections were applied to account for multiple comparisons (Rice 1989). We estimated null allele frequencies for each locus with the Charkraborty estimator (Chakraborty et al. 1992) in MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004), with 10,000 iterations. After accounting for the likely effect of inbreeding on homozygosity, five leopard frog loci (Rasp01, Rasp16, Rasp20, Rasp28, and Rasp42) were statistically out of Hardy-Weinberg (HW) equilibrium in five or more leopard frog populations even after Bonferroni Corrections and had evidence of significant null allele frequencies in eight to ten leopard frog populations. We therefore removed these loci from analysis. Rarefied allelic richness (r_g) was calculated in FSTAT v2.9.3.2 (Goudet 1995).

We used allelic richness as our genetic diversity parameter because of its parallel nature to species richness, which was the focal species diversity parameter of the theory of island biogeography. To estimate species richness, we used the program ESTIMATES (Colwell 2005). Parameter settings in ESTIMATES were as follows: 10,000 runs, randomize with replacement, classic estimator formula because of high variance due to limited sample size, upper abundance limit of rare or infrequent species was set to the sample size (number of days sampled) for each site (ESTIMATES recommends 10, however the maximum cannot be greater than the

sample size). We chose the Chao2 (Chao 1987) and Jackknife2 (Burnham and Overton 1978, 1979, Smith and van Belle 1984, Palmer 1991) estimates of species richness (S_{chao} and S_{jack}) because these estimators are known to perform best across a range of taxa (Walther and Moore 2005). We include observed species richness (S_{obs}) in our tabular results as a baseline for comparison with S_{chao} and S_{jack}, however all conclusions regarding species richness are based solely on S_{chao} and S_{jack} estimates as these have been shown to be more accurate estimates of species richness than S_{obs}, which tends to perform poorly as a predictor of species richness (Walther and Moore 2005).

5.3.5 LOCAL AND LANDSCAPE HABITAT CHARACTERIZATION

Local and landscape characterization were performed as described in McKee et al. (2012). We used the deepest point of our sample sites to designate the centers of the wetlands. Land cover surrounding wetlands was determined based on 2006 National Land cover Data (NLCD; www.mrlc.gov/nlcd_2006.php). In ArcMap 9 (ESRI), we created circle buffers with radii of 0.5, 1.0, and 2.5-km (Houlahan and Findlay 2003, Piha et al. 2007, Veysey et al. 2011) around the center of each wetland (Piha et al. 2007). Within the buffers, the percent area of each land cover feature was calculated in Hawth's Tools (www.spatialecology.com). We grouped land cover variables according to features of interest: development (DEVEL; sum of all development land cover types), forest (FOREST; sum of all forest land cover types), agriculture (AG; sum of pasture and row crop), and wetland (WTLND; sum of woody wetlands and emergent herbaceous wetlands). The land surrounding Ichauway is almost entirely center-pivot agriculture, and development within and adjacent to our study area consisted primarily of roads. Thus, DEVEL was essentially an indicator of road density rather than houses or urbanization. These percent land cover variables were arcsine square root transformed to meet assumptions of normality.

Variables we believed were of biological relevance to amphibians at the local scale were wetland area (AREA), isolation (ISO), and hydroperiod (HYDRO). Wetland area was estimated from survey contours (see Kirkman et al. 2012). These data were natural logarithmically transformed to meet assumptions of normality. Isolation was calculated with Hanski's isolation index (Si; Hanski and Thomas 1994) using relative distances

from all 90 wetlands on Ichauway as well as 34 wetlands within a 0.25-km buffer around Ichauway (Kirkman et al. 2012). HYDRO was calculated as the average number of days over a calendar year that a wetland was at least 25% full based on staff gauge data collected from 2000 – 2011 (Kirkman et al. 2012). To investigate spatial autocorrelation in our predictor and response variables, we calculated Moran's I values for all variables.

5.3.6 CORRELATIONS, MODEL SELECTION, AND MODEL AVERAGING

We used Pearson correlations coefficients to estimate the linear relationship between species and allelic richness. Inspection of the data indicated that a single data point was driving the linear relationships between species and allelic richness for both species. For the dwarf salamander, P58 (Figure 5.1) had significantly lower r_g than all other dwarf salamander sites (Table 5.1). For the southern leopard frog, P53 (Figure 5.1) had significantly greater species richness and significantly lower r_g than all other southern leopard frog sites (Table 5.1). As these sites were the most isolated for each respective species, we believed that isolation was an important driver of the diversity at these sites. We therefore included these data in all analyses, however we also performed a posthoc analysis with these data removed to understand how excluding them from analysis would have affected our results and interpretation. Our conclusions are based on the recognition that statistical significance does not necessarily correspond to ecological significance. Detailed results from the posthoc analysis are available in Appendix F and G.

Model selection and averaging were performed as described in McKee et al. (2012) with modifications. Briefly, Model selection was performed in SAM v4.0 (Rangel et al. 2010). We separated predictor variables into local variables (AREA, HYRO, and ISO) and landscape scale land cover variables (DEVEL, FOREST, AG, and WTLND). Only predictor variables from the same spatial scale (local, 0.5, 1.0, or 2.5km) were included for a given round of model selection. We performed model selection for allelic richness and all three estimators of species richness for the subsets of data from both dwarf salamander and southern leopard frog sites (eight and nine wetlands, respectively). Additionally, we performed model selection for species richness data from the dwarf salamander and southern leopard frog sites combined (15 wetlands). In total, 44 rounds of model selection were performed.

Top models were selected based on the lowest Akaike's Information Criteria value, corrected for small sample size (AICc) (Burnham and Anderson 2002). We assessed the predictive ability of our top models based on relative AICc and r^2 values and we used a condition number (CN) to determine how much multicollinearity was an issue within models (Lazaridis 2007). A CN less than 2.5 indicates multicollinearity is not an issue, a CN between 2.5 and 5.4 suggests that multicollinearity exists, but it is unlikely to affect estimates, and a CN greater than 5.4 suggests multicollinearity is likely to greatly affect parameter estimates (Lazaridis 2007). We calculated model averaged estimates and 95% confidence intervals for each predictor variable in SAM v4.0 (Rangel et al. 2010). When the 95% CI of the estimates did not cross zero, we considered this variable statistically significant and noted the direction of effect. For the species richness model selection and averaging, the results were nearly identical between Chao2 and Jackknife2 estimates. Because there were several differences between the two in terms of directional association with landscape scale land cover features, we considered predictor variables to have a significant directional association with species richness only if the results were consistent between the two estimators.

5.4 RESULTS

A total of 265 non-sibling dwarf salamanders was collected from 8 wetlands, and 246 non-sibling southern leopard frog larvae were collected from 9 wetlands (see McKee et al. 2012). Of the 19 species detected at Ichauway over the species survey period, S_{obs} ranged from 1 to 9, and species richness estimates ranged from approximately 3 to 12 (Table 5.1). Allelic richness in dwarf salamander populations ranged from 5.2 to 7.2, and 8.3 to 9.9 in southern leopard frog populations. Most predictor variables were spatially autocorrelated when investigated across the southern leopard frog subsets of sites and across all sites, suggesting the significance of habitat variables with overall species richness and allelic richness at southern leopard frog sites may be a statistical artifact as opposed to a true ecological relationship.

5.4.1 CORRELATIONS BETWEEN SPECIES AND ALLELIC RICHNESS

Dwarf salamander allelic richness had a null relationship with species richness (r_{Sobs} = 0.243, P = 0.532; r_{Schao} = 0.321, P = 0.405; r_{Sjack} = 0.299, P = 0.439; Figure 5.2a), whereas southern leopard frog allelic richness showed a significant negative correlation with species richness at southern leopard frog sites (r_{Sobs} = -0.625, P = 0.058; r_{Schao} = -0.707, P = 0.025; r_{Sjack} = -0.7, P = 0.028, Figure 5.2b). The relationships between species and allelic richness for both focal species appeared largely driven by the outlier sites (Figure 5.2c,d). This suggests the ecological significance of our results may differ from the statistical significance of our results.

5.4.2 ALLELIC AND SPECIES RICHNESS MODEL SELECTION

Dwarf salamander allelic richness was best predicted by $WTLND_{2.5}$ (β =12.07; AICc = 18.14; Table 5.2) indicating sites with greater surrounding wetland area had greater allelic richness. The goodness-of-fit of this model was relatively strong (r^2 = 0.62; Table 5.2). Allelic richness in southern leopard frogs was best predicted by $AG_{2.5}$ (β = 2.14; AICc = 17.63; Table 5.2), indicating that southern leopard frog sites surrounded by more agriculture had greater allelic richness. However, the goodness-of-fit of this model was not very good (r^2 = 0.31; Table 5.2), and the effect size $AG_{2.5}$ was not significant. Additionally, $AG_{2.5}$ was spatially autocorrelated across the southern leopard frog sites (Appendix H), suggesting the apparent statistical relationship between $AG_{2.5}$ and southern leopard frog allelic richness may have been caused by spatial dependency of the predictor variable. Species richness models for dwarf salamander and southern leopard frog subsets of sites in Appendix I.

Overall species richness was best predicted by $FOREST_{2.5}$ (β_{Chao} = 14.74, $AICc_{Chao}$ = 68.62; β_{Jack} = 13.90, $AICc_{Jack}$ = 65.42; Table 5.2). However, the ΔAICc, an indicator of the relative support of other models compared to the top model (Burnham and Anderson 2002), of ISO, which was the next best predictor of overall species richness and the top model of species richness at southern leopard frog sites, was less than two from $FOREST_{2.5}$, indicating some uncertainty as to which of these two models fit the data better (Burnham and Anderson 2002). The variables $FOREST_{2.5}$ and ISO were both significantly spatially

autocorrelated when investigated across all sites (Appendix H), however, when $FOREST_{2.5}$ was investigated across the dwarf salamander sites and ISO was investigated across southern leopard sites, these variables were not significantly spatially autocorrelated (Appendix H) suggesting spatial dependency did not greatly affect model selection for overall species richness. All of our top models contained only one variable, and therefore multicollinearity was not an issue.

5.4.3 Directional associations of local and land cover predictor variables with diversity

At the landscape scale, dwarf salamander allelic richness and species richness had the same general directional associations with the land cover features; allelic and species richness were both lower at sites with more DEVEL and AG, and greater at sites with more FOREST and WTLND (Table 5.3). However, associations with the landscape generally occurred at larger spatial scales for species richness whereas allelic richness had significant directional associations with all land cover features across all three landscape spatial scales (Table 5.3). The parallel directional associations with the landscape between species and allelic richness did not hold true at the local scale. Isolation was negatively associated with allelic richness and positively associated with species richness, indicating that more isolated sites had greater allelic richness in dwarf salamanders, but lower species richness. Dwarf salamander allelic richness was also lower at sites with shorter hydroperiods (Table 5.3). Directional associations between predictor variables and species richness at dwarf salamander and southern leopard frog subsets of sites are available in Appendix J.

Land cover variable directional associations with southern leopard frog allelic richness were generally opposite of those of dwarf salamander allelic richness. The only exception was DEVEL, which had a negative association with allelic richness of both species. Dwarf salamander allelic richness had significant associations across all landscape spatial scales, however each land cover feature was only significantly associated with southern leopard frog allelic richness at one to two spatial scales (Table 5.3).

At the local scale, overall species richness was greatest at the more isolated sites (Table 5.3). Associations between overall species richness and land cover features were congruent with results at the dwarf salamander and southern leopard frog subsets of sites, however, overall species richness was only

associated with features at the largest spatial scale (Table 5.3). Wetlands with greater WTLND and FOREST had greater species richness, whereas sites with greater DEVEL had lower species richness (Table 5.3).

5.4.4 POSTHOC ANALYSIS WITHOUT OUTLIER SITES

Pond 58 (P58; Figure 5.1), which was the most isolated dwarf salamander site, had significantly lower dwarf salamander allelic richness than the rest of the dwarf salamander sites. Pond 53 (P53; Figure 5.1), which was the most isolated southern leopard frog site, had significantly lower southern leopard frog allelic richness and significantly greater species richness than the rest of the southern leopard frog sites. Our posthoc analysis of the correlations between species and allelic richness for the dwarf salamander and southern leopard frog after removing these outlier sites suggested that without the outlier data, our hypotheses were supported. When we removed P58 from the dwarf salamander analysis, dwarf salamander allelic richness was no longer significantly associated with isolation (Appendix J), but was significantly positively correlated with species richness (Figure 5.2c). Similarly, removing P53 from the southern leopard frog analysis resulted in a null relationship between southern leopard frog allelic richness and species richness (Figure 5.2d) and a significant positive association between allelic richness and isolation (Appendix J).

5.5 DISCUSSION

Despite recognition that genetic diversity is essential for populations to adapt to changing environmental conditions, conservation efforts are generally more focused on protecting species diversity. If habitat area and isolation are the primary factors determining distributions of species and genetic diversity, then conservation efforts meant to protect species diversity will also help protect genetic diversity and vice versa. However, if selective and (or) species co-existence processes have a greater effect on species and/or genetic diversity, then conservation efforts aimed at protecting species will be less predictable, and may unintentionally decrease genetic diversity within populations. The aims of this study were to determine the relationship between species diversity in pond-breeding amphibian communities and genetic diversity of populations within these communities, to compare the habitat features associated with species versus genetic

diversity in pond-breeding amphibian communities, and to determine how these relationships differed when genetic diversity was measured in a highly vagile, habitat generalist species, versus a less vagile species with more habitat restrictions.

5.5.1 Correlations between species and allelic richness in species with differing vagilities

We hypothesized that based on differing vagilities and habitat restrictions of our focal genetic species we would find a positive correlation between species richness and dwarf salamander allelic diversity, and a null relationship between species richness and southern leopard frog allelic richness. Statistically, our results did not support our hypotheses. We did not find a statistically significant relationship between dwarf salamander allelic richness and amphibian community species richness, but we found a significant negative association between southern leopard frog allelic richness and amphibian community species. Graphical results, however, suggested that the statistical significance of our results might differ from the ecological significance, as single outlier sites largely drove linear relationships between species and allelic richness for both species. When the outlier sites driving the linear relationships were removed, our hypotheses about the relationships between species and allelic richness were supported; we found a positive strong relationship between species and dwarf salamander allelic richness, and a null relationship between species and southern leopard frog allelic richness.

Previous studies on species-genetic diversity correlations have suggested that species and genetic diversity are likely to be correlated when genetic diversity is measured in common versus rare species (Vellend 2005) and are somewhat common after disturbance events (Vellend 2004, Cleary et al. 2006, Evanno et al. 2009). Amphibian populations are known to fluctuate substantially from year-to-year (Marsh 2001), however southern leopard frogs have a large habitat range and are often considered abundant where they are found (Butterfield et al. 2011). Although dwarf salamanders have limited vagility and have more habitat restrictions, they were abundant at our study sites (unpublished data), suggesting we cannot make any inferences regarding the effect of relative local abundances on the relationships between species and allelic richness. In regards to possible effects of disturbances on correlations between species and genetic diversity, prescribed burns at

Ichauway may seem like a large disturbances, however results from studies on the short term (0-3 years post-treatment) effects of prescribed burns on amphibian species native to habitats where fire is a common recurrent disturbance have shown either no response in presence or abundance, or responded positively to fire (Steen et al. 2010, Brown et al. 2011, Westgate et al. 2012). While fire may not have caused a disturbance capable of affecting species and genetic diversity in amphibian communities at Ichauway, Baker County suffered severe drought conditions in 2006 when the species surveys were conducted, and 2007, the year before the genetic sampling began. Additionally, precipitation in 2008 was approximately 7.6 cm below average (www.georgiaweather.net). These events likely constituted severe disturbances to amphibian communities at Ichauway. Southern leopard frogs are found in nearly all freshwater body types within their habitat range, therefore they may have been able to seek refuge during the drought. However, dwarf salamanders are restricted to ephemeral water bodies, which may also help to explain the ecologically significant positive relationship between species richness and allelic richness in the dwarf salamander. Our results without the outlier sites also corroborate similar results found in bat communities in tropical forest fragments (Struebig et al. 2011). A positive relationship was found between species richness and allelic richness for bat species with more limited dispersal and more habitat restrictions compared to the relationships between species and allelic richness for two other bat species, one of which was highly mobile, the other of which was more of a habitat generalist.

5.5.2 Habitat associations with species and allelic richness

In the case of Struebig et al. (2011), the positive relationship between bat species and genetic diversity was driven primarily by habitat area. However in the case of the dwarf salamander and allelic richness, the ecologically significant relationship appeared to be caused by similar directional effects of surrounding land cover features, as neither species nor genetic diversity were related to wetland area, and isolation had opposing directional effects on the two scales of diversity. Dwarf salamander allelic richness was greater at less isolated wetlands, suggesting the importance of gene flow for maintaining genetic diversity in dwarf salamander populations. In contrast, species richness was greater at more isolated sites. We suggest two

possible explanations for this counterintuitive result. First, aggregated wetlands may have supported populations of predatory fish. A previous study on environmental correlates of species richness among taxa at Ichauway (Kirkman et al. 2012) also found greater amphibian species richness at more isolated sites. Their hypothesis was that the more aggregated wetlands in their study are often connected via ephemeral drains during times of heavy inundation, thereby enabling fish populations to disperse among them and thereby increasing the likelihood of establishment in the more aggregated sites (Battle and Golladay 2001, Liner 2006, Smith et al. 2006). Alternatively, amphibians may have accumulated at more isolated sites because of a lack of habitat options. In southeastern New Hampshire spotted salamander and wood frog breeding female population abundances (as indicated by egg mass abundance) were greater in more isolated wetlands, presumably because of fewer habitat options at more isolated sites (Veysey et al. 2011). The positive association between species richness and wetland area within 2.5km in our study seems to support the first hypothesis, as it suggests that abundance and proximity of wetlands within dispersal distance are still important for maintaining species diversity, however, when wetlands are too aggregated, they may be more likely to support populations of predatory fish.

Even though our measure of isolation had opposing effects on species versus dwarf salamander allelic richness, we believe the different land cover types had differing resistances to dispersal, meaning that the effective isolation of the wetlands may have differed from our measure of isolation (McIntyre and Barrett 1992, Manning et al. 2004, McGarigal and Cushman 2005). Many pond-breeding amphibian species require forests for non-breeding habitat (DeMaynadier and Hunter Jr 1999, Knutson et al. 1999, Semlitsch and Bodie 2003). For these species a lack of forest cover around wetlands may indicate a lack of necessary habitat (Trenham and Shaffer 2005). However, many pond-breeding amphibian species are believed to occur as metapopulations (Gulve 1994, Hecnar and M'Closkey 1996, Semlitsch 2000, Smith and Green 2005), which rely on occasional dispersal to prevent local extinction or to recolonize wetlands which have gone locally extinct. For these species, dispersing through forests may help prevent desiccation or predation (Rothermel and Semlitsch 2002, Rothermel and Luhring 2005). For amphibian species that do require forests for dispersal, agricultural landscapes are likely to be strong barriers to dispersal (Rothermel 2004). Additionally,

chemicals applied to agricultural fields are often detrimental to amphibian health and survival (Mann et al. 2009). Roads are also generally seen as a detrimental to amphibians (Cushman 2006, Eigenbrod et al. 2008) as studies have demonstrated that roads may lower dispersal rates (Gibbs 1998, DeMaynadier and Hunter 2000), increase mortality when dispersing (Carr and Fahrig 2001, Gibbs and Shriver 2005), and lower genetic diversity within populations (Reh and Seitz 1990). Therefore wetlands with more surrounding roads are likely to be effectively more isolated than wetlands with fewer surrounding roads.

Aside from negative associations with development (roads), the directional associations with land cover features differed substantially between southern leopard frog allelic richness and species richness. One distinguishing feature between southern leopards and endemic amphibians that may explain the differences in habitat associations is the differing breeding habitat requirements of southern leopard frogs versus longleaf pine endemic species. Southern leopard frogs are able to breed in nearly all freshwater habitats in their range, and are less palatable to a number of predatory fish that have been introduced into historically fishless breeding wetlands in the southeastern US (Gregoire and Gunzburger 2008, Butterfield et al. 2011). In contrast, endemic amphibians that evolved in historically fishless, semi-permanent wetlands may be more susceptible to predation than southern leopard frogs in wetlands with predatory fish. Therefore, southern leopard frogs are able to breed in a variety of freshwater habitats that are generally not utilized by longleaf pine endemics amphibians (Gregoire and Gunzburger 2008).

However, we believe the southern leopard frog allelic richness relationships with land cover features may have been largely driven by spatial autocorrelation, and the lack of strength in the models of southern leopard frog allelic richness suggests that it was not greatly affected by the land cover features we investigated (see McKee et al. 2012 for more in depth discussion on the southern leopard frog habitat associations). A similar lack of association between southern leopard frog abundance and habitat features (woodland proximity and wetland characteristics) was found by Babbitt and Brandt (2006) with their top model, which included conductivity and fish presence, only accounting for 4.6% of the variance.

One major limitation on the inferences that can be drawn from this study is that we measured alpha diversity (diversity within communities and populations), but not beta diversity (diversity between

communities and populations). While genetic frequency data were available, species frequency data were not. In the longleaf pine ecosystem, different amphibian communities are known to occur in different wetland types. Liner (2006) found that cypress-gum swamps tend to support a different amphibian assemblage than cypress-savannas or marshes. Therefore, although we did not find a significant difference in species richness among wetland types for the southern leopard frogs, it is entirely possible that they supported different assemblages. All dwarf salamander sites were cypress-gum swamps, and therefore the features that were associated with species diversity and dwarf salamander genetic diversity at this site may have been specific to species most strongly associated with cypress-gum swamps. However, as results were similar for species richness at southern leopard frog sites (Appendix I and J), we believe these results to be relatively robust against differences in wetland type.

5.5.3 Implications for Conservation Management

The best predictors of all richness types were at the 2.5km scale, which was the largest scale for land cover characterization. Hence, we cannot say that this is the scale at which management efforts should be targeted, as larger spatial scales may be of greater importance. However, other studies have also found that habitat features 2-4km away from breeding sites are important for pond-breeding amphibians (Reh and Seitz 1990, Findlay and Houlahan 1997, Houlahan and Findlay 2003, 2004). Spatial autocorrelation was an issue for land cover features when investigated across all sites and at the southern leopard frog subset of sites. However, spatial autocorrelation was not significant at dwarf salamander sites, and species richness directional associations when modeled for just dwarf salamander sites (Appendix J) were similar to results from overall species richness. As development (roads) was the only feature with a consistent directional association across species richness and allelic richness in both species, management efforts designed to benefit species and allelic richness across species with differing vagilities and habitat restrictions should focus on minimizing roads within at least 2.5km of breeding wetlands. The weak relationship between habitat variables and southern leopard frog allelic richness suggests that management actions meant to optimize species richness and dwarf salamander allelic richness may not benefit southern leopard frogs, however they

are also unlikely to be detrimental. If the species richness results are representative of amphibian communities in longleaf pine habitats, then management actions meant to optimize species richness should also help protect allelic richness in species with more restrictive habitat requirements and more limited vagility and vice versa. If we can apply the allelic richness results to amphibian species with corresponding habitat requirements and vagilities, then more information is needed as to determine how to protect allelic richness in generalist species with high vagility.

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5.7 LITERATURE CITED

Babbitt, K. J., M. J. Baber, and L. A. Brandt. 2006. The effect of woodland proximity and wetland characteristics on larval anuran assemblages in an agricultural landscapeThis is contribution No. 82 of the MacArthur Agro-Ecology Research Center. Canadian Journal of Zoology **84**:510-519.

Battle, J. and S. W. Golladay. 2001. Water quality and macroinvertebrate assemblages in three types of seasonally inundated limesink wetlands in southwest Georgia. Journal of freshwater ecology **16**:189-208.

Blaustein, A. R., D. B. Wake, and W. P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology **8**:60-71.

Brown, D. J., J. T. Baccus, D. B. Means, and M. R. J. Forstner. 2011. Potential Positive Effects of Fire on Juvenile Amphibians in a Southern USA Pine Forest. Journal of Fish and Wildlife Management **2**:135-145.

Burnham, K. P. and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, NY.

Burnham, K. P. and W. S. Overton. 1978. Estimation of the size of a closed population when capture probabilities vary among animals. Biometrika **65**:625-633.

Burnham, K. P. and W. S. Overton. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology **60**:927-936.

Butterfield, B. P., M. J. Lannoo, and P. Nanjappa. 2011. Rana sphenocephala. AmphibiaWeb: Information on amphibian biology and conservation. Berkeley, California: AmphibiaWeb, Berkeley, California.

Carr, L. W. and L. Fahrig. 2001. Effect of Road Traffic on Two Amphibian Species of Differing Vagility. Conservation Biology **15**:1071-1078.

Chakraborty, R., M. Andrade, S. Daiger, and B. Budowle. 1992. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Annals of Human Genetics **56**:45-57.

Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics **43**:783-791.

Cleary, D. F. R., C. Fauvelot, M. J. Genner, S. B. J. Menken, and A. Ø. Mooers. 2006. Parallel responses of species and genetic diversity to El Niño Southern Oscillation-induced environmental destruction. Ecology Letters **9**:304-310.

Colwell, R. K. 2005. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples (Software and User's Guide), Version 7.5.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation **128**:231-240.

Dalton, R. 2000. Biodiversity cash aimed at hotspots. Nature **406**:818-818.

DeMaynadier, P. G. and M. L. Hunter Jr. 1999. Forest canopy closure and juvenile emigration by pool-breeding amphibians in Maine. The Journal of Wildlife Management **63**:441-450.

DeMaynadier, P. G. and M. L. J. R. Hunter. 2000. Road effects on amphibian movements in a forested landscape. Natural Areas Journal **20**:56-65.

Dodd, C. K. 2003. Monitoring amphibians in Great Smoky Mountains National Park. Page 117. U.S. Geological Survey, Denver, Colorado, USA.

Eigenbrod, F., S. J. Hecnar, and L. Fahrig. 2008. The relative effects of road traffic and forest cover on anuran populations. Biological Conservation **141**:35-46.

Evanno, G., E. Castella, C. Antoine, G. Paillat, and J. Goudet. 2009. Parallel changes in genetic diversity and species diversity following a natural disturbance. Molecular Ecology **18**:1137.

Findlay, C. S. and J. Houlahan. 1997. Anthropogenic correlates of species richness in southeastern Ontario wetlands. Conservation Biology **11**:1000-1009.

Frost, C. C. 1993. Four Centuries of Changing Landscape Patterns in the Longleaf Pine Ecosystem. Pages 17-43 *in* The Longleaf Pine Ecosystem: Ecology, Restoration, and Management. Proceedings of the Tall Timbers Fire Ecology Conference, Tall Timbers Research Station.

Gibbs, J. P. 1998. Amphibian movements in response to forest edges, roads, and streambeds in southern New England. The Journal of Wildlife Management **62**:584-589.

Gibbs, J. P. and W. G. Shriver. 2005. Can road mortality limit populations of pool-breeding amphibians? Wetlands Ecology and Management **13**:281-289.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity **86**:485.

Gregoire, D. and M. Gunzburger. 2008. Effects of predatory fish on survival and behavior of larval gopher frogs (*Rana capito*) and southern leopard frogs (*Rana sphenocephala*). Journal of Herpetology **42**:97-103.

Gulve, P. S. 1994. Distribution and extinction patterns within a northern metapopulation of the pool frog, *Rana lessonae*. Ecology **75**:1357-1367.

Hanski, I. and C. D. Thomas. 1994. Metapopulation dynamics and conservation: a spatially explicit model applied to butterflies. Biological Conservation **68**:167-180.

Hecnar, S. J. and R. T. M'Closkey. 1996. Regional dynamics and the status of amphibians. Ecology **77**:2091-2097.

Hoffman, J. and W. Amos. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology **14**:599-612.

Houlahan, J. E. and C. S. Findlay. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences **60**:1078-1094.

Houlahan, J. E. and C. S. Findlay. 2004. Estimating the 'critical'distance at which adjacent land-use degrades wetland water and sediment quality. Landscape Ecology **19**:677-690.

Kirkman, L. K., P. C. Goebel, L. West, M. B. Drew, and B. J. Palik. 2000. Depressional wetland vegetation types: a question of plant community development. Wetlands **20**:373-385.

Kirkman, L. K., L. L. Smith, P. F. Quintana-Ascencio, M. J. Kaeser, S. W. Golladay, and A. L. Farmer. 2012. Is species richness congruent among taxa? Surrogacy, complementarity, and environmental correlates among three disparate taxa in geographically isolated wetlands. Ecological Indicators **18**:131-139.

Knutson, M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, and M. J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology **13**:1437-1446.

Lazaridis, A. 2007. A note regarding the condition number: the case of spurious and latent multicollinearity. Quality and Quantity **41**:123-135.

Liner, A. E. 2006. Wetland predictors of amphibian distributions and diversity within the Southeastern U.S. Coastal Plain. University of Georgia, Athens, GA.

MacArthur, R. H. and E. O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, New Jersey.

Mann, R. M., R. V. Hyne, C. B. Choung, and S. P. Wilson. 2009. Amphibians and agricultural chemicals: Review of the risks in a complex environment. Environmental Pollution **157**:2903-2927.

Manning, A. D., D. B. Lindenmayer, and H. A. Nix. 2004. Continua and Umwelt: novel perspectives on viewing landscapes. Oikos **104**:621-628.

Marsh, D. M. 2001. Fluctuations in amphibian populations: a meta-analysis. Biological Conservation **101**:327-335.

McGarigal, K. and S. A. Cushman. 2005. The gradient concept of landscape structure. Issues and perspectives in landscape ecology. Cambridge University Press, Cambridge:112-119.

McIntyre, S. and G. Barrett. 1992. Habitat variegation, an alternative to fragmentation. Conservation Biology **6**:146-147.

Mckee, A. M., S. L. Lance, K. L. Jones, C. Hagen, and T. C. Glenn. 2011a. Development and characterization of 12 microsatellite loci for the Dwarf Salamander, *Eurycea quadridigitata*. Conservation Genetics Resources **3**:1-3.

McKee, A. M., S. L. Lance, K. L. Jones, C. Hagen, and T. C. Glenn. 2011b. Development and characterization of 18 microsatellite loci for the Southern Leopard Frog, *Rana sphenocephala*. Conservation Genetics Resources **3**:267-269.

McKee, A. M., J. C. Maerz, L. L. Smith, and T. C. Glenn. 2012. Chapter 4: Local and Landscape Predictors of Genetic Diversity in Populations of Two Pond-breeding Amphibian Species with Differing Vagilities. University of Georgia, Athens, GA.

Means, B. 2006. Vertebrate Faunal Diversity. Pages 157-213 *in* S. Jose, E. J. Jokela, and D. L. Miller, editors. The Longleaf Pine Ecosystem: Ecology, Siviculture, and Restoration. Springer, New York, NY.

Palmer, M. W. 1991. Estimating species richness: the second-order jackknife reconsidered. Ecology **72**:1512-1513.

Pechmann, J. H. K., R. A. Estes, D. E. Scott, and J. W. Gibbons. 2001. Amphibian colonization and use of ponds created for trial mitigation of wetland loss. Wetlands **21**:93-111.

Piha, H., M. Luoto, M. Piha, and J. Merilä. 2007. Anuran abundance and persistence in agricultural landscapes during a climatic extreme. Global Change Biology **13**:300-311.

Rangel, T. F., J. A. F. Diniz Filho, and L. M. Bini. 2010. SAM: a comprehensive application for Spatial Analysis in Macroecology. Ecography **33**:46-50.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248.

Reh, W. and A. Seitz. 1990. The influence of land use on the genetic structure of populations of the common frog Rana temporaria. Biological Conservation **54**:239-249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution **43**:223-225.

Rothermel, B. B. 2004. Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. Ecological Applications **14**:1535-1546.

Rothermel, B. B. and T. M. Luhring. 2005. Burrow availability and desiccation risk of mole salamanders (Ambystoma talpoideum) in harvested versus unharvested forest stands. Journal of Herpetology **39**:619-626.

Rothermel, B. B. and R. D. Semlitsch. 2002. An experimental investigation of landscape resistance of forest versus old-field habitats to emigrating juvenile amphibians. Conservation Biology **16**:1324-1332.

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

Semlitsch, R. D. 2000. Principles for Management of Aquatic-Breeding Amphibians. The Journal of Wildlife Management **64**:615-631.

Semlitsch, R. D. and J. R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conservation Biology **17**:1219-1228.

Smith, E. P. and G. van Belle. 1984. Nonparametric estimation of species richness. Biometrics **40**:119-129.

Smith, L. L., W. J. Barichivich, J. S. Staiger, K. G. Smith, and C. K. Dodd Jr. 2006. Detection probabilities and site occupancy estimates for amphibians at Okefenokee National Wildlife Refuge. The American Midland Naturalist **155**:149-161.

Smith, M. A. and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography **28**:110-128.

Steen, D. A., A. E. R. McGee, S. M. Hermann, J. A. Stiles, S. H. Stiles, and C. Guyer. 2010. Effects of forest management on amphibians and reptiles: generalist species obscure trends among native forest associates. Open Environmental Sciences **4**:24-30.

Struebig, M. J., T. Kingston, E. J. Petit, S. C. Le Comber, A. Zubaid, A. Mohd-Adnan, and S. J. Rossiter. 2011. Parallel declines in species and genetic diversity in tropical forest fragments. Ecology Letters **14**:582-590.

Trenham, P. C. and H. B. Shaffer. 2005. Amphibian upland habitat use and its consequences for population viability. Ecological Applications **15**:1158-1168.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**:535-538.

Vellend, M. 2003. Island biogeography of genes and species. The American Naturalist **162**:358-365.

Vellend, M. 2004. Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. Ecology **85**:3043-3055.

Vellend, M. 2005. Species diversity and genetic diversity: parallel processes and correlated patterns. The American Naturalist **166**:199-215.

Vellend, M. and M. A. Geber. 2005. Connections between species diversity and genetic diversity. Ecology Letters **8**:767-781.

Veysey, J., S. Mattfeldt, and K. Babbitt. 2011. Comparative influence of isolation, landscape, and wetland characteristics on egg-mass abundance of two pool-breeding amphibian species. Landscape Ecology **26**:661-672.

Walther, B. A. and J. L. Moore. 2005. The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. Ecography **28**:815-829.

Westgate, M. J., D. A. Driscoll, and D. B. Lindenmayer. 2012. Can the intermediate disturbance hypothesis and information on species traits predict anuran responses to fire? Oikos **121**:1516-1524.

Wilson, K. A., M. F. McBride, M. Bode, and H. P. Possingham. 2006. Prioritizing global conservation efforts. Nature **440**:337-340.

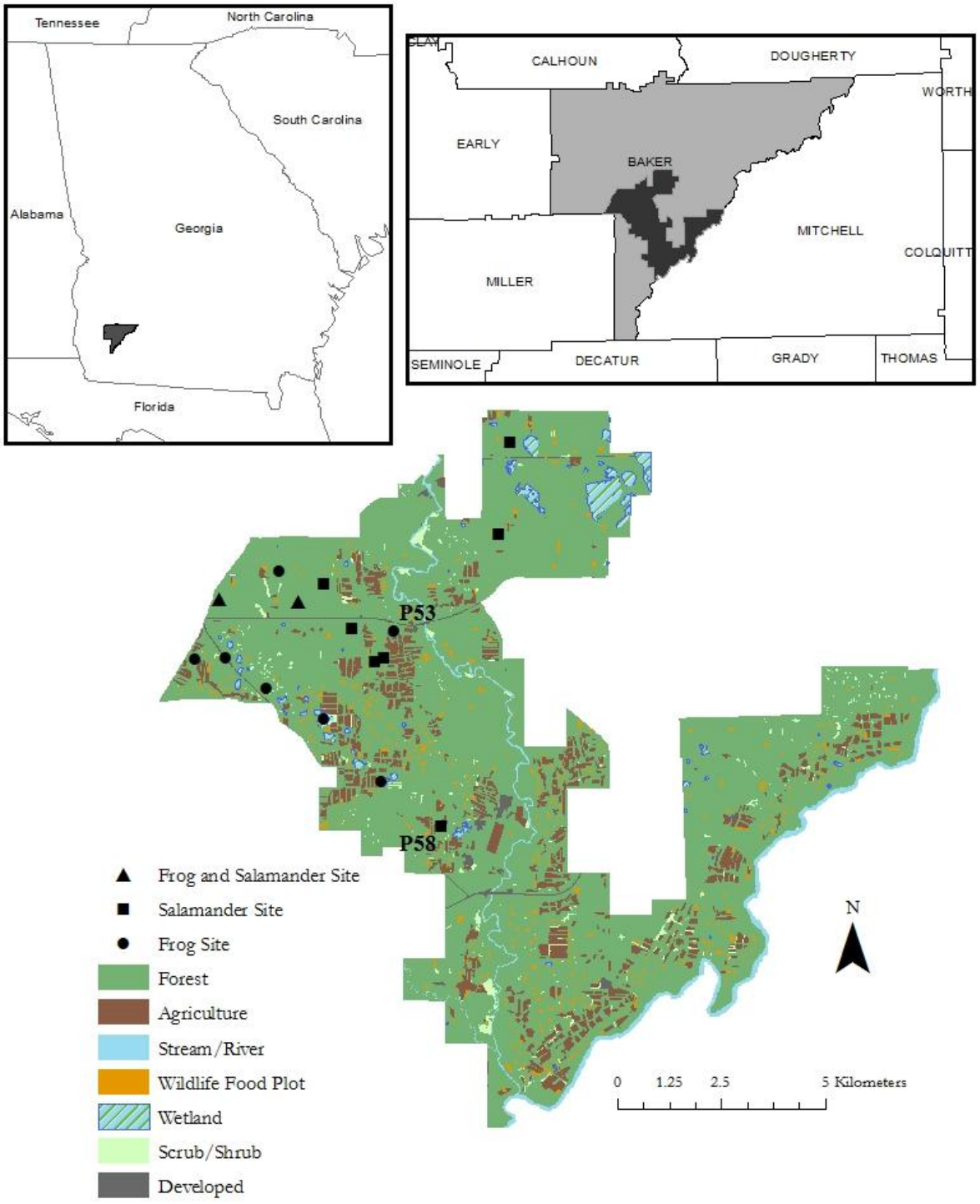

Figure 5.1. Ichauway, an 11,800 ha longleaf pine reserve located in Baker County, GA, and the location of our study sites.

Table 5.1. Modified from McKee et al. (2012); summary of population and community parameters at eight dwarf salamander (*Euqua quadridigitata*) sites and nine southern leopard frog (*Lithobates sphenocephalus*) sites. Genetic diversity parameters based on estimates from 12 microsatellite loci in the dwarf salamanders and 11 microsatellite loci in the southern leopard frog. Latitude/Longitude of sites are in UTM, *N* is the sample size by year before (and after) removing full siblings, r_g is the mean number of alleles rarefied to 24 individuals (min sample size, dwarf salamander) and 13 individuals (southern leopard frog) ± the interlocus standard error, S_{obs} represents the observed species richness during amphibian surveys between winter and spring 2006, S_{chao} represents estimates of species richness based on the Choa2 algorithm, S_{jack} represents estimates of species richness based on the Jackknife2 algorithm.

Site	Latitude/Longitude	N_{2008}	N_{2009}	r_g	S_{obs}	S_{chao}	S_{jack}
Dwarf Salamander							
P00	16 R 741137.17 m E / 3466868.20 m N	10 (10)	21 (20)	6.68 (±0.87)	4	6.07±1.44	6.69±1.59
P01	16 R 734138.75 m E / 3463106.12 m N	-	31 (31)	6.32 (±0.61)	1	2.73±0.80	3.45±1.30
P03	16 R 736048.12 m E / 3463054.93 m N	31 (30)	-	6.89 (±0.71)	6	6.37±1.72	6.62±2.02
P04	16 R 736666.06 m E / 3463489.00 m N	31 (30)	-	7.15 (±0.83)	6	8.49±3.05	8.14±3.10
P11	16 R740856.45 m E / 3464681.54 m N	-	31 (29)	6.11 (±0.63)	4	3.75±0.34	4.16±0.92
P52	16 R 737998.41 m E / 3461651.88 m N	31 (29)	-	6.81 (±0.69)	8	9.56±1.89	9.77±1.98
P58	16 R 739472.50 m E / 3457629.75 m N	-	30 (27)	5.21 (±0.45)	6	7.29±1.51	7.57±1.68
P68	16 R737330.65 m E / 3462395.76 m N	31 (31)	-	6.85 (±0.64)	7	7.44±1.84	7.81±1.82
Southern Leopard Frog							
P01	16 R 734138.75 m E / 3463106.12 m N	1 (1)	30 (30)	9.85 (±0.82)	1	2.73±0.80	3.45±1.30
P02	16 R 735598.04 m E / 3463772.73 m N	2 (1)	29 (23)	9.38 (± 0.87)	6	6.64±1.47	6.83±1.57
P03	16 R 736048.12 m E / 3463054.93 m N	18 (6)	17 (14)	9.19 (±0.84)	6	6.37±1.72	6.62±2.02
P27	16 R 733556.49 m E / 3461669.34 m N	16 (13)	15 (15)	9.38 (±0.76)	6	6.07±0.93	6.39±1.39
P41	16 R735278.02 m E / 3460955.73 m N	-	30 (19)	9.24 (±0.89)	3	3.82±0.56	4.24±1.12
P46	16 R736650.59 m E / 3460216.03 m N	6 (4)	26 (25)	9.72 (±1.00)	6	7.17±1.27	7.45±1.55
P53	16 R738359.54 m E / 3462353.85 m N	19 (15)	12 (12)	8.33 (±0.76)	9	11.27±1.64	11.5±1.93
P55	16 R738047.28 m E / 3458732.26 m N	14 (4)	26 (21)	9.77 (±0.84)	6	6.03±1.14	6.52±1.49
P96	16 R734309.96 m E / 3461709.40 m N	-	31 (25)	9.34 (±0.83)	4	4.07±1.49	4.42±1.60

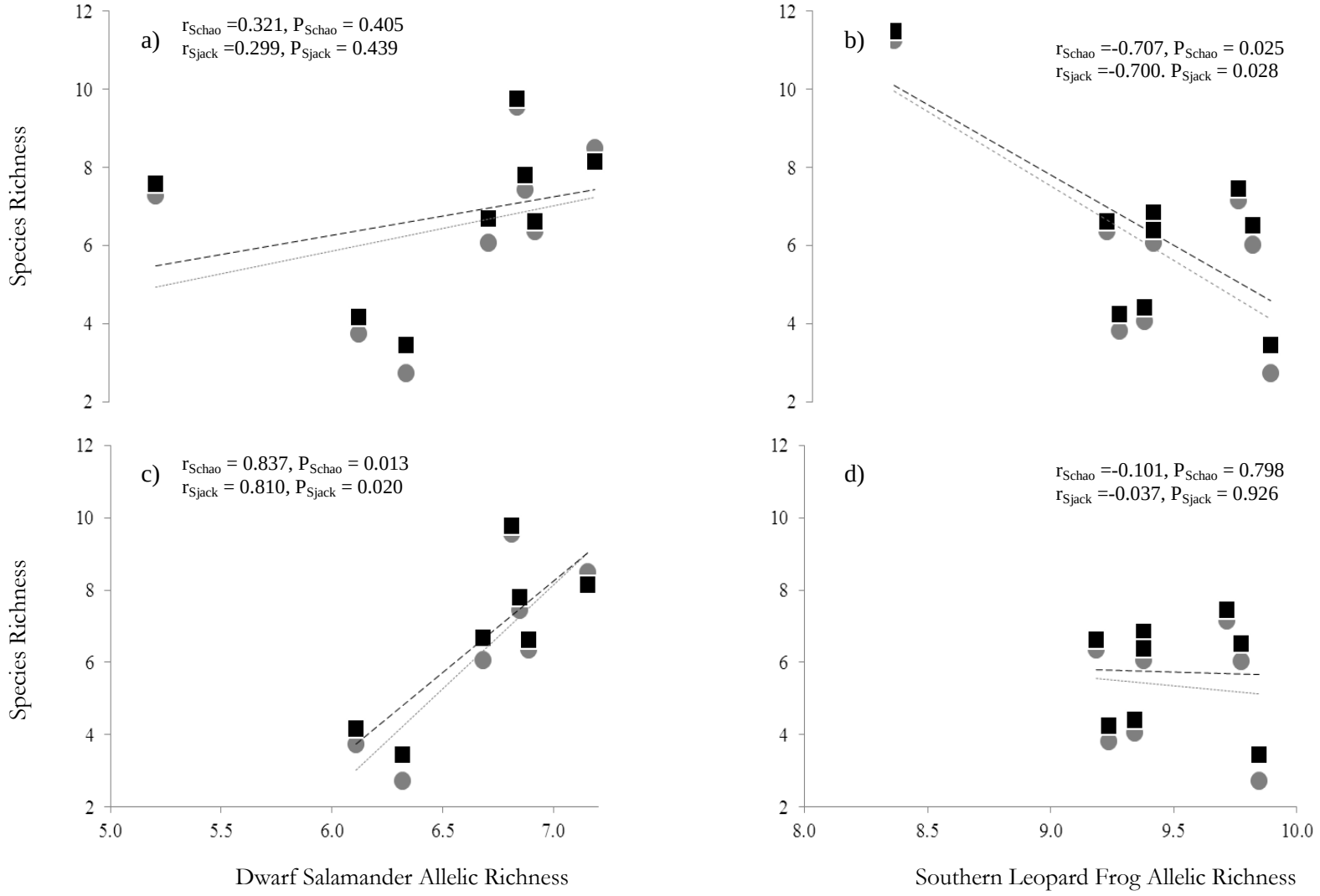

Figure 5.2. Species richness versus allelic richness for a) dwarf salamanders, N=8; b) southern leopard frogs, N=9; c) dwarf salamanders, N=7; d) southern leopard frogs, N=8. ● represent Chao2 estimates of species richness (S_{chao}), – – – – – – – – – – – – represents the S_{chao} linear trend, ■ represent Jackknife2 estimates of species richness (S_{jack}), and – – – – – represents the S_{jack} linear trend. Note the difference in x-axes scales.

Table 5.2. Top models of allelic richness and species richness. Rarefied allelic richness (r_g) model results are from McKee et al. (2012). The sample sizes for dwarf salamander r_g, southern leopard frog r_g, and species richness (S_{obs}, S_{chao}, and S_{jack}), were N = 8, 9, and 15, respectively. S_{obs} refers to the number of observed species, S_{chao} and S_{jack} represent the Chao2 and Jackknife2 species richness estimates. Condition number (CN) is the degree of multicollinearity in the model, when CN < 2, multicollinearity is not an issue in the model. AICcWi is the model weight relative to all other models for the same diversity measure at the same spatial scale. * Indicates the top model for a given parameter and number of populations. ‡ Indicates the 95% confidence interval of the variable does not cross 0.

Parameter/ Scale	Variable	Coeff.	SE	t	95% CI	r^2	CN	AICc	AICc Wi
Dwarf salamander r_g									
local	Constant‡	4.93	0.78	6.30	3.39 - 6.46	0.42	1.00	21.65	0.79
	ISO‡	-0.24	0.11	-2.07	-0.46 - -0.01				
0.5km	Constant‡	4.30	1.66	2.59	1.05- 7.55	0.23	1.00	23.86	0.33
	WTLND	5.84	4.37	1.34	-2.73- 14.41				
1.0km	Constant‡	4.10	0.86	4.79	2.42 - 5.77	0.58	1.00	19.06	0.83
	WTLND‡	7.54	2.64	2.86	2.38- 12.70				
2.5km*	Constant‡	2.95	1.14	2.60	0.73- 5.18	0.62	1.00	18.14	0.88
	WTLND‡	12.07	3.83	3.15	4.56- 19.59				
Southern leopard frog r_g									
local	Constant‡	9.35	0.16	59.32	9.04 - 9.66	0.05	1.00	20.58	0.34
	AREA	0.08	0.13	0.59	-0.18 - 0.34				
0.5km	Constant‡	9.69	0.40	24.16	8.90 - 10.48	0.11	1.00	20.02	0.32
	DEVEL	-1.44	1.59	-0.91	-4.55 - 1.67				
1.0km	Constant‡	10.43	1.19	8.75	8.09 - 12.77	0.11	1.00	20.01	0.29
	FOREST	-1.20	1.32	-0.91	-3.78 - 1.38				
2.5km*	Constant‡	8.17	0.68	12.09	6.84 - 9.49	0.31	1.00	17.63	0.33
	AG	2.14	1.20	1.79	-0.21 - 4.49				
S_{obs}									
local	Constant‡	8.38	1.35	6.20	5.73 - 11.03	0.29	1.00	65.38	0.54
	ISO‡	0.38	0.16	2.29	0.05 - 0.70				
0.5km	Constant‡	7.18	3.24	2.22	0.83 - 13.53	0.02	1.00	70.11	0.21
	FOREST	-1.88	3.49	-0.54	-8.72 - 4.97				
1.0km	Constant‡	6.54	1.48	4.41	3.63 - 9.44	0.04	1.00	69.77	0.22
	AG	-2.80	3.63	-0.77	-9.92 - 4.32				
2.5km*	Constant	-4.92	3.77	-1.31	-12.32 - 2.47	0.37	1.00	63.47	0.39
	FOREST‡	12.52	4.52	2.77	3.67 - 21.37				
S_{chao}									
local	Constant‡	10.03	1.51	6.66	7.08 - 12.98	0.33	1.00	68.62	0.65
	ISO‡	0.46	0.18	2.52	0.10 - 0.82				
0.5km	Constant‡	9.05	3.69	2.45	1.82 - 16.28	0.04	1.00	74.01	0.23
	FOREST	-2.84	3.98	-0.71	-10.64 - 4.96				
1.0km	Constant	4.60	2.54	1.81	-0.38 - 9.59	0.04	1.00	73.96	0.22

		WTLND‡	6.25	8.35	0.75	-10.13 - 22.62				
	2.5km*	Constant	-5.78	4.27	-1.36	-14.14 - 2.58	0.39	1.00	67.16	0.39
		FOREST‡	14.74	5.11	2.89	4.73 - 24.74				
S_{jack}										
	local	Constant‡	10.26	1.40	7.34	7.52 – 13.00	0.35	1.00	66.37	0.65
		ISO‡	0.45	0.17	2.65	0.12 - 0.78				
	0.5km	Constant‡	9.76	3.45	2.83	3.01 - 16.52	0.06	1.00	71.98	0.24
		FOREST	-3.27	3.72	-0.88	-10.56 - 4.02				
	1.0km	Constant‡	5.20	2.41	2.16	0.48 - 9.93	0.03	1.00	72.34	0.22
		WTLND	5.30	7.91	0.67	-10.21 - 20.82				
	2.5km*	Constant	-4.77	4.03	-1.19	-12.66 - 3.12	0.39	1.00	65.42	0.39
		FOREST‡	13.90	4.82	2.89	4.46 - 23.35				

Table 5.3. Modified from McKee et al. (2012); model averaged estimate directional associations between local and land cover features, and amphibian community biodiversity. The sample sizes for dwarf salamander r_g, southern leopard frog r_g, and species richness (S_{obs}, S_{chao}, and S_{jack}), were N = 8, 9, and 15, respectively. S_{obs} refers to the number of observed species, S_{chao} and S_{jack} represent the Chao2 and Jackknife2 species richness estimates.

	Local			Land cover			
Parameter/ Scale	AREA	HYDRO	ISO	DEVELOP	FOREST	AG	WTLND
Dwarf salamander r_g							
Local		-	-				
0.5km				-	+	-	+
1.0km				-	+	-	+
2.5km				-	+	-	+
Southern leopard frog r_g							
Local							
0.5km				-			
1.0km					-	+	-
2.5km							-
Species richness all sites							
S_{obs}							
Local	-		+				
0.5km							
1.0km						-	
2.5km					+	-	
S_{chao}							
Local			+				
0.5km							
1.0km							+
2.5km				-	+		+
S_{jack}							
Local			+				
0.5km					-		
1.0km							
2.5km				-	+		+

CHAPTER 6

NEUTRAL PROCESSES THAT REGULATE PATTERNS OF BIODIVERSITY: AN ACTIVITY FOR TEACHING UNDERGRADUATE STUDENTS ABOUT THE EFFECTS OF DRIFT AND DISPERSAL ON DISTRIBUTIONS OF SPECIES AND GENETIC DIVERSITY[9]

[9] A.M. McKee, Gary T. Green, and J.C. Maerz. Submitted to *Teaching Issues in Evolution and Ecology*. Formatting has been modified slightly and some information has been omitted to better meet The University of Georgia requirements for formatting.

6.1 Abstract

In this activity, undergraduate natural resource students simulate the neutral ecological and evolutionary processes of drift and dispersal in artificial communities to understand how these processes affect distributions of biodiversity in small (initially 10 individuals) versus large (initially 20 individuals) communities with varying degrees of isolation (well-connected, moderately-connected, and isolated). Individuals within a community are represented by Ziploc bags of candy, with the species noted on the outside of the bag and the genetic composition indicated by the presence of different colored candies or beads inside the bag. Plastic bins placed at different distances from each other represent communities [islands/patches]. Ten rounds of simulations were conducted. For each round, one bag was randomly removed from each community (ecological and genetic drift) and tossed toward another community (dispersal). Species richness and allelic richness were recorded after each round, data were graphed, and results were discussed to understand how community size and isolation affect the rate at which species richness and allelic richness decline.

6.2 Logistical Information for Instructors

6.2.1 Course Context

This activity was developed for an undergraduate upper level course for natural resources majors. Classes of 20 – 30 students work best for the activity. However it may be conducted with as few as ten students and has been conducted with as many as 40 students. For larger classes, two sets of islands could be created, or additional islands could be added to the layout. This activity requires no special equipment or settings, therefore it could be used at any other institution. A basic background in evolution and ecology is helpful for students to obtain the most out of this activity. However, if this material has been covered previously in the semester, this activity could be appropriate for non-majors as well.

6.2.2 Class Time and Setting

The activity alone may be completed during a two-hour lab session. However, additional time is recommended for the introduction of the theoretical concepts (which may be done during lecture and/or lab)

and for the concluding discussion. If students calculate species richness and allelic richness on their own in class, additional time should be allowed. Students will need an additional one to two hours to complete the discussion questions, locate and review journal article(s), and summarize their findings. Extension activities will require an extra 30 minutes to two hours depending on which activities are performed. This lab may be conducted inside or outside. However, sufficient room for separating bins is necessary (~400 – 900 ft^2) as well as room for students to stand out of the way of tossed bags.

6.2.3 Equipment/ Logistics Required

Supplies:

- 5 large bins (18" x 26" x 6" or something similar in dimensions)
- 5 small bins (12" x 18" x 6" or something similar in dimensions)
- 150 (+ 1 extra for each student in the class) zip-closure sandwich bags – 20 per large bin, 10 per small bin
- 10 (+ extras) copies of the animal pictures sheet, Appendix K
- 300 (+ 2 extra per student in the class) Starbursts® of various flavors – 2 per bag
- 300 (+ 2 extra per student in the class) Jolly Ranchers® of various flavors – 2 per bag
- Packaging tape
- 10 copies of the species richness data sheet (1 per group), 30 copies of the allelic richness data sheet (3 per group), 1 copy of each island characteristic sheet, Appendix L
- Computer with Microsoft Excel
- 1 handout copy per student in the class

6.2.4 Lab Preparation (This should be done in advance)

- Follow the layout of bins as shown in Figure 6.2 to set up the islands.
- Cut out each of the animals and individually tape one to each plastic bag.

- Place two starburst and two jolly ranchers into each bag.
- Randomly select 20 bags to place in each large bin, and 10 bags to place in each small bin.
- Place 1 copy of the datasheet packet into each bin - make sure these datasheets have the island number and characteristics written in.

6.3 Synopsis of the Activity

The principal ecological question addressed in this activity is, "how do the neutral ecological and evolutionary processes of drift and dispersal affect biodiversity?"

6.3.1 What Happens

Students take turns simulating drift and dispersal in animal communities, represented by bags of candy. Each round, students randomly remove one individual and its genes (one bag of candy) from the community, representing ecological and genetic drift. The student then attempts to toss the individual into another community (or island/patch represented by a plastic bin). Successful dispersal occurs if the bag of candy lands in the bin. Bags that miss an intended target are removed from the game. Intuitively and consistent with island biogeography theory, students are more likely to be successful tossing bags of candy into larger or closer bins, which should reflected by greater species and allelic richness relative to the smaller, more isolated bins.

6.3.2 Activity Objectives

After completing this activity, students should be able to

- Understand how the processes of dispersal and ecological/genetic drift affect species and genetic diversity on islands and/or habitat patches
- Apply the concepts of island biogeography to alternative systems
- Understand how habitat size and isolation play into decisions regarding habitat reserves for conserving biodiversity

- Understand the main forces that influence distributions of biodiversity

6.3.3 Summary of What is Due:

Students submit responses to discussion questions that compare class results to student hypotheses, gauge student understanding of the application of the material to alternative systems and conservation management, as well as when the material may not be applicable to conservation management. Students also turn in a summary of a literature review on articles that investigate the effects of habitat area and isolation on species and/or allelic richness. Along with the summary, students must state and justify their opinion concerning what the results of their review suggest in terms of the importance of drift and dispersal on species/genetic diversity distributions.

6.4 Detailed Description of the Activity (audience students)

6.4.1 Introduction

Processes that regulate biodiversity are central foci of ecology and evolutionary biology, and the conservation of biodiversity is something that most people recognize as a contemporary issue and major management priority. This activity will focus on two of the three components of biodiversity – species and genetic diversity. Because the processes that regulate species and genetic diversity include biogeographic neutral forces related to space and scale (patch size and isolation), management for species and genetic diversity requires understanding how patch size and the relative isolation or connectivity of patches affects these scales of diversity within communities and populations. See Table 6.1 for a definition of important terms.

Ecological communities gain species through speciation and immigration (also commonly referred to as dispersal). Relative to immigration, speciation is rare and generally contributes little to community diversity over an ecological timeframe. The process of immigration is referred to as a neutral process, because it occurs independently among species regardless of their ecological differences. In contrast to the positive effects of immigration on species diversity, extinction (i.e., ecological drift) causes the loss of species diversity.

Ecological drift due to random fluctuations in population sizes, environmental variations, and mortality (as opposed to species and population interactions) is also considered a neutral process, because it occurs independent of ecological differences among species.

Island biogeography is one of the more prominent ecological theories that are applied to explain patterns of community diversity, and more recently to design and manage habitats and reserves for conservation (Sax and Gaines 2011). This theory, which was developed by E.O. Wilson and Robert MacArthur in the 1960's (MacArthur and Wilson 1967), is based on the idea that immigration and extinction serve as balancing forces on species diversity within a community. Larger islands are expected to have more species and more individuals within those species (*i.e.*, larger populations) than smaller islands because larger islands are likely to have a greater variety of habitat types to exploit and more resources for more individuals within those populations. Larger populations of any given species reduces the risk of extinction of that species due to random chance. Islands that are located closer to a source of immigrants (a mainland or other islands) are expected to have higher species richness because immigrants are likely to be more successful dispersing over a short distance versus a long distance, and higher recolonization rates, which reduces the chance of prolonged extinction. Eventually, an equilibrium level of species richness in a community will be reached due to the balancing effects of extinction (i.e., ecological drift) and immigration (i.e., dispersal).

A similar neutral theory has been developed for populations (Kimura 1968). Instead of drift and dispersal affecting species diversity, genetic drift (e.g., random loss of alleles in a population) and gene flow (i.e., dispersal) affect genetic diversity (Vellend 2004, Hu et al. 2006). Similar to the theory of island biogeography, an equilibrium value of allelic richness is reached through a balance between the additive processes of mutations and dispersal and the subtractive process of genetic drift. Larger populations often have higher allelic richness than small populations because they are less likely to lose alleles from the population due to random chance. Populations that are located closer to a source of immigrants (the mainland or other islands) are expected to have higher allelic richness because immigrants are more likely to be successful dispersing over a short distance versus a long distance.

The similar effects of drift (ecological and genetic) and dispersal on species and genetic diversity, suggest that genetic and species diversity should be correlated if drift and dispersal are the primary processes regulating distributions of biodiversity. This is important for conservation because in situations where this is the case, similar management strategies could be used to optimize biodiversity at the species and genetic scales.

6.4.2 Overview of Data Collection and Analysis Methods

You will be assigned to an island community (see Figure 6.2 for island layout), represented by a tub (the island) and bags of candy (individuals in the community). The islands have two distinguishing characteristics, their size and degree of connectivity/isolation (Table 6.2).

The bags of candy in your bin represent individuals within your community. Within the bags, you have various candies – these represent the allelic composition of your individuals. Note on the datasheets the species richness of your community, and allelic richness of your populations.

Calculate initial species richness prior to the first round. For each species in your community, record the number of individuals on the *Species Richness Datasheet* as demonstrated in Figure 6.3. Calculate initial allelic richness. Different colors of the same candy are considered unique alleles, e.g., a red jolly rancher and a green jolly rancher are different. Count the number of copies of each allele that are present within each species. Record this information on the *Allelic Richness Datasheet*. Using the *Average Allelic Richness Datasheet* and calculate the average number of alleles in the community (out of the total number of species included in the game) as demonstrated in Figure 6.4; the colored dots in the squares represent different alleles.

At the beginning of each round, create a mortality event by randomly selecting an individual to remove from your community. This represents the process of drift. This individual no longer exists from your community's perspective. Although not biologically realistic, the randomly removed individual from your community now acts as an immigrant to another community. Create a dispersal event by tossing your removed individual toward any community within an uninterrupted linear path (if you have to throw over

someone's head, then it is off limits – review Figure 6.5 to double-check which islands you can toss to). This represents the process of dispersal.

The random selection of an individual to remove from your community represents the ecological process of drift – the random loss of biodiversity from the community. Toss your randomly selected individual into another tub. To the receiving tub, this represents immigration. If your tossed individual does not make it to the island (if it does not land in the tub), the individual is removed from the meta-community.

After one individual from each island has dispersed, record the species and alleles that were removed from the community in the *Species Richness Datasheet* and *Allelic Richness Datasheet*. Similarly, for any new immigrants into the community, record the species and alleles gained as shown in Figures 6.6 (species) and 6.7 (alleles). Repeat this process until you have completed ten rounds. The class data will be compiled and provided to you in graphs. Part of your homework assignment will be comparing your hypotheses with the class results.

6.4.3 Questions for Further Thought and Discussion after the activity

The following topics are discussed in class after the activity is over:

- Give two additional examples of systems, besides islands, to which these concepts of drift and dispersal could be applied.
    - Example answers include mountaintops, heads of coral, forest fragments, wetlands, national parks, etc.
    - Purpose: Help students understand neutral ecological forces on islands behave the same as neutral ecological forces in habitat patches/islands
- Refer to Figure 6.2. Assuming you have no information about the islands besides their size and location, If you were a natural resources manager and responsible for selecting three islands to protect for biodiversity conservation a) which three would you select and b) why would you select these three?

    - Islands 1, 3, & 5 are the largest and most well-connected islands, and would minimize drift while maximizing dispersal among the islands.
    - Purpose: Ensure students understand how the concepts of drift and dispersal can be applied to habitat reserve design

- In the real world, other factors besides drift and dispersal affect distributions of biodiversity. a) What factors besides drift and dispersal affect distributions of species and allelic richness? b) How might you determine whether or not these other factors have stronger effects on species and/or genetic diversity compared to the neutral processes of drift and dispersal?
    - Selective forces e.g., species interactions and habitat preferences, etc.
    - If species and/or allelic richness were not positively correlated with area and/or were not negatively correlated with increasing isolation, this would suggest that other selective factors had stronger effects on the distributions of species and genetic diversity
    - Question purpose: Help students recognize that drift and dispersal are not the only forces that affect the distributions of species and genetic diversity. However, they are the only forces that lead to predictable outcomes across tax

6.5 Tools for Assessment of Student Learning Outcomes

Grading answers to discussion questions that are answered outside of class assesses student mastery of the material. To reinforce primary concepts and test for students' ability to apply the material to different systems, the first three questions for the homework assignment are similar to the questions that are discussed after the activity in class. Additionally, the material (terminology and/or concepts) may be covered on exams. The questions below add to a total of 15 points.

- Compare your hypotheses to the class results for how species and allelic richness changed over time on small versus large islands. If the results were different than what you expected, explain (and justify) whether you think this was a matter of sampling error or whether you think your hypotheses were incorrect. (1 point)

    - 1 point – This answer will depend on whether or not the results agreed with the original hypothesis. However, the response should make sense given the class results.
    - Question purpose: Help students interpret graphical data to understand the class results. Additionally, students can also compare their original graphical hypothesis to the class results, to help remind them of what the graphical relationship should look like.
- Compare your hypotheses to the class results for how species and allelic richness changed over time on isolated versus well-connected islands. If the results were different than what you expected, explain (and justify) whether you think this was a matter of sampling error or whether you think your hypotheses were incorrect. (1 point)
    - 1 point – This answer will depend on whether or not the results agreed with the original hypothesis. However, the response should make sense given the class results.
    - Question purpose: Help students interpret graphical data to understand the class results. Additionally, students can also compare their original graphical hypothesis to the class results, to help remind them of what the graphical relationship should look like.
- Explain why the concepts of drift and dispersal can be applied to other systems besides islands. (1 point)
    - 1 point – Movement among habitat patches (habitat islands) requires dispersal across suboptimal habitat, and larger patches are likely to have more resources than smaller patches – similar characteristics that describe islands.
    - Question purpose: Help students understand the basis for the application of island biogeography to alternative systems.

- Refer to Figure 6.12. Pretend you're working as an environmental consultant to a resort development company. The company is building the resort in an undeveloped area and wants to minimize the impact of construction on biodiversity in the area. The company has proposed three optional designs for habitat reserves on the property, and you are responsible for selecting which design will optimize species and allelic. Without any other information besides relative size and location, which reserve arrangement would you recommend and why? (1 point)
    - 1 point –A –This reserve design has the largest habitat patches (lower rates of drift) and greatest connectivity (greater rates of dispersal).
    - Question purpose: Ensure students understand how the concepts of drift and dispersal can be applied to habitat reserve design.
- In the real world, other factors besides drift and dispersal affect distributions of biodiversity. a) What factors besides drift and dispersal affect distributions of species and allelic richness? And, b) How might you determine whether or not these other factors have stronger effects on species and/or allelic richness compared to the neutral processes of drift and dispersal? (2 points)
    - 1 point – a) Selective forces e.g., such as species interactions and habitat preferences, etc.
    - 1 point - b) If species and/or allelic richness were not positively correlated with area and/or negatively correlated with increasing isolation, this would suggest that other selective factors were influencing the distributions of species and genetic diversity.
    - Question purpose: Help students recognize that drift and dispersal are not the only forces that affect the distributions of species and genetic diversity. However, they are the only forces that lead to predictable outcomes across taxa.

The purposes of the following exercise is to get students looking through the primary literature and help them understand that the relationships between richness and area/isolation are not nearly as clean or predictable in the real world as they were in the activity.

- Work with the members of your group to find five to seven peer-reviewed journal articles that investigate the relationship between habitat area and richness (species OR allelic) and the relationship between habitat isolation and richness (species OR allelic). (9 points total). For each paper:
- Include the citation (1 point)
    - 1 point – Citations for each article on the effects of area and/or isolation on species and or allelic richness.
    - Question purpose: Give students practice with citing references
- Describe the taxa investigated (e.g., amphibians, birds, vertebrates, etc.) and what the habitat patches were (e.g., islands, wetlands, mountain tops, national parks, etc.) (1 point)
    - 1 point – Descriptions of the taxa and habitat types in the studies.
    - Question purpose: Emphasize that a variety of habitat types can often effectively function as habitat islands.
- Write a summary -in full sentences- that includes 1) which predictor variables of species and/or allelic richness were investigated (e.g., area, isolation, elevation, forests, etc.); 2) the results of the study in terms of which predictor variables were most strongly and/or closely associated with richness; and 3) the directional relationship (positive or negative) between richness and these predictor variables. If area and/or isolation were not found to be associated with richness, please include this in your summary as well. (3 points)
    - 2 points – Summaries of the variables investigated and which variables were most closely associated with the measure of diversity.
    - Question purpose: Ensure students understand that are and isolation are not the only factors that can influence distributions of diversity.

- 1 point – Explanations of the directional associations between the top variables and diversity.
- Question purpose: Make students aware that associations between diversity and area/isolation may be contrary to their expectations, which would indicate that drift and/or dispersal were not the primary processes affecting diversity.

- Based on the results of the five to seven articles, how important do you think the processes of drift and dispersal are compared to other factors (e.g., habitat selection, competition) for determining distributions of species or genetic diversity? Justify your opinion with examples from the results of the studies (e.g., Species richness had a stronger association with habitat variables compared to area or isolation in four of the five studies, which suggests habitat is more important than drift or dispersal for determining distributions of richness). (4 points)
  - 2 points –Students provide their opinion about the importance of drift and dispersal, relative to some other factors or processes, for determining distributions of species and/or genetic diversity.
  - 2 points – Students back up their opinions with supporting evidence from the articles that they read.
  - Question purpose: Give students practice making informed opinions based on scientific evidence.

See Appendix M for Sample Exam Questions.

6.5.1 Extension Activities for Advanced Students

Collect all the data sheets from the students after the activity, graph the results, and provide these graphs to students for interpretation. The calculations may be a bit confusing and some students may have a difficult time understanding this within the lab period. Advanced students may have more success doing the

calculations and graphing on their own, possibly making this an appropriate additional activity for advanced students.

6.6 Comments to Instructors of the Activity

We have not tried alternatives to candy as representatives as alleles. However, any colored objects that can fit inside the plastic baggies (e.g., beads, marbles or buttons) should work. If candy is used, we bring extra candy for students to eat and make sure that the students understand that the candies in the bags are being used in the activity and are not for their consumption.

Be sure to include enough variation at the genetic level that students can see changes in allelic richness, but not so much variation that it is difficult to keep up with outgoing and incoming alleles. This approach is also recommended at the species level. We generally use nine to ten different species (Figure 2) and nine to ten different alleles, as they are manageable levels for students to record.

Remind students to be aware of others when they are tossing the candy. We recommend, prior to the activity, stating consequences of hitting someone with a bag of candy – on purpose or by accident –to avoid potential injuries. Our students have found data recording easier if they keep their bags outside of the bins, so that when they receive an immigrant, they do not have trouble keeping track of which individual entered the community.

As the objectives of this activity are related to student understanding of the material, as opposed to data entry and learning graphing programs, we collected the student data sheets and compiled and graphed the results, to make the most effective use of student time. However, if this activity were conducted for an advanced level class, requiring the students to perform their own species and allelic richness calculation and graph the results would add an element of complexity to the activity.

6.6.1 Challenges to Anticipate and Solve

As with any new class activity, unexpected challenges will arise. Below are listed several challenges we encountered while conducting the activity and how we ameliorated them.

- CONFUSION ABOUT HOW TO RECORD THE DATA AND CALCULATE RICHNESS (SPECIES AND ALLELIC): Some students have a difficult time keeping track of allelic richness for each species after the first round. To keep the activity moving along and minimize work outside of the class, students were only asked to record the species and alleles entering and leaving communities and we calculated species and allelic richness and provided students with the graphs. However, if you would prefer for your students to perform the calculations and create their own graphs, this could be done after the lab, following the examples in the handout. Students could also just graph the results from their own island, and predict what the results from islands with differing characteristics (size and isolation) would look like graphically relative to their results.
- OCCUPYING SPARE TIME: The well-connected and moderately-connected islands are likely to receive more immigrants than the isolated islands (if the activity is working as it should). Therefore students at these more connected islands will require more time to record data than the students at the isolated populations. To keep students at these isolated islands engaged while other students are busy recording data, provide them with either the questions for further discussion and ask them to brainstorm answers while they wait, or to ask additional questions, such as what sort of adaptations have species developed to facilitate long-distance dispersal events? Alternatively, these students could work on calculating average allelic richness after each round. Similarly, if you are working with a large class and have three to four students per group, students can rotate through the roles of recording species data, recording genetic data, removing and tossing the randomly selected baggie, and brainstorming responses to the discussion questions.
- THEORETICAL VERSUS REAL SYSTEMS: This activity ignores the ecological differences among species, such that there are no consequences of selection or species interactions. This could be incorporated into the activity, for example by selectively removing certain species or alleles from the communities each round. Instead of incorporating a selection into the game, we chose to include discussion questions that encourage students to think about other forces that determine distributions of species and genetic diversity. Additionally, with the literature review, students are likely to

encounter studies where species and/or allelic richness were associated with habitat variables as opposed to habitat size and/or isolation.

6.6.2 Introducing the Activity to Your Students

We generally precede this activity with one or two days of lecture on evolution and island biogeography. In addition to covering mutation and natural selection in the evolution material, extra time was spent discussing the effects of genetic drift and gene flow (i.e., dispersal + reproduction) on genetic diversity in populations so that students are comfortable with these terms going into the activity. We also assign several readings from the book*, Song of the Dodo* (Quammen 1996), a non-fiction book on island biogeography (Appendix N).

Although it is not essential to understanding the concepts of neutral evolutionary and ecological processes, we generally begin the lab with a discussion on the constituents of biodiversity (ecosystem, species, and genetic diversity; "biodiversity lab component") and the importance of biodiversity to human existence. This approach puts the relevance of the activity to the students' own existence into context. We give students the following assignments and have them work in groups to answer questions about what biodiversity is and why it is important:

The first part of lab is a "pair-think-share" activity. In groups of three to four the students brainstorm answers to questions and share their answers with the class. Answers are then discussed as a class and any confusion that groups may have is clarified. As a group, students brainstorm three examples of biodiversity. Each group reads their answers; a brief summary of what they list is written on the board. Generally this list does not include examples of genetic diversity. The definition of biodiversity is then posted, one that includes something about species, genetic, and ecosystem diversity. With the definition of biodiversity in front of them, the students then brainstorm three more examples of biodiversity. If students have trouble brainstorming examples, then they are given several examples:

- Ecosystem
    - Coral reef ecosystems.

    - Longleaf pine and tidal marsh ecosystems in Georgia.
    - Rainforests, cloud forests, and dry pacific forests in Costa Rica.
- Species
    - The various coniferous trees in the Colorado Rockies.
    - The composition of species that occur on the Hawaiian Islands.
    - A community of pond-breeding amphibians.
- Genetic
    - A population of lizards, some of which have blue eyes and some of which have green eyes.
    - A plant species that has diploid (two copies of each chromosome) and triploid (three copies of each chromosome) variants.
    - Different DNA sequences among three individuals from the same species.

In the same group, students brainstorm answers to the following question, "if you were moving to a new planet, what three species would you bring with you to make it habitable? Explain/justify each species that you list." Each group then shares three of their examples and explains why they were selected. Sample answers include:

- Plants for photosynthesis – oxygen, carbohydrates
- Plants and filter feeders that clean water
- Animals to eat
- Insects to pollinate
- Plants and animals for clothing
- Medicine
- Shelter

The students are then asked to assume there is no limitation to the number of species or the number of individuals of each species that you may bring to this new planet and they reselct which species/individuals to they would bring, keeping in mind that they must account for the stochastic (unpredictable) environment (e.g.

new diseases, changing climate, extended periods of drought, etc.) on the new planet. Example responses include:

- Species that are not of great importance to humans on earth could be important on the new planet (species that are not important now could be important in the future).
- Combinations of species often required to perform ecosystem services (e.g., nutrient cycling).
- Genetic diversity for disease resistance (e.g., potato blight)

During lab, but prior to the activity, we discuss the basic concepts of island biogeography and the effects of island size and isolation on species diversity. We also review the concepts of genetic drift and dispersal and their effects on genetic diversity in populations. Students are asked to write out and graph their hypotheses (see Figure 6.9 for form that students fill out) of how species and genetic diversity changes over time in communities and populations of varying sizes (small versus large) and with varying degrees of isolation (isolation versus well-connected).

6.6.3 Data Collection and Analysis Methods Used in the Activity

Prior to starting the activity, we strongly recommend working through an example of how to record outgoing and incoming species and alleles. Also, we recommend making sure students understand why they are recording the cumulative number of individuals for a given species, and cumulative number of alleles for a given species (so each round they only have to count the individuals that are removed or added to the community, as opposed counting all individuals and alleles each round). By keeping track of the number of individuals per species and the number of each allele for each species, students know when that species or allele has become extinct from the community or population, respectively. After the first round in the activity, check with students to make sure they're comfortable with how to record the data.

We used pre and post surveys to test whether the activity was helping students to meet the objectives. We suggest that this be done in other classrooms as well, to ensure students are gaining knowledge through the activity, and that students are meeting the objectives outlined in the activity.

6.7 Formative Evaluation of this Activity[10]

This activity was conducted in two semesters of an upper level undergraduate ecology course, and one semester in a split-level (upper level undergraduate and graduate) course (N = 89, Table 6.3). During the development of this activity, we created a survey with questions in a quiz-like format (True/False and Multiple Choice) to test the effectiveness of this activity at meeting the outlined objectives, to gauge changes in student confidence in their knowledge of the material, and to obtain feedback from students about the activity and areas for improvement (Appendix 6.5). We were interested in the constructs of the effects of drift and dispersal on species and genetic diversity, as well as designing habitat reserves based on patch/island layouts that minimize drift and maximize dispersal. The survey was also designed to test students' knowledge of the constituents of biodiversity (ecosystem, species, and genetic diversity), as this is a standard part of the lab in these classes. We include survey results to demonstrate the effectiveness of this additional lab component at increasing student knowledge and confidence in their understanding of the constituents of biodiversity. At least three questions were developed for each construct to test reliability and validity of the constructs. Students were asked to rate how confident they were with their response to each of the questions using a Likert scale (one = Unconfident, five = Confident).

Between species and genetic concepts, we hypothesized that students would initially be least familiar with the idea of genetics as a component of genetic diversity, as well how population isolation (dispersal) and population size (drift) would affect genetic diversity. However, since species and genetic diversity are not the only constituents of biodiversity (also includes ecosystems), we were interested in assessing how familiar students were with biodiversity, and whether they could distinguish what is versus isn't biodiversity.

Pre-activity surveys were given to all students present during lecture on the designated survey day. Treatment and control groups were self-selected based on the lab section in which the students were enrolled. Members of the treatment group re-took an identical survey after participating in the activity, whereas members of the control group either retook the survey prior to participating in the activity or after participating in a different lab activity.

[10] We present only results from consenting undergraduate students, as the number of graduate students was insufficient for comparing results, who were present for both the first and second round of surveys.

We calculated Cronbach's alpha and performed Principle Components Factor Analysis with Varimax raw rotation summary of factors from post-activity survey responses in STATISTICA 6 (StatSoft; Tulsa, OK) to assess the reliability and validity of the questions within specific constructs (i.e., knowledge and confidence). Student overall knowledge scores for the surveys were calculated as the proportion of questions answered correctly out of the number of questions that were answered. We calculated scores for each construct in the same manner. Similarly, confidence scores were calculated as the average confidence value that students reported. Confidence scores for each construct were calculated in a similar manner as overall confidence scores.

We performed factorial ANOVAs to check for differences in pre-activity confidence and knowledge scores among classes and between control and treatment groups. We performed ANCOVAs to test for differences in overall post-activity confidence and knowledge scores between control and treatment groups, controlling for pre-activity score. We also tested for effects of gender and age, however these were not significant and hence not include in any further analysis. We then combined the results of the classes to test for effects of the activity and biodiversity lab component on confidence and knowledge scores the each of the different constructs.

6.7.1 SURVEY RELIABILITY

Cronbach's alpha analysis of the confidence results from the survey confirmed the reliability of the questions within the constructs (Table 6.4). Cronbach's alpha analysis from the knowledge portion of the survey generally supported the reliability of the questions within the constructs (if we accept alpha >= 0.5; Table 6.4), however two of the constructs (effects of dispersal on genetic diversity and species as a constituent of biodiversity) were not reliable (Cronbach's alpha < 0.5). As this may have been a result of the small number of questions per construct (3 questions) we also consulted expert opinions, which supported that the questions effectively tested knowledge of the constructs.

Factor analysis of the confidence questions suggested one primary factor (Table 6.5). Although this factor did contain all three of the questions about habitat reserves, it also contained two additional questions

of different constructs. We believe the grouping of this factor was a result of the relative difficulty of the question in terms of number of answer options. It was composed of questions with five to six possible answers, as opposed two to three possible answers such was the case with the majority of all other questions. The increase in possible answers likely decreased the student confidence in their answers.

Factor analysis of the knowledge questions grouped a number of the constructs, including dispersal (two of the genetics questions and two of the species questions; Table 6.5), the different constituents of biodiversity (as well as the construct that abiotic factors are not constituents of biodiversity), effects of drift on genetic diversity (all three questions; Table 6.5). The last factor included all three reserve design questions as well as a question on the effects of drift on species diversity (Table 6.5). All of these questions had five possible answers, suggesting that this factor may have been grouped due to difficulty (more possible answers, higher probability of getting the question incorrect. This may also explain why the construct of effects of drift on species richness did not result as a factor (due to the differing degrees of difficulty).

Despite some statistical disagreements due to varying difficulty of questions, we believe based on expert opinions, overall Cronbach's Alpha scores, and factor analysis that our survey questions were sufficiently reliable and valid to gauge changes in student confidence in and knowledge of the constituents of biodiversity and the effects of drift and dispersal on species and genetic diversity. However, for future surveys and/or tests, we recommend that the questions for the constructs of species as constituents of biodiversity and the effects of dispersal on genetic diversity be modified and number of answers be reduced to ensure students are not confused by the questions.

One example of how the species as constituents of biodiversity questions may be modified would be to reword the questions so that they phrased to sound more similar. For example, instead of, "The various coniferous trees in the Colorado Rockies are an example of biodiversity," the statement could be reworded as, "Co-occurring coniferous tree species in the Colorado Rockies are an example of biodiversity," so that students recognize the word species and understand the question is in reference to species diversity. Similarly, the statement, "A community of pond breeding amphibians is an example of biodiversity," may be rewritten as, "Co-occurring pond-breeding amphibian species are an example of biodiversity."

Consistency with wording may also have been a problem for questions pertaining to the effects of dispersal on genetic diversity. We tended to use the "dispersal" during the lab, whereas we used the term "immigration" on the survey, we recommend using one term throughout the lab and survey to avoid confusion.

6.7.2 Pre-Activity Overall Knowledge and Confidence Scores

Average pre-activity knowledge scores were not significantly different among classes or between control and treatment groups (Figure 6.10a). Although there was a significant difference in pre-activity confidence among classes, there was no significant difference between the control and treatment groups within classes (Figure 6.10b).

6.7.3 Pre-Activity Constituents of Biodiversity Construct Knowledge and Confidence Scores

As predicted, students were initially less familiar (had lower knowledge scores) with genetic diversity as a constituent of biodiversity compared to their knowledge of species and ecosystem diversity as constituents of biodiversity (Figure 6.11a). Similarly, prior to the activity and the additional lab component, students were least confident in their answers about genetic diversity as a component of biodiversity and were significantly more confident in their answers to questions about ecosystem and species diversity as constituents of biodiversity (Figure 6.11b).

6.7.4 Pre-Activity Effects of Neutral Factors on Species and Genetic Diversity Construct Knowledge and Confidence Scores

Because students tend to be more familiar with the concept of species diversity relative to genetic diversity, we hypothesized that students would be more familiar and confident with questions pertaining to the effects of neutral factors on species diversity compared to genetic diversity. However, the results did not support our hypothesis. Initially, students scored significantly higher on questions pertaining to the effects of

dispersal on genetic diversity compared to questions pertaining to the effects of dispersal on species diversity (Figure 6.11b). Prior to the activity, students were also more confident in their knowledge about the effects of drift and dispersal on genetic diversity versus species diversity (Figure 6.11b).

6.7.5 Post-Activity Changes in Overall Knowledge and Confidence Scores

Across classes, students who participated in the activity and additional lab component had significantly greater knowledge and confidence scores compared to students in the control groups (Figure 6.12, Table 6.6-6.7). The activity was more effective at increasing knowledge in some classes over others (Figure 6.12a, Table 6.6). Strangely, the FANR3200 Spring 2010 control group had significantly reduced knowledge scores overall on the post-activity survey (Figure 6.12a). One explanation for this may be that students learned about these topics prior to the first survey and those in the control group had forgotten the material by the time the second survey was administered. Confidence scores increased similarly across treatment groups in all classes (Figure 6.12b, Table 6.7).

6.7.6 Post-Activity Changes in Constituents of Biodiversity Construct Knowledge and Confidence Scores

Participants of the biodiversity lab remained confused that abiotic factors are not constituents of biodiversity (Figure 6.13a). However, students who participated in the lab and activity did have greater knowledge about ecosystem, species, and genetic diversity as constituents of biodiversity (Figure 6.13a). Changes in students' confidence about their knowledge of the constituents of biodiversity did not entirely correspond to the changes in knowledge scores across questions regarding abiotic factors, and ecosystem, species, and genetic diversity as constituents of biodiversity. Students who participated in the lab and activity had increased knowledge and increased confidence about their knowledge of ecosystem, species, and genetic as constituents of biodiversity (Figure 6.13b). However, students' confidence in their understanding that abiotic factors are not constituents of biodiversity increased (Figure 6.13b) despite a lack of change in knowledge scores about abiotic factors. This suggests that while students were more confident in their overall

understanding of what constitutes biodiversity, the additional activity was not successful at teaching students that abiotic factors are not constituents of biodiversity.

6.7.7 Post-Activity Changes in Effects of Neutral Factors on Species and Genetic Diversity Construct Knowledge and Confidence Scores

With regard to questions of the effects of neutral factors on species and genetic diversity, the scores of students who participated in the activity only increased for questions on the effect of drift and dispersal on species richness (Figure 6.14a), whereas students' confidence scores increased across all constructs (Figure 6.14b). The only knowledge scores that improved after participating in the activity were those for the questions pertaining to the effects of neutral factors on species richness (Figure6.14a). Similar to the overall reduced knowledge scores in the FANR3200 Spring 2011 control group, knowledge scores for the effect of drift on species richness were also significantly reduced in the control group (Figure 6.14a).

The activity and biodiversity lab component were undoubtedly successful in improving student confidence in their understanding of the constituents of biodiversity and the effects of neutral forces on species and genetic diversity. The biodiversity lab component significantly improved student knowledge that ecosystems, species and genetic diversity are the constituents of biodiversity. However, the lab did not improve students' abilities to recognize that abiotic factors are not examples of biodiversity. We suggest that slight modifications be made to the biodiversity lab component such that more emphasis is placed on helping students recognize what is and is not biodiversity.

Surprisingly, students were relatively familiar with the effects of drift and dispersal on genetic diversity regardless of whether or not they participated in the activity. Although the activity only significantly increased student scores on questions pertaining to the effects of dispersal on species richness, we believe based on the improvement in confidence scores that students understanding of the effects of neutral forces on biodiversity is better internalized such that students are more confident in their understanding of these forces on species and genetic diversity.

6.8 Translating the Activity to Other Institutional Scales or Locations

This activity could be modified for smaller classes by decreasing the number of islands, or be modified for larger classes by either increasing the number of islands or by breaking the class up into two or more groups. If islands are either added or removed, you may wish think about the arrangement of the islands/bins such that the arrangement has large and small bins distributed at scales of differing connectivity levels. This activity could also be modified to fit other systems that exhibit patchy distributions, or systems that occur as metapopulations. Although we have only conducted this activity in upper level undergraduate ecology courses, we believe the activity may translate to lower level undergraduate courses or possibly even be suitable for high school courses, to teach concepts of island biogeography and effects of neutral factors on genetic diversity.

For more advanced classes, the activity could be repeated multiple times, with slight modifications each time to demonstrate effects of differing scenarios, within a class period. An example of a modification that could be made to the activity would be to incorporate a selective component that affects dispersal abilities. Heavier bags are easier to toss farther and more accurately, so one way to represent this in the activity would be to add extra weight to the bags of some species, but not others.

6.9 Student Collected Data and Examples

We include examples of student-recorded data on the species and allelic richness datasheets (Figures 6.15 and 6.16), graphical representations of results from one class (Figures 6.17 and 6.18), as well as example responses to two different types of homework assignments (Figures 6.19 and 6.20).

Students were assigned the following, "Find 5 peer-reviewed journal articles that investigate the relationship between habitat area and richness (species OR allelic) and the relationship between habitat isolation and richness (species OR allelic). For each paper: a) describe the taxa investigated - amphibians, birds, vertebrates, etc., b) describe what the habitat patches were - islands, wetlands, mountain tops, national parks, etc., and c) summarize the results of the study in terms of whether or not correlations were found between richness and area/isolation. If correlations were found, be sure to include whether they were positive

or negative. Based on the results of the 5 articles, how important do you think the processes of drift and dispersal are for determining distributions of species OR genetic diversity?" One student's responses are listed below:

Phylogenetic and phylogeographic analysis of Iberian lynx populations

This paper investigated the impact of population fragmentation on the genetic richness and diversity of the Iberian lynx (*Lynx pardinus*). The population fragments examined were current national parks as well as historical areas in which the lynx was known to have lived but has since been extirpated. They used museum specimens/remains to collect genetic data on these individuals. The study found that there was low genetic diversity among all of the populations, and the investigators determined that the lack of genetic diversity was due to population bottlenecking caused by habitat fragmentation. This fragmentation allowed for genetic drift and haplotype fixation in certain individual populations. The loss of some of the historical populations was also attributed to the lack of genetic diversity and the inability to immigrate between populations. This shows that the smaller the habitat, the less diversity and overall fitness a population exhibits.

The relative effects of habitat loss and fragmentation on population genetic variation in the red-cockaded woodpecker (Picoides borealis)

This paper studied the impact of habitat loss and fragmentation on the red-cockaded woodpecker (*Picoides borealis*). The habitat areas of interest were forested areas of either high-quality habitat, low-quality habitat, or unforested areas, and the amount of fragmentation ranged from highly fragmented to perfectly contiguous habitats (using simulation modeling). The study found that fragmentation was the leading cause of low genetic diversity compared to population size, although it also showed that a low population size compounded these genetic problems. Population fragmentation had an increasingly

negative impact on the amount of genetic diversity as the fragments became smaller and more isolated.

Effects of habitat loss, habitat fragmentation, and isolation on the density, species richness, and distribution of ladybeetles in manipulated alfalfa landscapes

This paper looked at the effects of habitat fragmentation and isolation on the species richness and density of species of ladybeetles/coccinellids (*Eriopis connexa*, *Hyperaspis sphaeridioides*, *Hippodamia variegata*, and *Hippodamia convergens*). The habitat patches that were studied were alfalfa microhabitats with varying degrees of fragmentation and isolation. This study found that habitat loss had varying impacts on the species richness and abundance in the remaining habitat, and that in this case habitat fragmentation actually had a positive impact on species richness and the density of several coccinellids. The level of isolation that the populations exhibited also did not show any correlation to richness.

Impact of anthropogenic habitat fragmentation on population health in a small, carnivorous marsupial

The species of interest in this study was the agile antechinus (*Antechinus agilis*), and it was examined to determine the effect of anthropogenic habitat fragmentation on the health of the population. The study area was a region in southeastern Australia called South Gippsland, and the sites were fragmented by agricultural activities that isolated the native forested areas. This study found that ectoparasite loads were greater in the fragmented habitat areas than in the contiguous forest sites. The study found that this decrease in health was most likely due to habitat fragmentation and that the smaller the area, the more likely the population's health in that area was to decline. This is a negative relationship between fragmentation and species abundance and genetic diversity.

Impacts of rain forest fragmentation on butterflies in northern Borneo: species richness, turnover and the value of small fragments

This study was conducted on fragmented populations of butterflies (Order Lepidoptera). The study areas were in the tropical rain forest of Sabah, Borneo and included rain forest, agricultural land, and mangrove forests. This study showed that the habitat fragment size had a positive effect on butterfly species richness and diversity, while isolation had a negative impact. There was greater species diversity when the fragments were large and close together.

The results of these five articles show that for the majority of species and habitats, fragmentation and isolation lead to a decline in both species and genetic richness. The opposing findings from the third study on the coccinellids proves that although one ecological theory, such as that of island biogeography, may be true in many situations, it will not be fitting for every species or population. The largely negative effects of increased isolation on richness within populations supports the determination that dispersal is an important factor in maintaining both species and genetic richness. In most situations, the closer the fragmented populations were together, the greater their richness, and this is due to the ability of individuals to travel between metapopulations, sharing both individuals and genetic material between populations. Although isolation, and therefore dispersal, were important factors in maintaining richness and biodiversity, drift within the population fragments seemed to have an even greater impact. The small population sizes in fragments led to increased chances of haplotype fixation, inbreeding, and random loss of individuals from a community. All of these factors reduce the fitness of individuals, caused smaller populations to have greater rates of extinction, and reduce population sizes further. Drift, genetic and otherwise, is a key factor habitat fragments that reduces genetic and species richness over time.

6.10 ACKNOWLEDGEMENTS

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6.11 LITERATURE CITED

Hu, X. S., F. He, and S. P. Hubbell. 2006. Neutral theory in macroecology and population genetics. Oikos **113**:548-556.

Kimura, M. 1968. Evolutionary rate at the molecular level. Nature **217**:624-626.

MacArthur, R. H. and E. O. Wilson. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, New Jersey.

Quammen, D. 1996. The Song of the Dodo: Island Biogeography in an Age of Extinctions. Simon & Schuster, New York, NY.

Sax, D. F. and S. D. Gaines. 2011. The equilibrium theory of island biogeography. Chicago: University of Chicago Press.

Vellend, M. 2004. Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. Ecology **85**:3043-3055.

a)

b)

Figure 6.1. Students participating in the biodiversity activity. a) One student creating a "dispersal event" by tossing a bag of candy to another island community. b) Students recording species richness and allelic richness in their island community. I took the photos and own the copyrights.

Table 6.1 Important ecological and evolutionary terms for students to understand.

Term	Definition
Allele	A unique variant of a DNA sequence at a gene
Allelic richness	The number of alleles (genetic variants) in a population
Average allelic richness	The average number of alleles per species in a community
Community	The composition of species within the same geographic location
Connectivity	The relative proximity of an island to sources of immigrants. The opposite of isolation (greater isolation = lower connectivity)
Dispersal	For this activity, we are using the term 'dispersal' as a synonym for *immigration* – the movement of an individual into a population – and/or *gene flow* – the movement of genetic material from one population in to another. The definition of dispersal in other contexts is generally the movement of an individual from one population into another population.
Drift	In this activity, 'drift' refers to the dual process of losing a random individual from a community, as well as losing that individual's genes
Ecological drift	The stochastic (random) extinction of species from a community
Gene	The DNA sequence that codes for a protein
Gene flow	Movement of genetic material from one population to another, through dispersal followed by reproduction in the receiving population. In this activity, we refer to this process simply as *dispersal*.
Genetic drift	The stochastic loss of alleles (genetic variants) from a population
Immigration	The movement of an individual into a population
Island biogeography	A theory that attempts to describe the processes responsible for distributions of species richness across islands. This theory has since been applied to habitat islands, such as mountaintops, isolated wetlands, heads of coral, etc.
Neutral process	An ecological or evolutionary process that theoretically has the same directional effect on all species regardless of their ecological differences
Population	An interbreeding group of the same species, within the same geographic location
Species richness	The number of species in a community

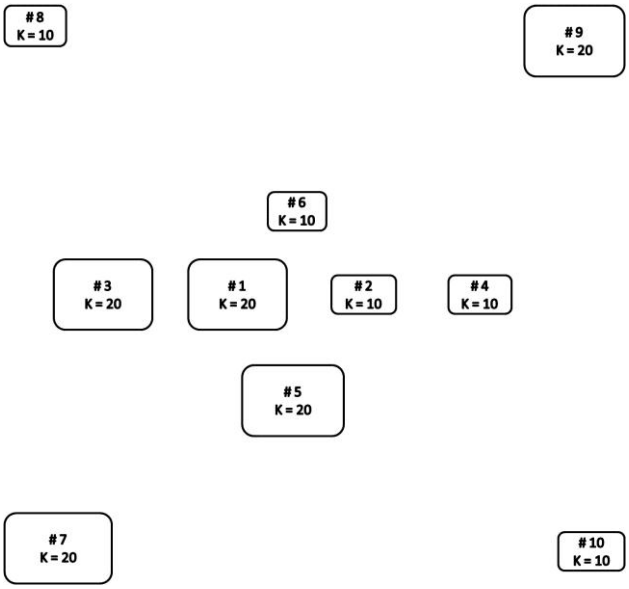


Figure 6.2. Layout of the islands, represented by rounded rectangles. The top number within each rectangle represents the island ID. The bottom number represents the carrying capacity (K) of the island, which is based on the island size and K is also the initial size of the community.

Table 6.2. Characteristics of islands in the activity. Each number corresponds with a specific island. Small islands have an initial community size of 10 individuals, whereas large islands have an initial community size of 20 individuals.

Island Size		Island Connectivity		
Large	Small	Well connected	Moderately connected	Isolated
1	2	1	3	7
3	4	2	4	8
5	6		5	9
7	8		6	10
9	10			

Round 1

Ants + Spiders + Bird + Tortoise → 4 Species → Species richness = 4

Round	Ant	Bear	Bird	Frog	Snake	Spider	Tortoise	Species richness
1	4		1			2	1	4

Figure 6.3. Calculating initial species richness and recording initial species richness in the *Species Richness Datasheet.*

Round 1

Species	Round	Red	Orange	Yellow	Green	Blue	Purple	Allelic richness
Ant	1	1	0	0	2	7	2	4
Bear	1	0	0	0	0	0	0	0
Bird	1	2	0	0	0	0	1	2
Frog	1	0	0	0	0	0	0	0
Snake	1	0	0	0	0	0	0	0
Spider	1	0	0	0	3	0	3	2
Tortoise	1	0	0	0	0	3	0	1

Round	Ant	Bear	Bird	Frog	Snake	Spider	Tortoise	Sum	# Species	Avg. Allelic Richness
1	4	0	2	0	0	2	1	9	9	=9/9=1.00

Figure 6.4. Calculating and recording initial allelic richness in the *Allelic Richness Datasheet* and the *Average Allelic Richness Datasheet*.

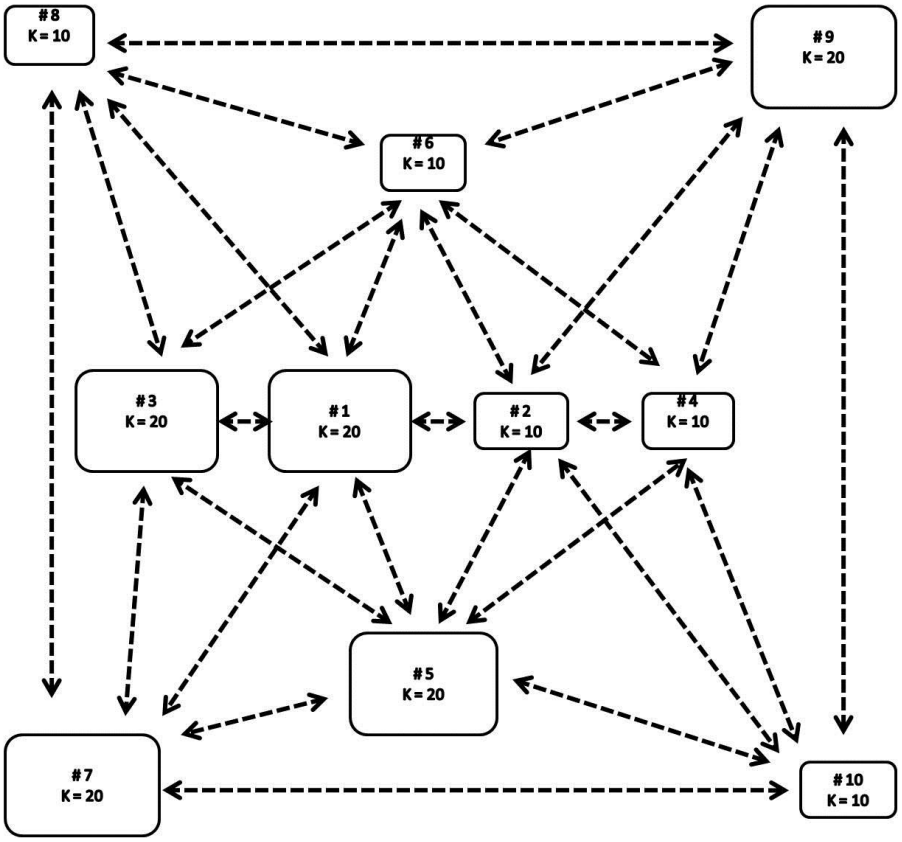
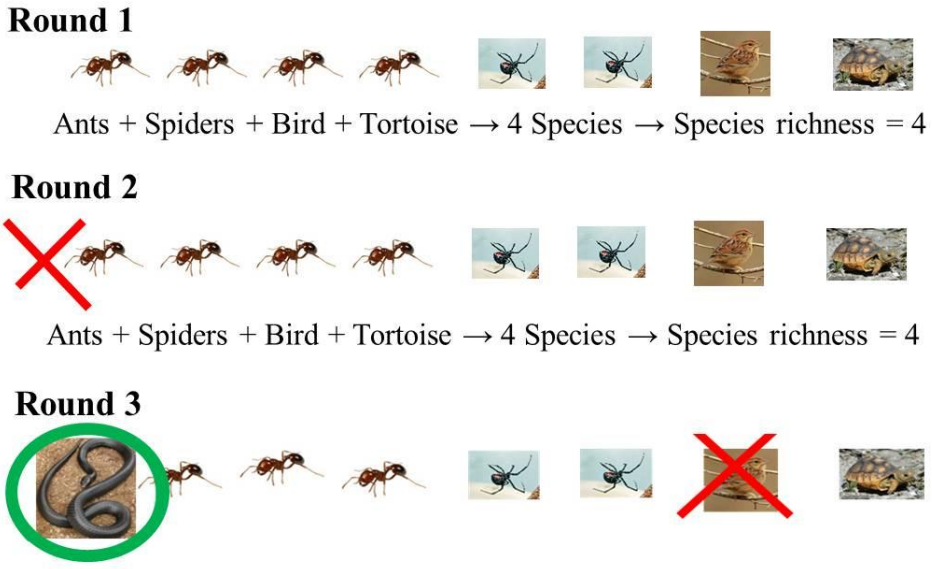
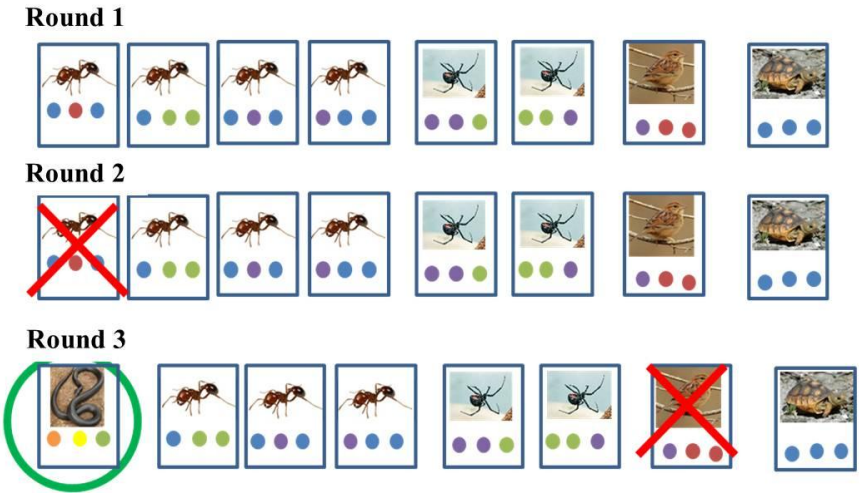


Figure 6.5. Acceptable dispersal routes.



Round	Ant	Bear	Bird	Frog	Snake	Spider	Tortoise	Species richness
1	4		1			2	1	4
2	-1 (3)		1			2	1	4
3	3		-1(0)		+1 (1)	2	1	4

Figure 6.6. Calculating species richness and recording species richness in the *Species Richness Datasheet*.



Species	Round	Red	Orange	Yellow	Green	Blue	Purple	Allelic richness
Ant	1	1	0	0	2	7	2	4
Bear	1	0	0	0	0	0	0	0
Bird	1	2	0	0	0	0	1	2
Frog	1	0	0	0	0	0	0	0
Snake	1	0	0	0	0	0	0	0
Spider	1	0	0	0	3	0	3	2
Tortoise	1	0	0	0	0	3	0	1
Ant	2	-1 (0)	0	0	2	-2 (5)	2	3
Bird	3	-2 (0)	0	0	0	0	-1 (0)	0
Snake	3	0	+1 (1)	+1 (1)	+1 (1)	0	0	3

Round	Ant	Bear	Bird	Frog	Snake	Spider	Tortoise	Sum	# Species	Avg. Allelic Richness
1	4	0	2	0	0	2	1	9	9	= 9/9 = 1.00
2	3	0	2	0	0	2	1	8	9	= 8/9 = 0.89
3	3	0	0	0	3	2	1	9	9	= 9/9 = 1.00

Figure 6.7. Calculating allelic richness and recording allelic richness in the *Allelic Richness Datasheet* and the *Average Allelic Richness Datasheet.*

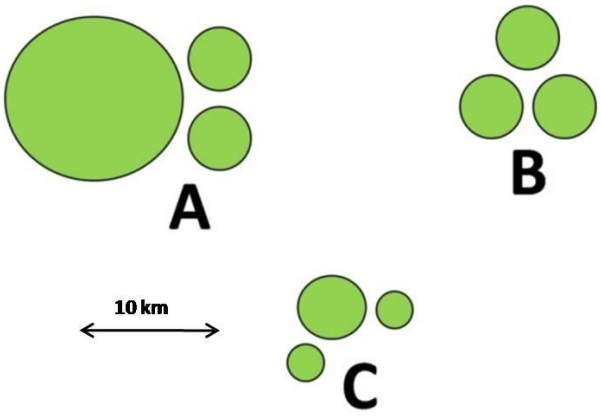
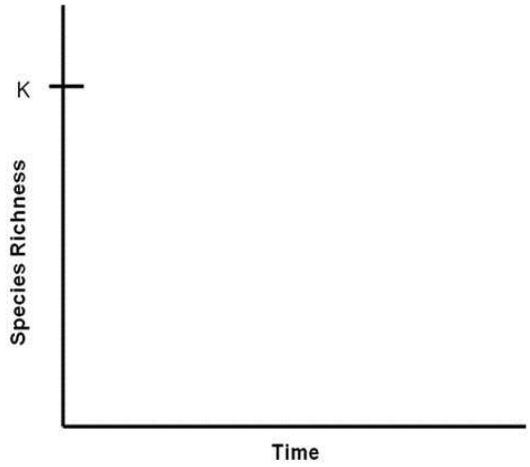
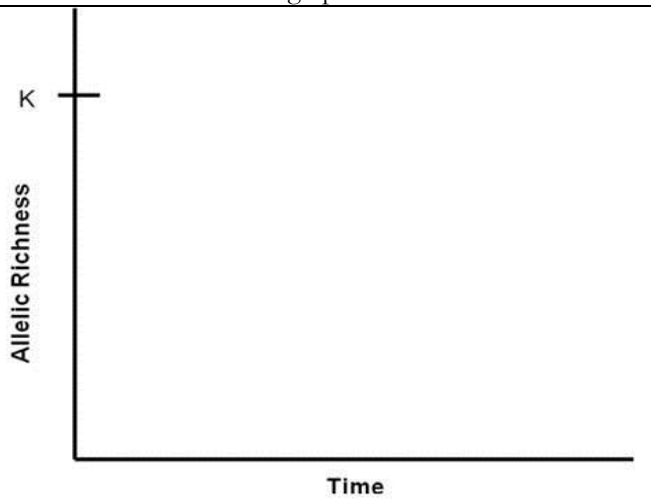


Figure 6.8. Potential island layouts for conservation.

Small Versus Large Islands	
Starting with two islands, a small and a large island, both at carrying capacity with the maximum number of species/individuals possible in the community/population:	
Species Richness	Allelic Richness
Predict how species richness will change over time on small versus large islands. Describe verbally and graphically.	Predict how allelic richness will change over time on small versus large islands. Describe verbally and graphically.
Written hypothesis: *Species richness will decline faster in the community on the small islands compared to large islands*	Written hypothesis: *Allelic richness will decline faster in the community on the small islands compared to large islands*
Graphical Hypothesis: Label both curves on the graph.	Graphical Hypothesis: Label both curves on the graph.
 Species richness by time graph	 Allelic richness by time graph

Isolated Versus Well-Connected Islands	
Starting with two islands of the same size, one in close proximity to another inhabited island and one very far from any other inhabited islands, both at carrying capacity with the maximum number of species/individuals possible in the community/population:	
Species Richness	Allelic Richness
Predict how species richness will change over time on the isolated versus well-connected island. Describe verbally and graphically.	Predict how allelic richness will change over time on the isolated versus well-connected island. Describe verbally and graphically.
Written hypothesis: *Species richness will decline faster on the isolated island*	Written hypothesis: *Allelic richness will decline faster on the isolated island than on*

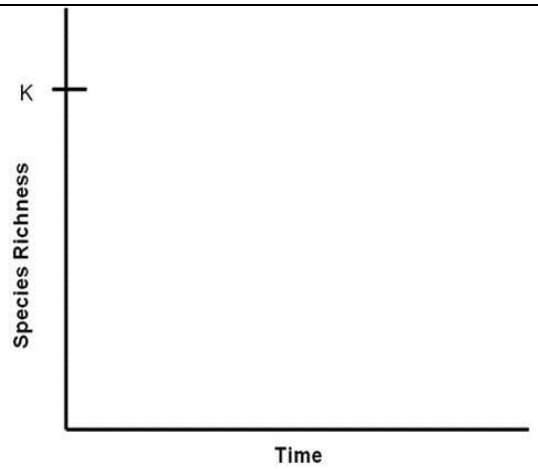
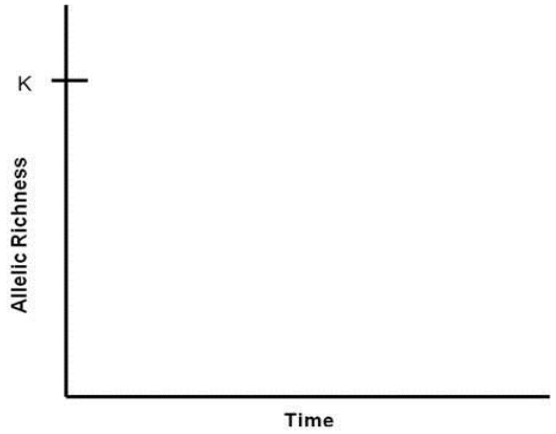
than on the well-connected island.	*the well-connected island.*
Graphical Hypothesis: Label both curves on the graph.	Graphical Hypothesis: Label both curves on the graph.
 Species richness by time graph	 Allelic richness by time graph

Figure 6.9. Student handout for the written and graphic hypotheses of the relationships of island size and connectivity versus species and genetic diversity over time.

Table 6.3. Sample Size by Control and Treatment Per Group (only includes undergraduates who gave consent for inclusion in the study and were present for both pre and post activity surveys).

Group	Control	Treatment
FANRA3200 Fall 2010	9	20
FANR3200 Spring 2011	27	20
WILD4550 Spring 2011	3	10

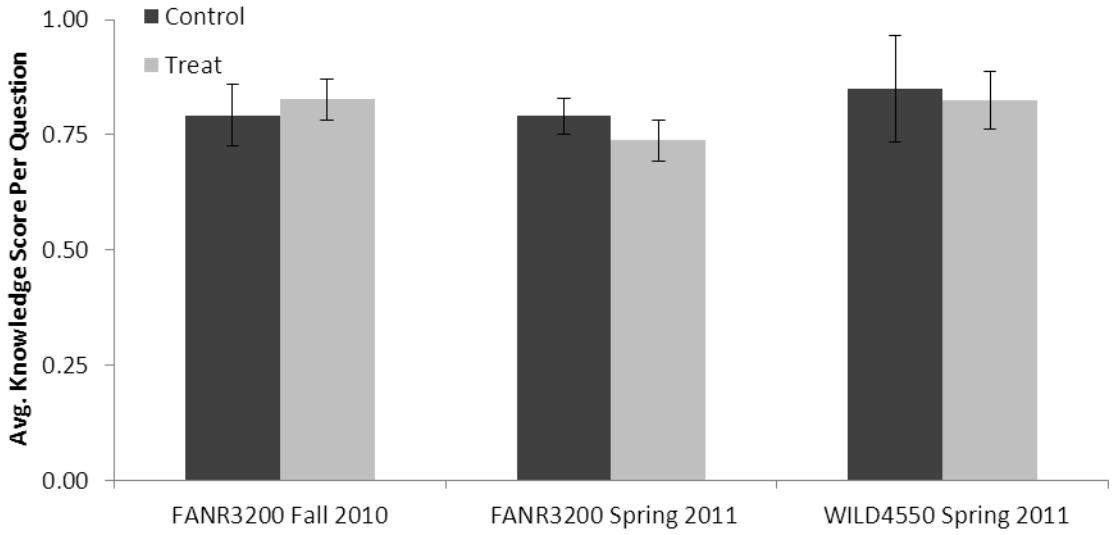
Table 6.4. Cronbach's Alphas (CA) for knowledge and confidence questions for constructs of the constituents of biodiversity and the effects of neutral factors on species and genetic diversity. ‡Indicates that Cronbach's Alpha was below 0.50.

Construct	Question #	/Question	Knowledge CA	Question Type	Confidence CA
Biodiversity (Abiotic)	#4	The variation in temperature over the winter is an example of biodiversity.	0.72	True/False	0.81
	#10	Variation in water chemistry across two streams is an example of biodiversity.		True/False	
	#13	Igneous, sedimentary, and metamorphic rock types are examples of biodiversity.		True/False	
Biodiversity (Ecosystem)	#8	Coral reef ecosystems are examples of biodiversity.	0.58	True/False	0.74
	#11	Longleaf pine and tidal marsh ecosystems in Georgia are examples of biodiversity.		True/False	
	#14	Rainforests, cloud forests, and dry pacific forests in Costa Rica are examples of biodiversity.		True/False	
Biodiversity (Genetic)	#2	A population of lizards, some of which have blue eyes and some of which have green eyes is an example of biodiversity.	0.84	True/False	0.84
	#6	A plant species that has diploid (2 copies of each chromosome) and triploid (3 copies of each chromosome) variants is an example of biodiversity.		True/False	
	#12	Different DNA sequences among three individuals from the same species are an example of biodiversity.		True/False	
Biodiversity (Species)‡	#1	The various coniferous trees in the Colorado Rockies are an example of biodiversity.	0.34	True/False	0.77
	#5	The composition of species that occur on the Hawaiian Islands is an example of biodiversity.		True/False	
	#9	A community of pond-breeding amphibians is an example of biodiversity.		True/False	
Dispersal (Genetic)‡	#17	Immigration into a population helps maintain genetic diversity in that population.	0.24	True/False	0.74
	#21	Genetic diversity within populations tends to _____ with a (n) _____ in immigration rates.		Multiple Choice	
	#24	Rank the following islands in terms of expected genetic diversity (most diverse to least diverse).		Multiple Choice	
Dispersal (Species)	#16	Immigration decreases species richness over time.	0.66	True/False	0.70
	#19	Species richness in communities without immigration will _____ over time.		Multiple Choice	
	#20	Immigration tends to have a _____ effect on the species richness of an island		Multiple Choice	
Drift (Genetic)	#18	Larger populations tend to have more genetic diversity than small populations.	0.54	True/False	0.76
	#23	Small populations lose genetic diversity _____ larger populations.		Multiple Choice	
	#29	Which population of Easter bunnies would you predict would lose genetic diversity faster?		Multiple Choice	
Drift (Species)	#15	Given the same number of species on two islands, the extinction rate of the larger islands will tend to be higher than the extinction rate on the smaller island.	0.50	Multiple Choice	0.81
	#22	Large islands will generally have _____ species and _____ extinction rates compared to small islands.		Multiple Choice	
	#25	Rank the following islands in terms of expected species richness (1 = most species, 3 = least species).		Multiple Choice	
Optimal Reserve Design	#26	Which of the following reserve designs would most likely maximize species and genetic diversity?	0.58	Multiple Choice	0.76
	#27	Which reserve design would maximize genetic diversity?		Multiple Choice	

Table 6.5. Principal Component Factor Analysis with Varimax Row Rotation for post-activity confidence and knowledge questions.

	Knowledge			Confidence		
Factor	Question #	Eigenvalue	Explained Variance	Question #	Eigenvalue	Explained Variance
1	16, 17, 20, 21	4.21	2.72	24, 25, 26, 27, 28	17.01	4.81
2	2, 6, 12	2.86	2.95	30, 31,32, 33, 34, 35, 36, 37	2.66	6.41
3	4, 10, 13	2.24	2.27	3, 7, 16	1.89	2.19
4	18, 23, 29	2.08	2.09	9, 18, 23, 25	1.60	4.17
5	1, 8, 9, 11, 14,	1.83	2.10	6, 10, 13, 14	1.35	4.14
6	25	1.53	1.87	5, 8, 17	1.11	2.97
7	22, 26, 27, 28	1.52	2.26	19, 22	1.08	2.00

a)



b)

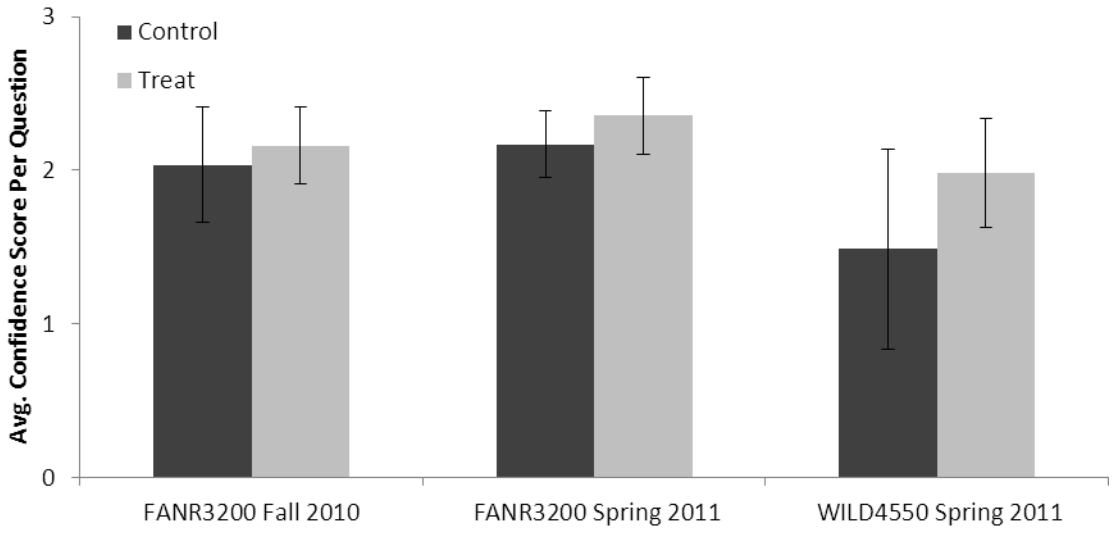
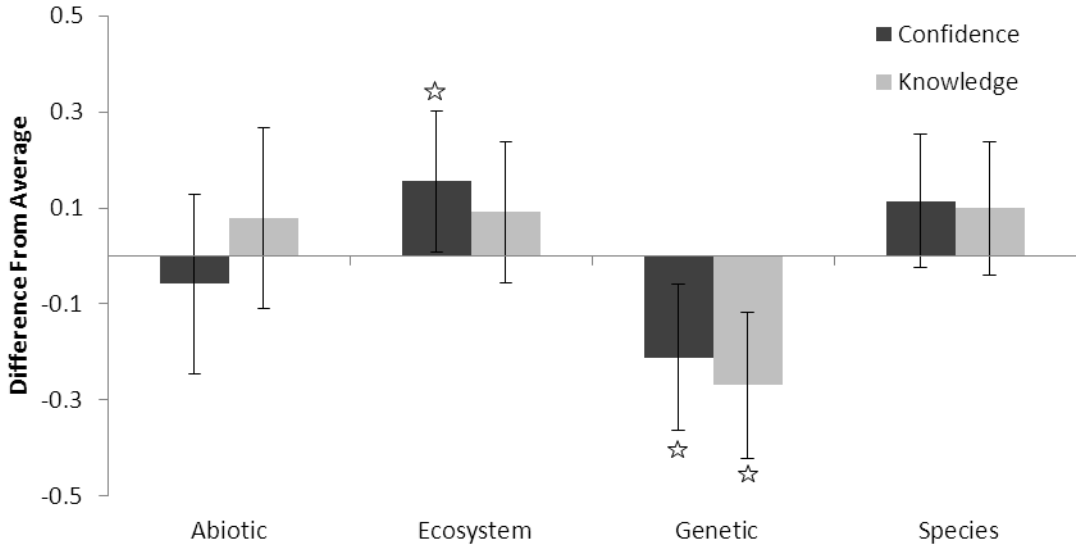


Figure 6.10. Pre-activity average a) knowledge and b) confidence score per question by class and treatment.

a)



b)

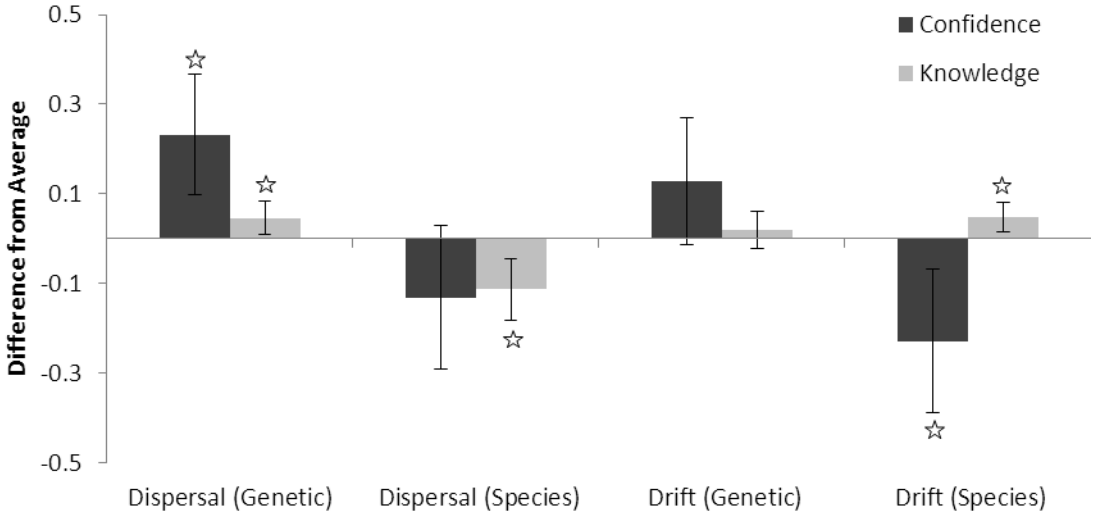
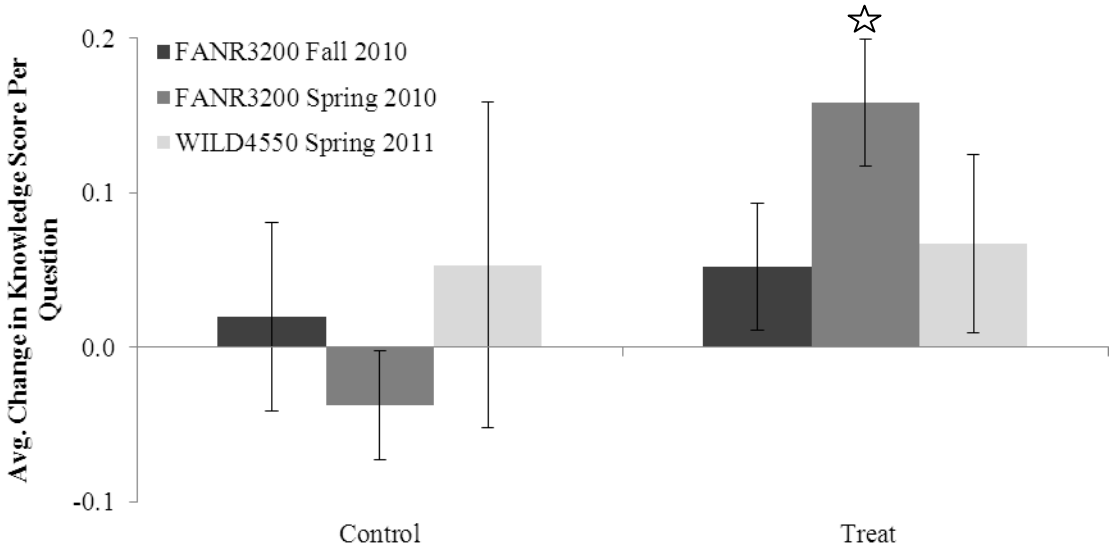


Figure 6.11. Pre-activity knowledge and confidence differences from the average scores for questions pertaining to a) the constituents of biodiversity and b) effects of dispersal and drift on genetic and species diversity. Bars represent 95% confidence intervals. Stars indicate a significant difference from the mean.

a)



b)

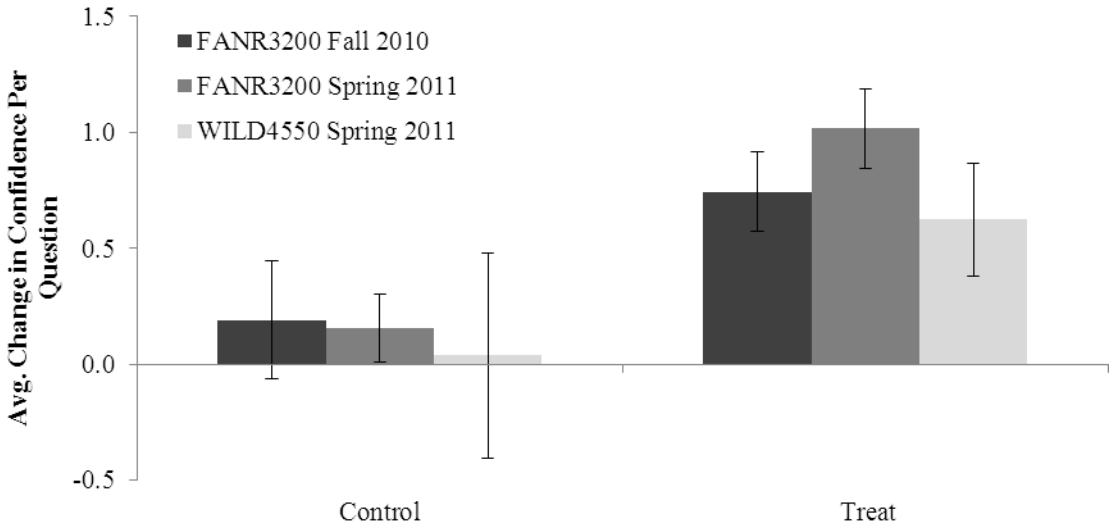


Figure 6.12. Average change in a) knowledge score per question and b) confidence score per question by class for control versus treatment groups. ANCOVA result of effect of group and treatment on post-activity scores, with pre-activity score as a covariate.

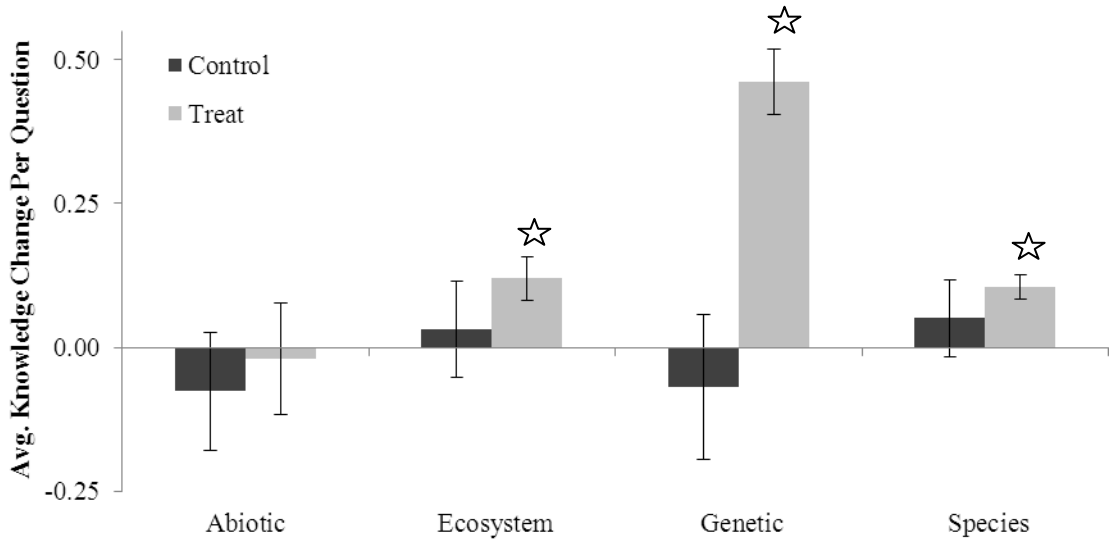
Table 6.6. ANCOVA results testing for the effect of the activity and the additional biodiversity lab component on per question knowledge scores, controlling for the effect of pre-activity scores and class.

Variable	SS	Degrees of freedom	MS	F	p
Intercept‡	0.31	1	0.31	35.94	0.00
Pre-activity Score‡	0.18	1	0.18	21.01	0.00
Class	0.01	2	0.01	0.71	0.50
Treatment‡	0.07	1	0.07	7.99	0.01
Class x Treatment‡	0.09	2	0.04	4.89	0.01
Error	0.71	82	0.01		

Table 6.7. ANCOVA results testing for the effect of the activity and the additional biodiversity lab component on per question confidence scores, controlling for the effect of pre-activity scores and class.

Variable	SS	Degrees of freedom	MS	F	p
Intercept‡	2.97	1	2.97	19.25	0.00
Pre-activity Confidence‡	4.90	1	4.90	31.79	0.00
Class	0.04	2	0.02	0.12	0.89
Treatment‡	3.35	1	3.35	21.73	0.00
Class x Treatment	0.56	2	0.28	1.81	0.17
Error	12.65	82	0.15		

a)



b)

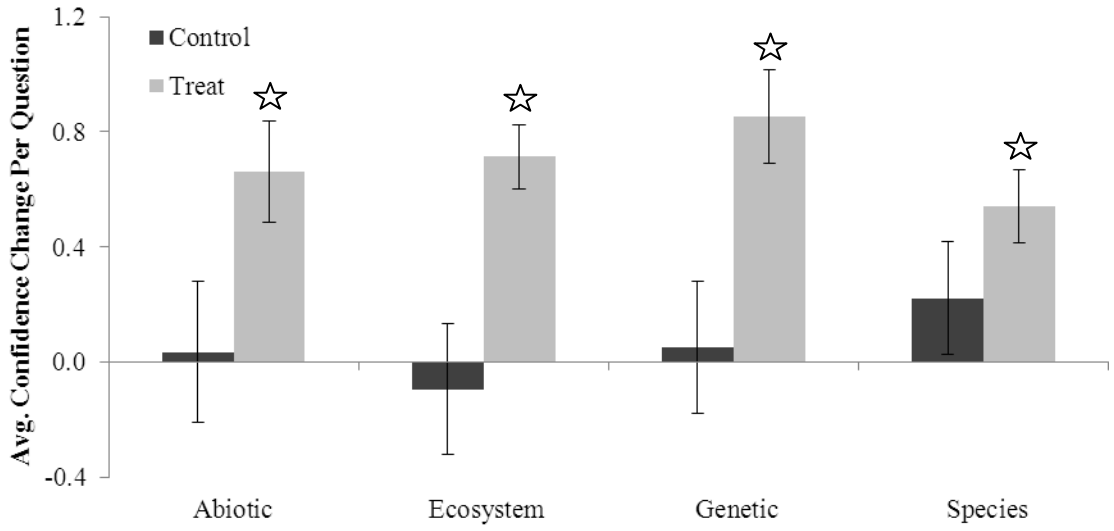
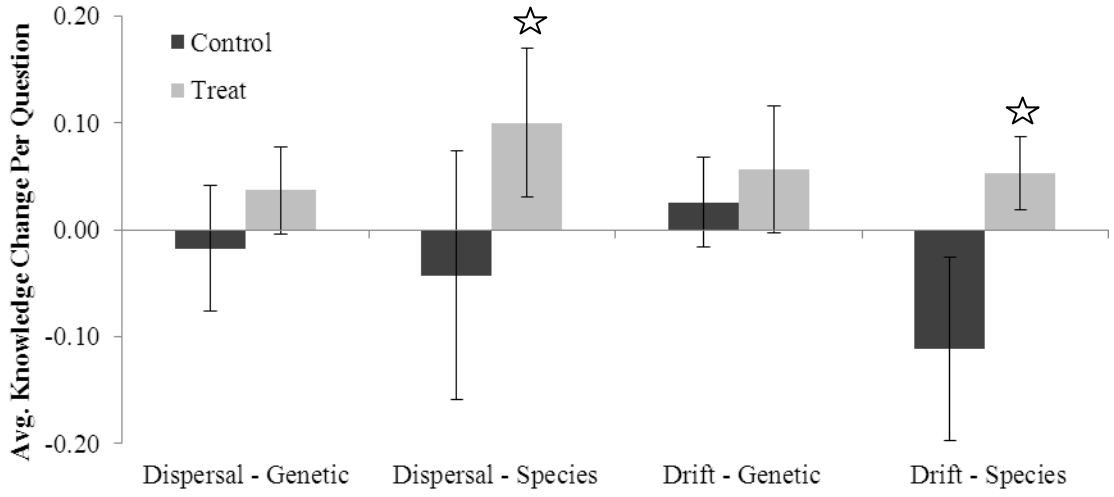


Figure 6.13. Average change in a) knowledge and b) confidence scores compared to pre-activity averages for survey questions regarding the different constituents of biodiversity. We included questions about abiotic factors in the survey to test if students could distinguish between biodiversity and abiotic factors. Bars represent 95% confidence intervals. Stars indicate significant differences between the control and treatment scores, at alpha = 0.05, after accounting for students' pre-activity confidence and knowledge scores.

a)



b)

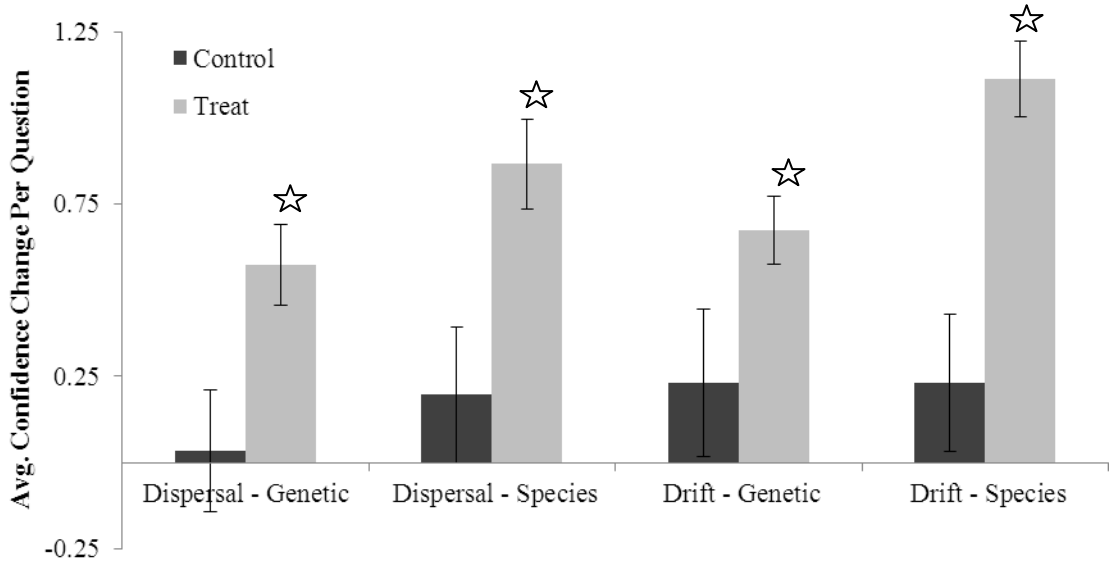


Figure 6.14. Average change in a) knowledge and b) confidence scores compared to pre-activity averages for survey questions regarding the effects of dispersal and drift on species and genetic diversity. Bars represent 95% confidence intervals. Stars indicate significant differences between the control and treatment scores, at alpha = 0.05, after accounting for students' pre-Activity confidence and knowledge scores.

Island #: 7 **Island size: Large** **Island Isolation: Isolated**

Species Richness Datasheet

Round	American Shad	Bluegill	Carp	Flathead Catfish	Hybrid Bass	Redfin Pickerel	Striped Bass	Walleye	White Bass	Yellow Perch	Species Rich
0	1	3	2	3	4	1	1	3		2	
1	1	3	2	3	4	1	1	-1 (2)		2	
2	1	3	-1 (1)	3+1(4)	4	1	1	2		2	
3	1	3	1	4	-1 (3)	1	1	2		2	
4	1	3	1	4	3	1	1	2		2	
5	1	-1 (2)	1	4	3	1	1	2		2	
6	1	2	1	4	3	1	1	-1 (1)		2	
7	-1 (0)	2	1	4	3	1	1	1		2	
8	0	2	1	4	3	1	1	1		-1 (1)	
9	0	2	1	-1 (3)	3	1	1	1		1	
10	0	2	1	3	3	1	1	1		-1 (0)	
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											

Figure 6.15. Student completed species richness datasheet.

Island #: 7 **Island size: Large** **Island Isolation: Isolated**

Allelic Richness Calculation Sheet

Species	Round	Ornge star brst	Pnk star brst	Red star brst	Ylw star brst	Apple JR	Blue Rspbry JR	Cherry JR	Grape JR	Wtrmln JR	Allelic Rich
Bluegill	0	1		3		2		2			4
Striped Bass	0		2				2				2
Flathead Catfish	0	1	1	2				3		1	5
Bluegill	1	-1		-1		-1		-1			
Carp	1	+2				+1		+1			
American Shad	**0**	1		1						2	
Bluegill	**0**	3	1	2			1	2		3	
Carp	**0**	1	1		2	1		1		2	
Flathead Catfish	**0**		3	2	1	1		2	2	1	
Hybrid Bass	**0**	2		4	2	1	2	2	2	1	
Redfin Pickerel	**0**	1		1					1	1	
Striped Bass	**0**			1	1		1		1		
Walleye	**0**	3			3	2	1	3			
Yellow Perch	0	1	3			1	1	1		1	
Walleye	1	-1			-1		-1	-1			
Carp	2	-1	-1		[illegible]			-1		-1	
Flathead Catfish	2		+1		+1			+1	+1		
Hybrid Bass	3	-1		-1		-1		-1			
Flathead Catfish	4			-1	-1			-2			
Flathead Catfish	4	+1		[illegible]	+1	+1		+1			
Bluegill	5	-1	-1							-2	
Walleye	6				-2	-1		-1			
American Shad	7	-1		-1						-2	
Yellow Perch	8	-1		-1				-1		-1	

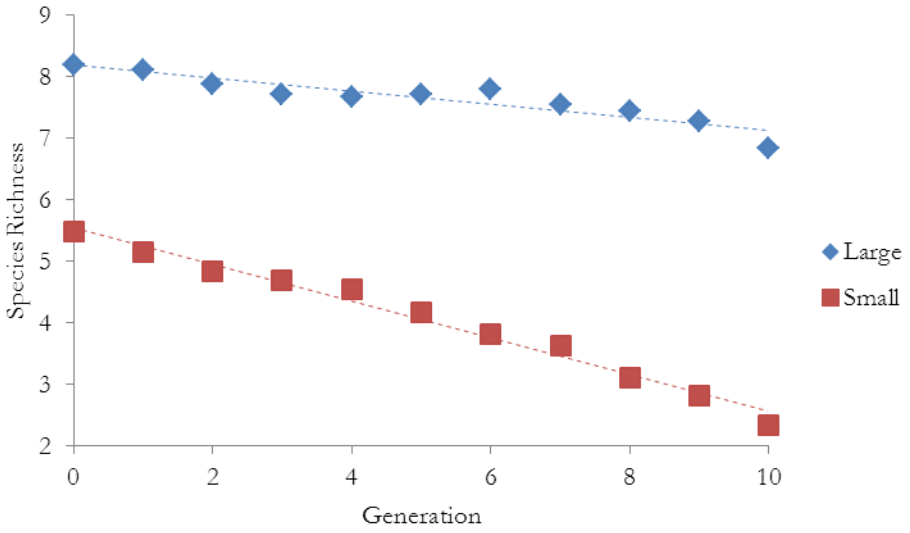
Island #_____ **Island size_____** **Island Isolation_____**

Allelic Richness Calculation Sheet

Species	Round	Ornge star brst	Pnk star brst	Red star brst	Ylw star brst	Apple JR	Blue Rspbry JR	Cherry JR	Grape JR	Wtrmln JR	Allelic Rich
Flathead Catfish	9		-1		-1			-1	-1		
Yellow Perch	10			-2		-1	-1				

Figure 6.16. Student completed allelic richness datasheets.

a)



b)

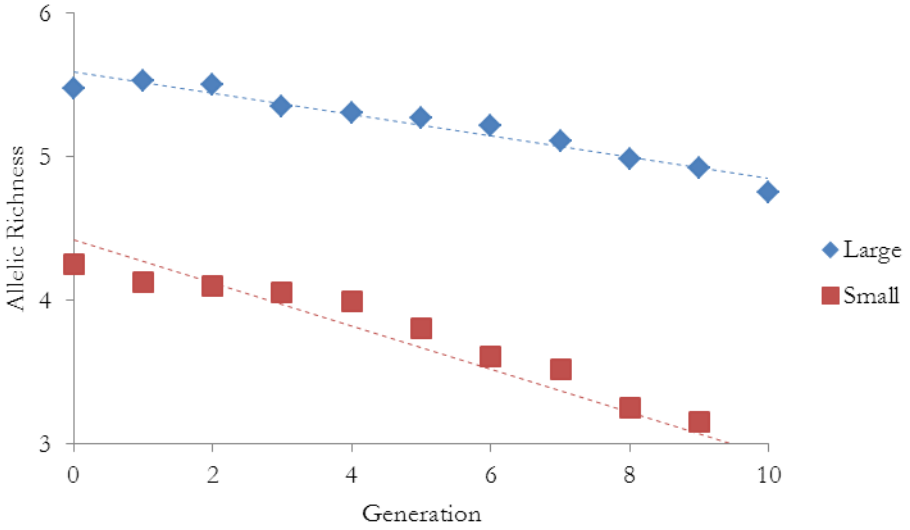
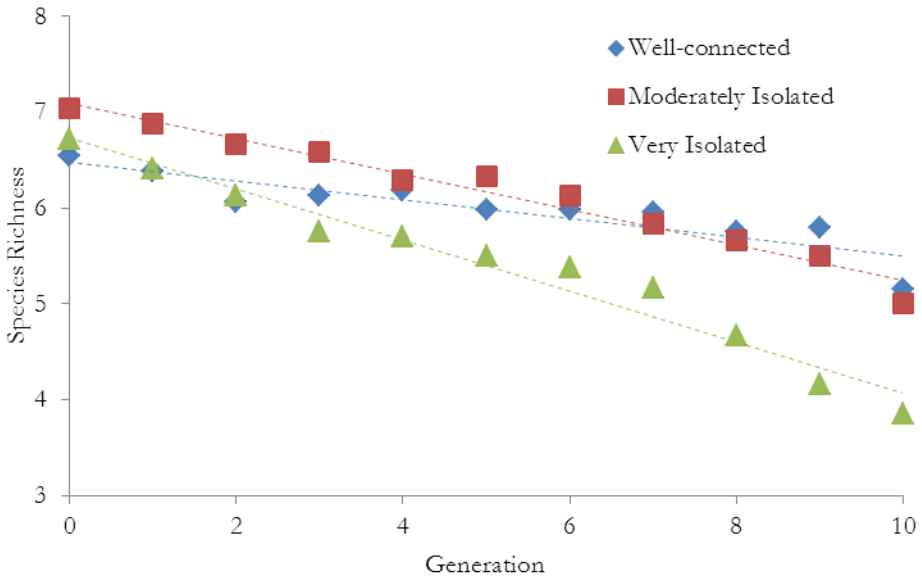


Figure 6.17. Results from the biodiversity activity in terms of a) species and b) allelic richness over time on islands of differing sizes.

a)



b)

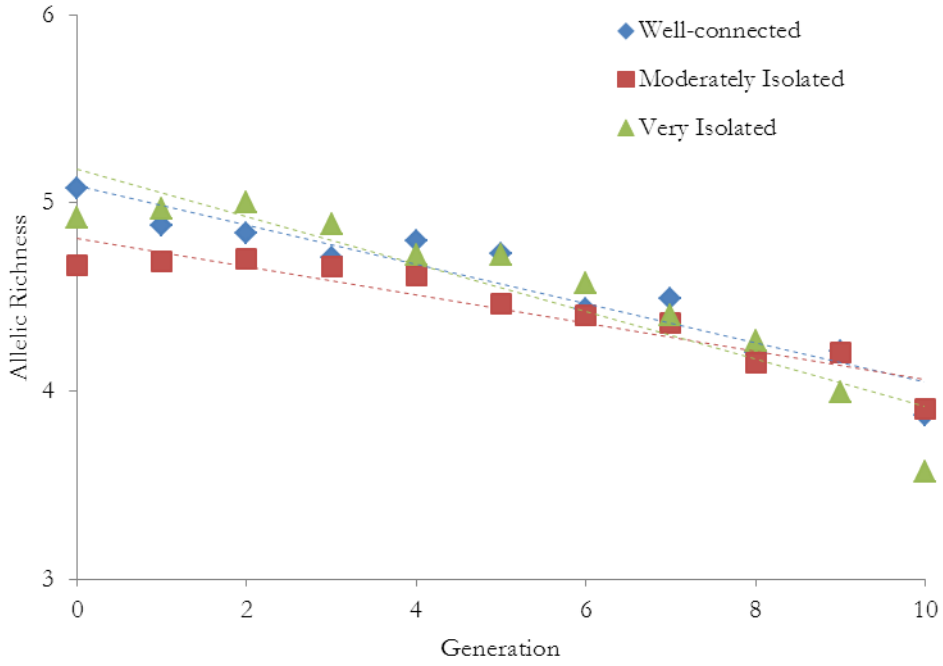


Figure 6.18. Results from the biodiversity activity in terms of a) species and b) allelic over time on islands with differing degrees of isolation.

2. Our hypothesis was that over time, species richness would decrease linearly in both the small and large islands. In our experiment, we did see this happen but not on such a steep scale. Our hypothesis was over much more than 10 generations—which is how long we continued our experiment—which means that eventually our experimental population will reach our hypothesized levels. As we take species out of our population and "throw" them into new islands, we lose species richness, and say if we are a small island where it is very hard for species to make it to, then you are losing species and not gaining any to replace it, thus the species richness for the smaller island drops at a higher rate than the larger island.

3. Our hypothesis in regards to allelic richness stated that the larger islands (or ponds) would have a higher allelic richness initially and that they would lose alleles from their population slower than the small islands. This hypothesis was supported by our experimental data. As the generations go on, the allelic richness of the small islands drops steadily over time, whereas the allelic richness of the large population falls slightly and then remains rather constant. This goes back to the explanation from question 2. When the small island loses a species, it loses those alleles from the population, and then it is harder for a small island to receive more or new species, thus they are giving away alleles from their population and are not receiving any new ones to take their places.

4. The effect of island size on both allelic richness and species richness is profound. Thhe smaller islands start out with fewer species and thus fewer alleles in their population. When the small island loses a species, their species richness decreases, and that lost species also means that those alleles are gone from the gene pool as well, decreasing the islands allelic richness also. Because the larger island starts out with more species and thus more alleles, it can stand to go a few generations without any new species coming into the population, but the small islands cannot. Also, the small islands are hard for new species to get to, thus they give away species and alleles and they get none in return because nothing can get to their island.

5. When looking at the species richness over time, one has to take into account the condition and location of the study area. For example, we hypothesized that both the isolated and the well connected islands would start out with the same level of species richness(max # species) and that after 10 generations, the isolated island would peter out first, coming to rest at a species richness of zero after only ~7 generations, whereas the well connected island makes it to a specie richness of zero on the 10^{th} generation. We see a similar trend in our experimental data however it is a bit different. None of our experimental data actually reached a species richness of zero, and they did not all start at the exact same species richness level. The overall theme is the same however, the most well connected islands lose species and gain species rapidly, thus their species richness is fairly constant, the low connectivity islands lose species rapidly and do not seem to gain any new species to fill the void, thus they have quickly declining species richness.

6. The allelic richness of isolated islands versus non-isolated islands is not quite what we had hypothesized. We guessed that isolated islands would have fewer immigrants and thus fewer alleles introduced. We also stated that the connected islands would have more immigrants and thus would have more alleles introduced to the population. Somehow in our experiment the islands with the lowest isolation ended up with a lower allelic richness than the highly isolated islands. This could have been due to human error in calculations, or maybe all of the species in the connected island have very similar alleles, thus they are not changing much because they are all the same (there is an unnaturally high amount of the same allele in the population).

7. Island isolation plays a very large role in species richness as well as allelic richness. Think about it, if your island is cut off from 8 of the 10 other islands, that doesn't give you much of a gene-pool to be choosing from when it comes to new species entering the population. In an isolated population, it is very hard for other species to get onto your island and it is also hard for you to get off of the island, thus the allelic richness is low as is the species richness. In the case of well connected islands, the opposite effect is noted. A well connected island means that species can leave the island and enter the island easily, meaning that species richness is high along with a high allelic richness.

Figure 6.19. Example of one student's response to the questions: 2) Compare your hypotheses to the class results for how species richness changed over time on small versus large islands. If the results were different than what you expected, explain (and justify) whether you think this was a matter of sampling error or whether you think your hypotheses were incorrect. 3) Compare your hypotheses to the class results for how allelic richness changed over time on small versus large islands. If the results were different than what you expected, explain (and justify) whether you think this was a matter of sampling error or whether you think your hypotheses were incorrect. 4) Based on your hypotheses and/or the class results from the activity, what do you think the relationship is between the effect of island size on species richness and the effect of island size on allelic richness? 5) Compare your hypotheses to the class results for how species richness changed over time on isolated versus well-connected islands. If the results were different than what you expected, explain (and justify) whether you think this was a matter of sampling error or whether you think your hypotheses were incorrect. 6) Compare your hypotheses to the class results for how allelic richness changed over time on isolated versus well-connected islands. If the results were different than what you expected, explain (and justify) whether you think this was a matter of sampling error or whether you think your hypotheses were incorrect. 7) Based on your hypotheses and/or the class results from the activity, what do you think the relationship is between the effect of island isolation on species richness and the effect of island isolation on allelic richness?

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1 INTRODUCTION

As mentioned throughout this dissertation, genetic diversity is essential for long-term population persistence . However, resources for conservation are limited and are often prioritized for protecting species diversity. Many pond-breeding amphibians exist as metapopulations (Sinsch 1990, Smith and Green 2005), which rely on occasional dispersal events for rescuing populations from extinction, or recolonizing habitats where populations have gone extinct (Lande 1988, Semlitsch and Bodie 1998, Hanski 1999, Rowe et al. 1999, Semlitsch 2002, Cushman 2006). Therefore, conservation efforts meant to protect pond-breeding amphibian species diversity by maximizing connectivity among communities should also help protect genetic diversity within populations of these communities.

The results from my dissertation suggest that upland habitats, which serve as dispersal habitat for many species at my study sites, surrounding breeding sites should be protected, that maintaining wetlands within dispersal distances of breeding sites may help to facilitate gene flow in amphibian species with limited vagility, and that roads, paved and dirt, appear to be barriers to dispersal regardless of vagility and should be minimized within dispersal distance of amphibian breeding sites. These results are similar to those from previous studies that have investigated the relationships between habitat features and species or genetic diversity in amphibians (for review see Cushman 2006). However, my dissertation research contributes novel information in that it is the first study which has essentially investigated which habitat features are simultaneously associated with amphibian species and genetic diversity.

Additionally, my dissertation has contributed important results for empirical tests of the theoretical correlation between species and genetic diversity. My results suggest that positive correlations occur between species and genetic diversity when genetic diversity is measured in species with limited vagility and more restricted habitats compared to species with greater vagility and more general habitat requirements. Struebig

et al (2011) found similar results in bat species in tropical forest fragments. While more studies are obviously needed to make any strong statements regarding the relationship between species diversity and genetic diversity in species with limited vagility and restricted habitats, similar results between my research and the Struebig et al (2011) study do provide compelling reasons to conduct more studies to better understand how vagility and habitat restrictions affects relationships between species diversity and genetic diversity.

7.2 Conservation of focal genetic species

Although I have purposefully minimized any emphasis on global amphibian declines in my dissertation, given the extent of the crisis (Stuart et al. 2004) and the likelihood that further declines will continue, the implications of this research for the conservation the focal genetic species should not be ignored. A number of hypotheses of the underlying causes of amphibian declines have been proposed (Collins and Storfer 2003), including habitat loss (Hecnar 1997, Achard et al. 2002), diseases, and climate change (Rohr et al. 2008). As genetic diversity is essential for populations' abilities to adapt to changing environmental conditions and important for minimizing the susceptibility of populations' to diseases, long term conservation plans must make genetic diversity protection a priority. The results from my dissertation suggest that genetic diversity of dwarf salamanders, my species with the characteristics (limited vagility, more habitat restrictions, and a smaller habitat range) that make it more susceptible to population declines than the southern leopard frog, is most strongly associated with wetlands, which are important for both breeding and non-breeding habitat for this species, within 2.5km. While more studies on species with similar characteristics are necessary to make any generalizations, these results suggest that conservation efforts for protecting genetic diversity in dwarf salamanders should focus on maximizing connectivity among wetlands, as they are important for all stages of the dwarf salamander life cycle.

7.3 Study Limitations

The climate conditions in 2006 and 2008, the year that the amphibian community surveys were conducted and the first year that amphibian tissue samples were collected, were relatively extreme (rainfall

was 11.2 cm and 7.7 cm, respectively, below average; www.GeorgiaWeather.net). This indicates that results of a similar study, even at the same location, may vary substantially depending on the climate conditions. Dwarf salamanders have been documented at wetlands besides those included as dwarf salamander sites in my research. Some of the wetlands where dwarf salamanders have been found in the past include cypress savannas and marshes, which tend to have shorter hydroperiods than cypress gum swamps. Given that I was unable to find them at cypress savannas or marshes over a relatively dry spring and summer may have been because of the stochastic nature of metapopulation, or alternatively, the wetlands where I was able to collect dwarf salamanders may have been able to support the populations because of their longer hydroperiods (Kirkman et al. 2000, Subalusky et al. 2009, Kirkman et al. 2012), making them potential source populations. Had dwarf salamander populations from cypress savannas and(or) marshes been included in my study, the results from my analyses may have been different as wetland type is known to influence amphibian species assemblages (Liner et al. 2008) and may affect genetic diversity in dwarf salamander populations.

Results from the southern leopard frog habitat modeling suggested a weak association with the landscape, and a side project on population genetic structure in southern leopard frogs suggested that the scale of my study may not actually have fully encompassed the spatial extent of a metapopulation of southern leopard frogs. Therefore, a larger tract of longleaf pine may be necessary to determine which land cover features facilitate versus inhibit dispersal in the highly vagile species. However, in terms of area, Ichauway is one of the largest remaining tracts of reference quality longleaf pine. This suggests that if Ichauway is not large enough to encompass a southern frog metapopulation, then determining the scale at which southern leopard frogs do exhibit metapopulation structure in longleaf pine forests may not be relevant to management.

7.4 Future research avenues

One obvious avenue for future research includes continuing this study at Ichauway over years with a range of precipitation levels, and adding populations of dwarf salamanders from other wetland types. Additionally, given that my research was only conducted on one tract of longleaf pine habitat, I am limited in

my ability to make inferences to other tracts. Therefore, repeating this study at other longleaf pine reserves, such as those at Fort Stewart and Fort Benning, would help determine whether the results from my research are robust across longleaf pine reserves or if correlations between and habitat predictors of amphibian species and genetic diversity are location dependent.

A related research avenue would be the incorporation of next generation sequencing technology into the aforementioned studies. As next-generation, high–throughput sequencing becomes less expensive and more accessible, the potential ecological and evolutionary questions that can be investigated increases exponentially. For example, I measured genetic diversity at approximately 11-12 neutral microsatellite loci in southern leopard frogs and dwarf salamanders as surrogates for genome-wide genetic diversity, which also includes genetic diversity at markers under selection. As more information about gene sequences and function become available for more species, research projects such as mine, will be able to measure genetic diversity at more loci, as well as loci that are ecologically relevant.

One recent advancement that I am particularly excited about incorporating into my research is the use of environmental DNA (eDNA) for species and allelic inventories. While this technique has been used for bacteria in soil and seawater (Valentini et al. 2009) for several years (Oline 2006, Herrera et al. 2007), only recently has this technique been used to detect vertebrate species in aquatic systems, which was a major breakthroughs in amphibian monitoring (Goldberg et al. 2011). Not only does eDNA provide the ability to detect amphibians at the species level, but as next-generation sequencing becomes cheaper and more accessible, measuring genetic diversity at the community scale in pond-breeding amphibians may also soon be possible (Thomsen et al. 2012). [11]

[11] A sidenote, I will be assisting Todd Pierson, an undergraduate student, with his research developing eDNA to detect *Urspelerpes* in streams.

7.5 LITERATURE CITED

Achard, F., H. D. Eva, H. J. Stibig, P. Mayaux, J. Gallego, T. Richards, and J. P. Malingreau. 2002. Determination of deforestation rates of the world's humid tropical forests. Science **297**:999.

Collins, J. P. and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions **9**:89-98.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological Conservation **128**:231-240.

Goldberg, C. S., D. S. Pilliod, R. S. Arkle, and L. P. Waits. 2011. Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders. PLoS One **6**:e22746.

Hanski, I. 1999. Metapopulation Ecology. Oxford University Press, USA.

Hecnar, S. 1997. Amphibian pond communities in southwestern Ontario. Society for the Study of Amphibian and Reptiles, St. Louis, MO.

Herrera, A., M. Héry, J. E. M. Stach, T. Jaffré, P. Normand, and E. Navarro. 2007. Species richness and phylogenetic diversity comparisons of soil microbial communities affected by nickel-mining and revegetation efforts in New Caledonia. European journal of soil biology **43**:130-139.

Kirkman, L. K., P. C. Goebel, L. West, M. B. Drew, and B. J. Palik. 2000. Depressional wetland vegetation types: a question of plant community development. Wetlands **20**:373-385.

Kirkman, L. K., L. L. Smith, P. F. Quintana-Ascencio, M. J. Kaeser, S. W. Golladay, and A. L. Farmer. 2012. Is species richness congruent among taxa? Surrogacy, complementarity, and environmental correlates among three disparate taxa in geographically isolated wetlands. Ecological Indicators **18**:131-139.

Lande, R. 1988. Genetics and demography in biological conservation. Science **241**:1455.

Liner, A. E., L. L. Smith, S. W. Golladay, S. B. Castleberry, and J. W. Gibbons. 2008. Amphibian Distributions within Three Types of Isolated Wetlands in Southwest Georgia. The American Midland Naturalist **160**:69-81.

Oline, D. K. 2006. Phylogenetic comparisons of bacterial communities from serpentine and nonserpentine soils. Applied and environmental microbiology **72**:6965-6971.

Rohr, J. R., T. R. Raffel, J. M. Romansic, H. McCallum, and P. J. Hudson. 2008. Evaluating the links between climate, disease spread, and amphibian declines. Proceedings of the National Academy of Sciences **105**:17436.

Rowe, G., T. Beebee, and T. Burke. 1999. Microsatellite heterozygosity, fitness and demography in natterjack toads Bufo calamita. Animal Conservation **2**:85-92.

Semlitsch, R. D. 2002. Critical Elements for Biologically Based Recovery Plans of Aquatic Breeding Amphibians. Conservation Biology **16**:619-629.

Semlitsch, R. D. and J. R. Bodie. 1998. Are small, isolated wetlands expendable? Conservation Biology **12**:1129-1133.

Sinsch, U. 1990. Migration and orientation in anuran amphibians. Ethology Ecology & Evolution **2**:65-79.

Smith, M. A. and D. M. Green. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography **28**:110-128.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science **306**:1783.

Subalusky, A. L., L. A. Fitzgerald, and L. L. Smith. 2009. Ontogenetic niche shifts in the American Alligator establish functional connectivity between aquatic systems. Biological Conservation **142**:1507-1514.

Thomsen, P., J. Kielgast, L. L. Iversen, C. Wiuf, M. Rasmussen, M. T. P. Gilbert, L. Orlando, and E. Willerslev. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology **21**:2565-2573.

Valentini, A., F. Pompanon, and P. Taberlet. 2009. DNA barcoding for ecologists. Trends in Ecology & Evolution **24**:110-117.

APPENDIX A.

Top models of allelic richness (r_g) and observed heterozygosity (H_o) for the dwarf salamander (*Eurycea quadridigitata*) and the southern leopard frog (*Lithobates sphenocephalus*) when the outlier site, Psk, was included. Condition number (CN) is the degree of multicollinearity in the model, when CN < 2, multicollinearity is not an issue in the model. AICc Wi is the model weight relative to all other models tested for the same number of populations at the same spatial scale. * Indicates the top model for a given parameter and number of populations. ‡ Indicates the 95% confidence interval of the variable does not cross 0.

Parameter/ Scale	# Pops	Variable	Coeff.	SE	t	95% CI	r^2	CN	AICc	AICc Wi
Dwarf Salamander										
r_g										
local	9	Constant‡	4.08	0.62	6.53	2.85 - 5.30	0.66	1.00	23.72	0.737
		ISO‡	-0.35	0.10	-3.69	-0.54 - -0.17				
0.5km	9	Constant ‡	3.23	0.86	3.75	1.54 - 4.92	0.65	1.00	23.92	0.475
		WTLND‡	8.61	2.38	3.62	3.95 - 13.27				
1km	9	Constant ‡	3.59	0.51	7.00	2.58 - 4.59	0.81	1.00	18.50	0.728
		WTLND‡	9.06	1.66	5.45	5.80 - 12.31				
2.5km	9*	Constant ‡	3.36	0.50	6.77	2.38 - 4.33	0.84	1.00	16.89	0.866
		WTLND‡	10.74	1.77	6.08	7.28 - 14.20				
H_o										
local	9	Constant‡	0.57	0.03	20.04	0.52 - 0.63	0.03	1.00	-31.82	0.332
		ISO	<-0.01	<0.01	-0.47	-0.01 - 0.01				
500m	9*	Constant ‡	0.54	0.04	15.45	0.47 - 0.60	0.23	1.00	-33.87	0.429
		WTLND	0.14	0.10	1.44	-0.05 - 0.33				
1km	9	Constant ‡	0.55	0.03	18.88	0.50 - 0.61	0.15	1.00	-32.95	0.311
		WTLND	0.10	0.10	1.09	-0.08 - 0.29				
2.5km	9	Constant ‡	0.56	0.03	17.42	0.50 - 0.62	0.09	1.00	-32.40	0.288
		WTLND	0.10	0.11	0.84	-0.13 - 0.32				
Southern Leopard Frog										
r_g										
local	10	Constant‡	6.12	1.37	4.47	3.44 - 8.81	0.35	1.00	45.59	0.354
		ISO‡	-0.32	0.15	-2.08	-0.62 - -0.02				
500m	10	Constant‡	10.32	1.06	9.74	8.25 - 12.40	0.27	1.00	46.85	0.387
		AG	-3.68	2.17	-1.70	-7.92 - 0.57				
1km	10	Constant	2.63	3.15	0.84	-3.54 - 8.81	0.33	1.00	45.95	0.342
		FOREST‡	7.10	3.59	1.98	0.06 - 14.13				
2.5km	10*	Constant‡	18.49	5.32	3.48	8.07 - 28.90	0.83	1.67	38.10	0.671
		DEVEL‡	-72.44	24.89	-2.91	-121.21 - -23.66				
		WTLND‡	25.36	4.31	5.88	16.91 - 33.81				
H_o										
local	10	Constant‡	0.72	0.01	75.55	0.71 - 0.74	0.12	1.00	-33.78	0.42

		AREA	0.01	0.01	1.04	-0.01 - 0.03				
500m	10*	Constant‡	0.76	0.02	31.44	0.72 - 0.81	0.27	1.00	-35.58	0.373
		DEVEL	-0.16	0.10	-1.70	-0.35 - 0.03				
1km	10	Constant‡	0.64	0.05	11.77	0.53 - 0.75	0.24	1.00	-35.29	0.358
		FOREST	0.10	0.06	1.60	-0.02 - 0.22				
2.5km	10	Constant‡	0.79	0.04	18.08	0.71 - 0.88	0.23	1.00	-35.18	0.23
		AG	-0.12	0.08	-1.57	-0.26 - 0.03				

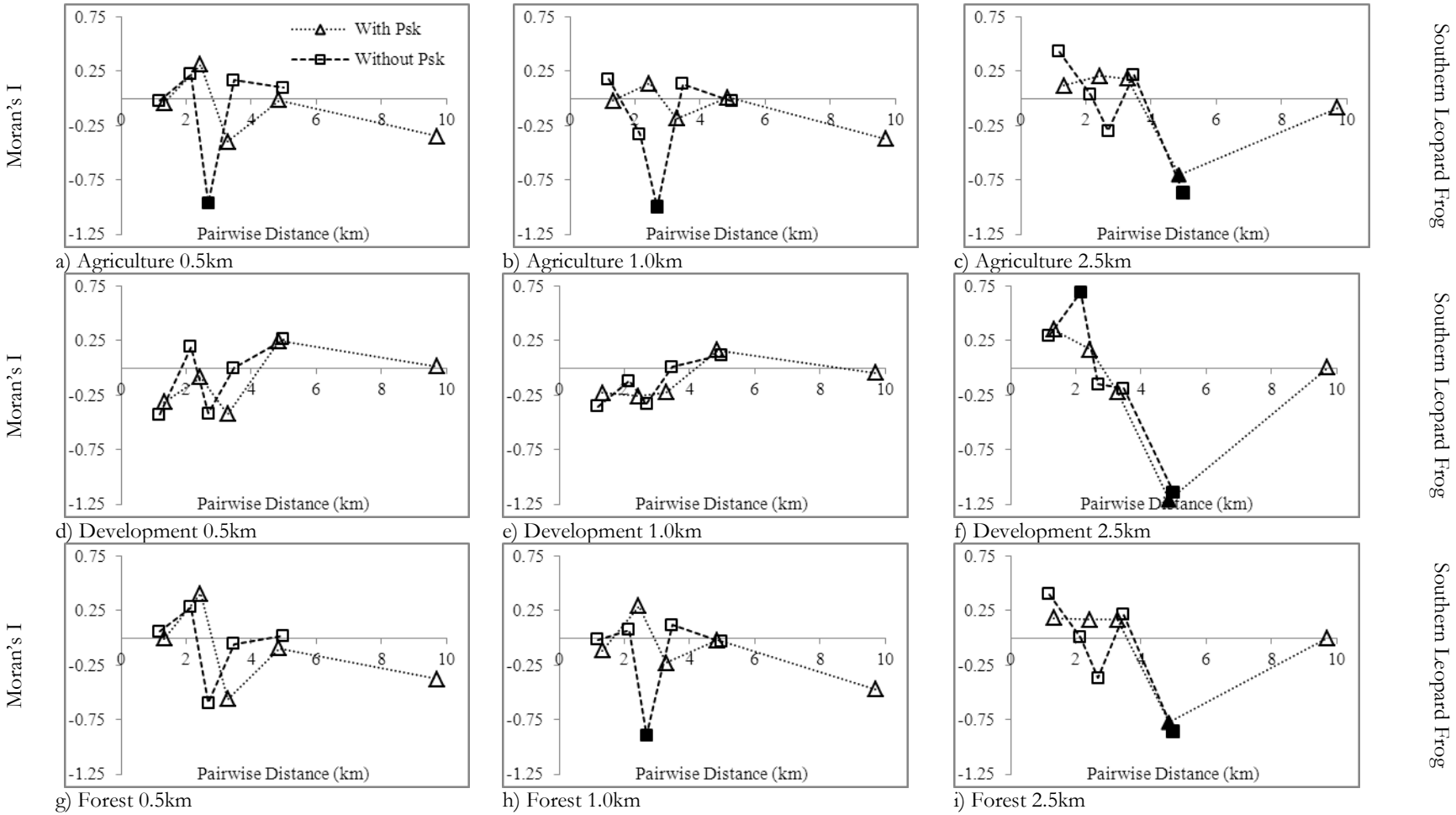
APPENDIX B.

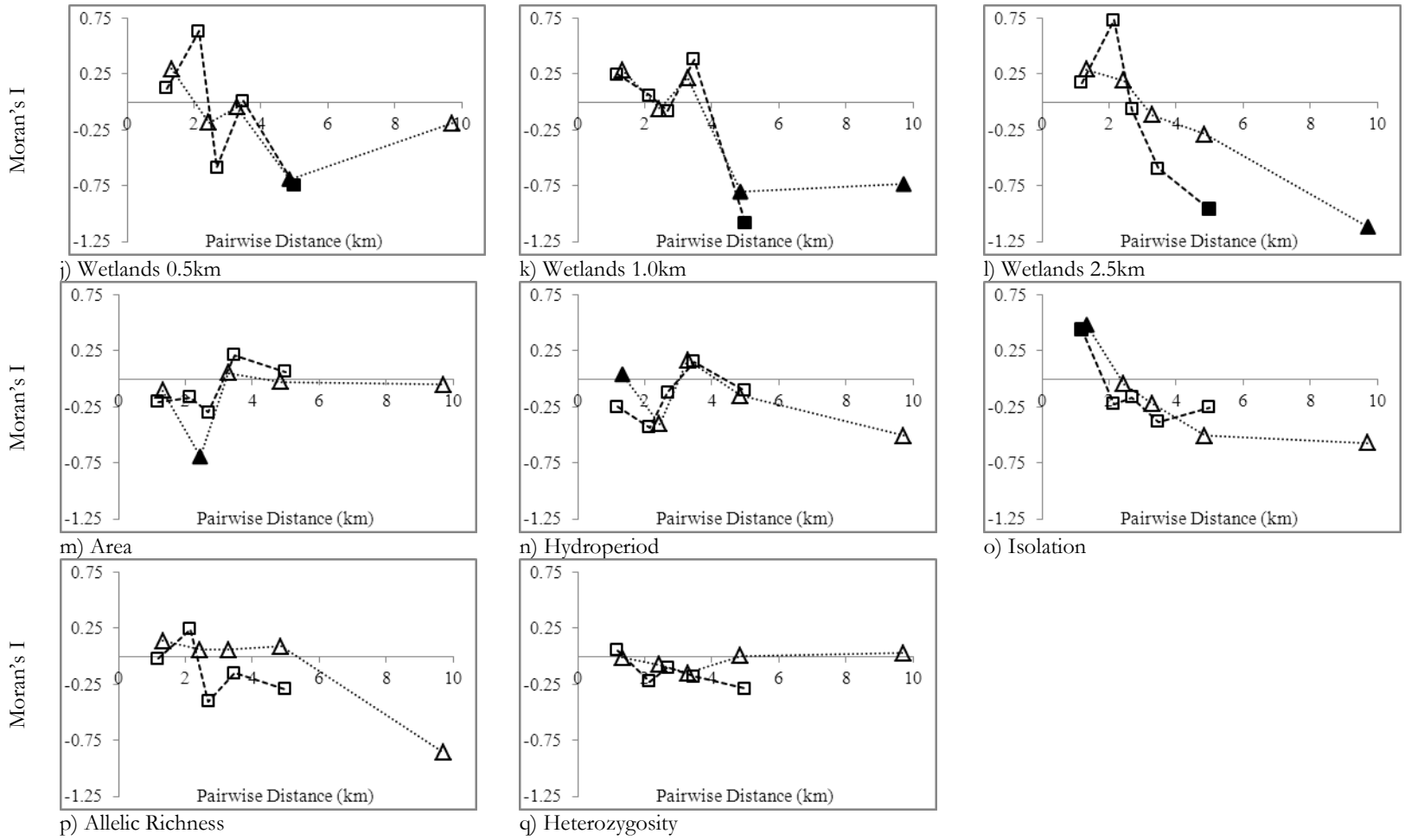
Model averaged estimate directional effects of local and landscape scale predictor variables of allelic richness (r_g) and observed heterozygosity (H_o) in the dwarf salamander *(E. quadridigitata*) and the southern leopard frog *(L. sphenocephalus*) with the outlier site, Psk, included.

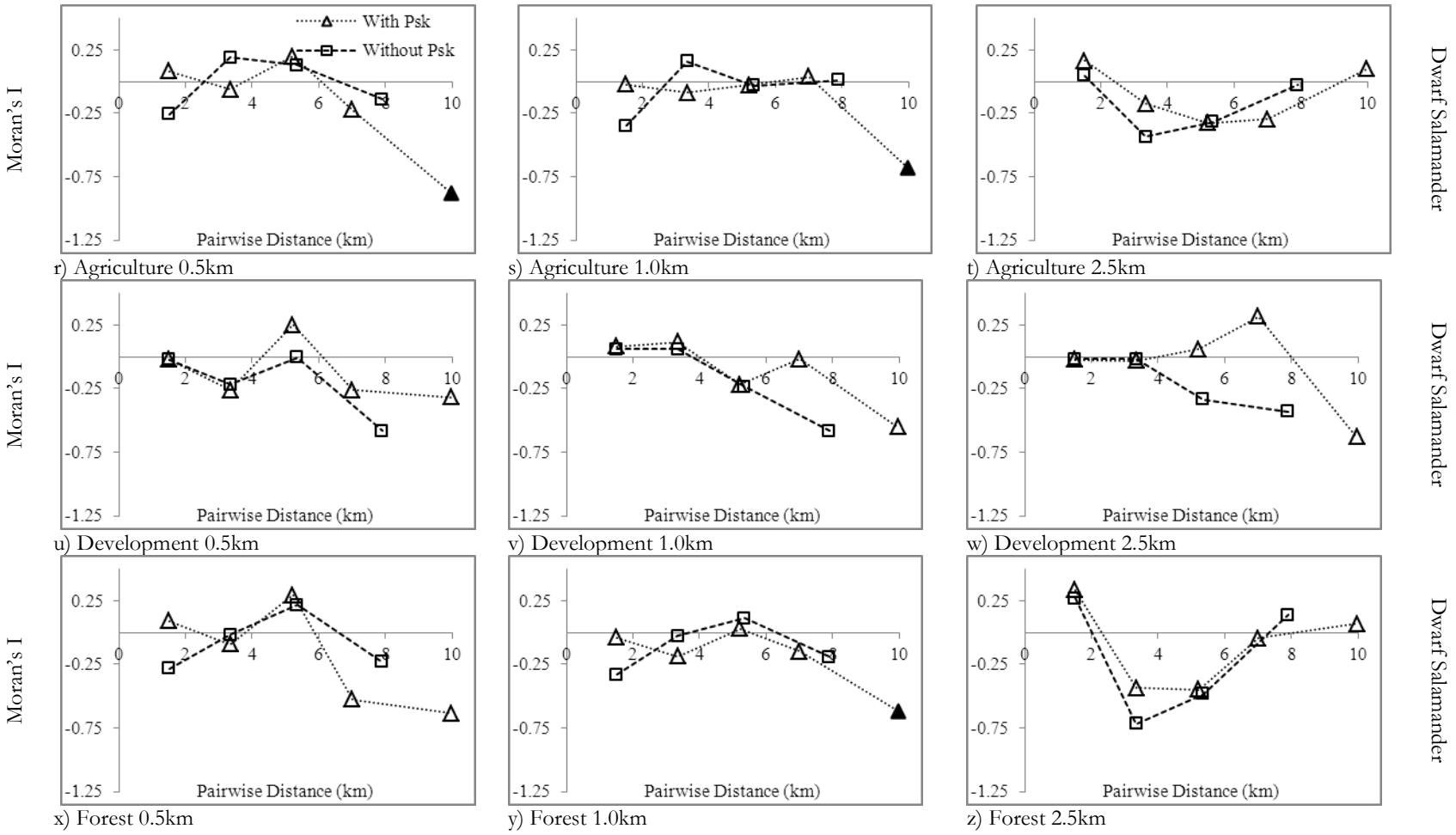
Parameter/ Scale	AREA	HYDRO	ISO	DEVEL	FOREST	AG	WTLND
Dwarf Salamander							
r_g							
local	+	+	-				
0.5km				-	+	-	+
1.0km				-	+	-	+
2.5km				-	+	-	+
H_o							
local							
0.5km				-		-	+
1.0km					+	-	+
2.5km					+		+
Southern Leopard Frog							
r_g							
local	+	+	-				
0.5km				-	+	-	
1.0km				+	+	-	+
2.5km				-	-	-	+
H_o							
local	+						
0.5km				-	+	-	-
1.0km				-	+	-	-
2.5km				-	+		+

APPENDIX C.

Moran's I correlograms of southern leopard frog (a – q) and dwarf salamander (r – ah) predictor and response variables, both with Psk (Δ) and without (□) Psk. Moran's I values that significantly deviate from 0 when alpha = 0.05 are filled in (▲ or ■)



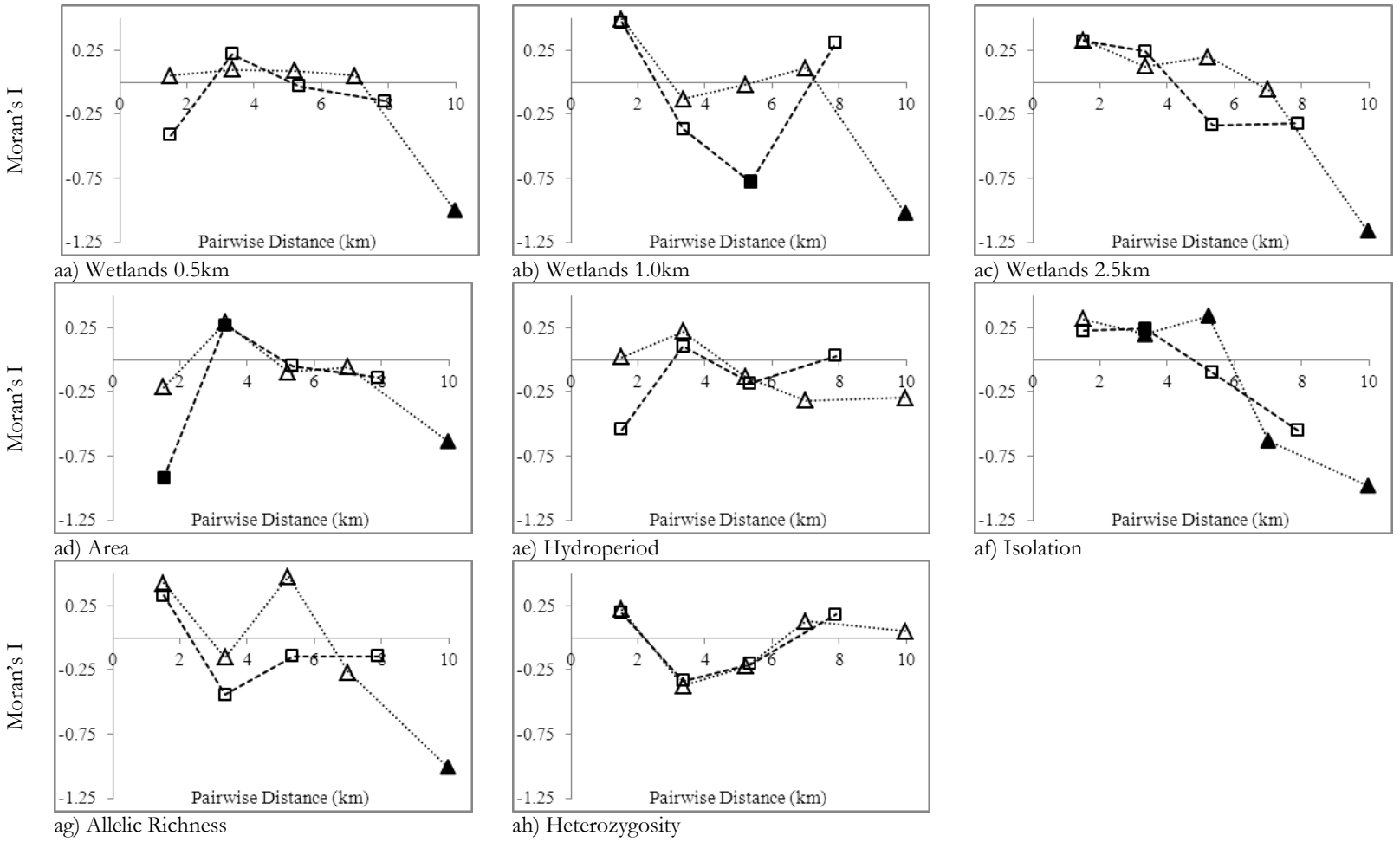

Moran's I
0.75
0.25
-0.25
-0.75
-1.25
0
2
4
6
8
10
Pairwise Distance (km)
j) Wetlands 0.5km
k) Wetlands 1.0km
l) Wetlands 2.5km
m) Area
n) Hydroperiod
o) Isolation
p) Allelic Richness
q) Heterozygosity
Southern Leopard Frog
Southern Leopard Frog
Southern Leopard Frog



r) Agriculture 0.5km s) Agriculture 1.0km t) Agriculture 2.5km

u) Development 0.5km v) Development 1.0km w) Development 2.5km

x) Forest 0.5km y) Forest 1.0km z) Forest 2.5km



aa) Wetlands 0.5km ab) Wetlands 1.0km ac) Wetlands 2.5km

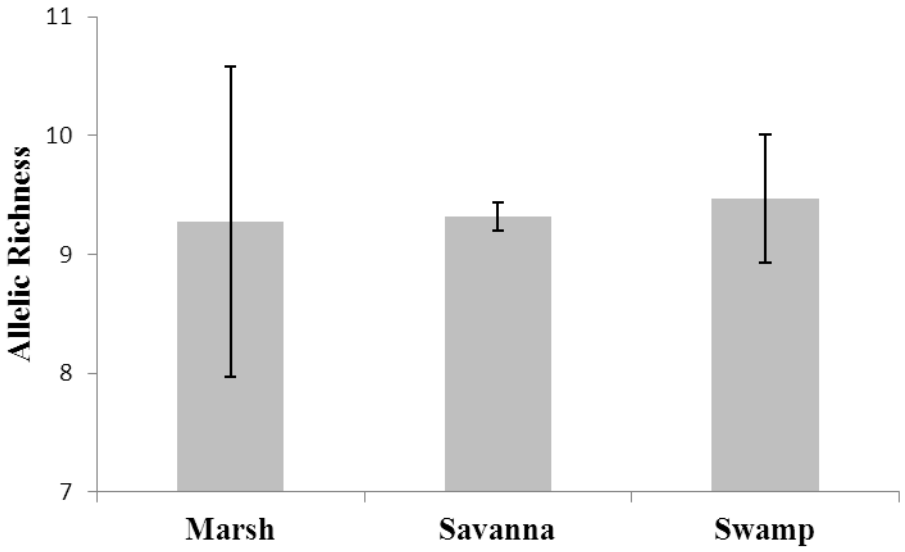
ad) Area ae) Hydroperiod af) Isolation

ag) Allelic Richness ah) Heterozygosity

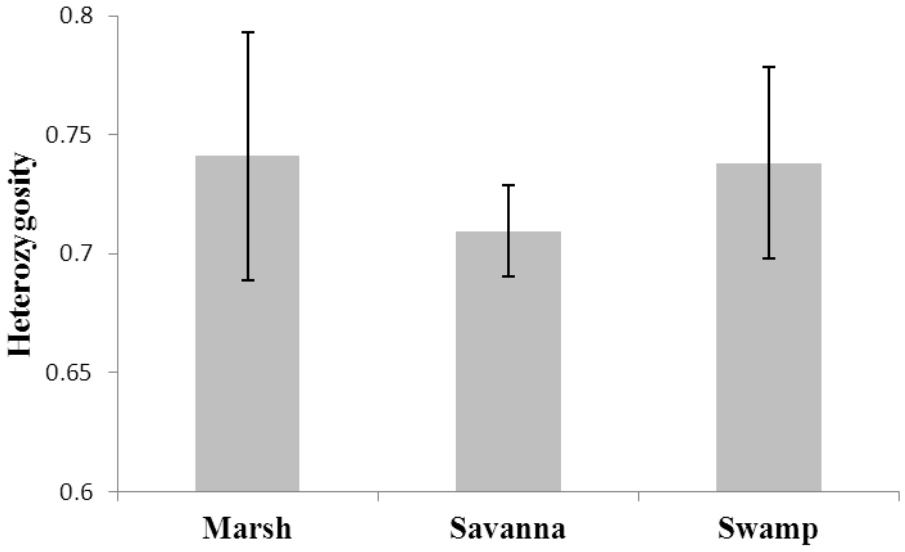
APPENDIX D.

Southern leopard frog rarefied allelic richness (a) and heterozygosity (b) by wetland type, excluding Psk. N=3 for all wetland types. Error bars are 95% CI (1.96*SD).

a)

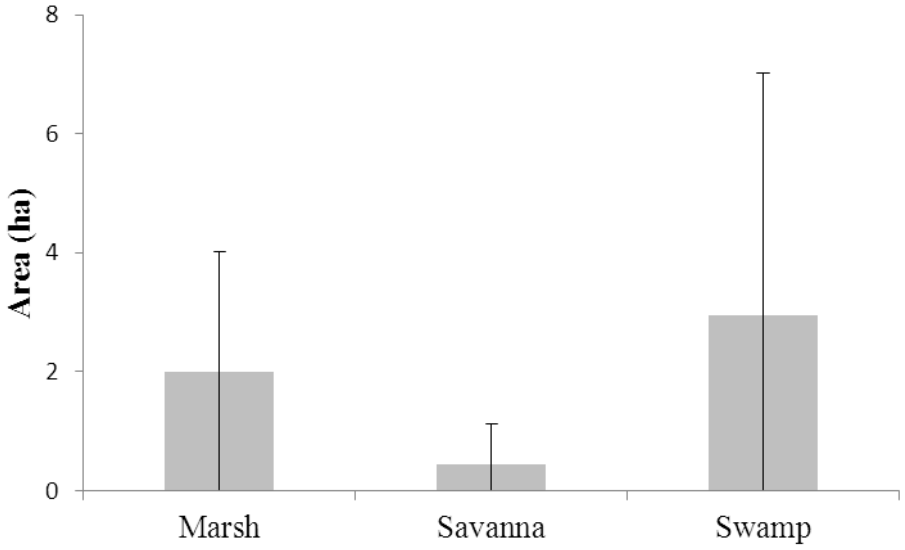


b)



APPENDIX E.

Wetland area by wetland type for southern leopard frog sample sites, excluding Psk. N=3 for all wetland types. Error bars are 95% CI (1.96*SD). No significant difference among wetland types.



APPENDIX F.

Top models of dwarf salamander allelic richness without P58, southern leopard frog allelic richness without P53, and species richness estimates without P53. The sample sizes for dwarf salamander r_g, southern leopard frog r_g, and species richness (S_{obs}, S_{chao}, and S_{jack}), were N=7, 8, and 14, respectively. S_{obs} refers to the number of observed species, S_{chao} and S_{jack} represent the Chao2 and Jackknife2 species richness estimates. Condition number (CN) is the degree of multicollinearity in the model, when CN < 2, multicollinearity is not an issue in the model. AICcWi is the model weight relative to all other models for the same diversity measure at the same spatial scale. * Indicates the top model for a given parameter and number of populations. ‡ Indicates the 95% confidence interval of the variable does not cross 0.

Parameter/ Scale	Variable	Coeff.	SE	t	95% CI	CN	r^2	AICc	AICc Wi
Dwarf salamander r_g									
Local	Constant‡	7.69	1.37	5.60	5.00 - 10.38	1.00	0.10	17.68	0.41
	HYDRO	-0.01	0.01	-0.73	-0.02 - 0.01				
0.5km	Constant‡	5.23	0.94	5.55	3.39- 7.08	1.00	0.33	15.64	0.54
	WTLND	3.82	2.46	1.56	-0.99- 8.63				
1.0km*	Constant‡	5.22	0.64	8.14	3.97- 6.48	1.00	0.52	13.31	0.73
	WTLND‡	4.43	1.92	2.31	0.68- 8.19				
2.5km	Constant‡	4.68	1.09	4.29	2.54- 6.82	1.00	0.41	14.75	0.54
	WTLND	6.63	3.59	1.85	-0.39- 13.66				
Southern leopard frog r_g									
Local*	Constant‡	10.45	0.26	39.89	9.94 - 10.96	2.85§	0.83	7.09	0.53
	AREA‡	0.28	0.06	4.93	0.17 - 0.39				
	HYDRO‡	-0.01	0.00	-3.75	-0.01 - -0.00				
0.5km	Constant‡	10.25	0.37	27.75	9.52 - 10.97	1.00	0.43	7.45	0.47
	FOREST‡	-0.84	0.40	-2.11	-1.62 - -0.06				
1.0km	Constant‡	10.59	0.56	18.88	9.49 - 11.69	1.00	0.40	7.81	0.52
	FOREST‡	-1.24	0.62	-2.00	-2.45 - -0.03				
2.5km	Constant‡	10.55	0.55	19.12	9.47 - 11.64	1.00	0.39	7.93	0.45
	WTLND‡	-3.59	1.83	-1.96	-7.17 - -0.01				
Species richness estimates all sites									
S_{obs}									
Local	Constant‡	7.62	1.36	5.60	4-95 – 10.29	1.00	0.23	60.05	0.55
	ISO	0.30	0.16	1.87	-0.02 – 0.62				
0.5km	Constant‡	5.70	0.91	6.24	3.91 – 7.48	1.00	0.03	63.18	0.23
	DEVEL	-2.62	4.18	-0.63	-10.80 – 5.57				
1.0km	Constant‡	6.33	1.34	4.74	3.72 – 8.95	1.00	0.06	62.72	0.22
	AG	-2.93	3.26	-0.90	-9.33 – 3.46				
2.5km*	Constant	-3.28	3.79	-0.86	-10.71 – 4.16	1.00	0.30	58.69	0.38
	FOREST‡	10.33	4.59	2.25	1.34 – 19.32				
S_{chao}									
Local	Constant‡	8.95	1.41	6.34	6.19 – 11.72	1.00	0.28	61.06	0.55

	ISO‡	0.36	0.17	2.13	0.03 – 0.69				
0.5km	Constant‡	7.03	0.94	7.46	5.18 – 8.87	1.00	0.10	64.07	0.32
	DEVEL	-5.01	4.31	-1.16	-13.46 – 3.44				
1.0km	Constant‡	7.91	1.74	4.55	4.50 – 11.32	1.00	0.09	64.25	0.24
	DEVEL	-8.32	7.66	-1.09	-23.34 – 6.70				
2.5km*	Constant	-3.32	4.00	-0.83	-11.16 – 4.51	1.00	0.32	60.17	0.40
	FOREST‡	11.47	4.83	2.37	2.00 – 20.95				
S_{jack}									
Local	Constant‡	9.20	1.28	7.21	6.70 – 11.71	1.00	0.31	58.25	0.59
	ISO‡	0.35	0.15	2.29	0.05 – 0.65				
0.5km	Constant‡	7.21	0.88	8.21	5.49 – 8.93	1.00	0.08	62.10	0.29
	DEVEL	-4.22	4.02	-1.05	-12.10 – 3.66				
1.0km	Constant‡	8.08	1.61	5.03	4.93 – 11.24	1.00	0.09	62.05	0.25
	DEVEL	-7.61	7.09	-1.07	-21.50 – 6.28				
2.5km*	Constant	-2.31	3.69	-0.63	-9.53 – 4.92	1.00	0.32	57.90	0.39
	FOREST‡	10.63	4.46	2.39	1.90 – 19.37				

APPENDIX G.

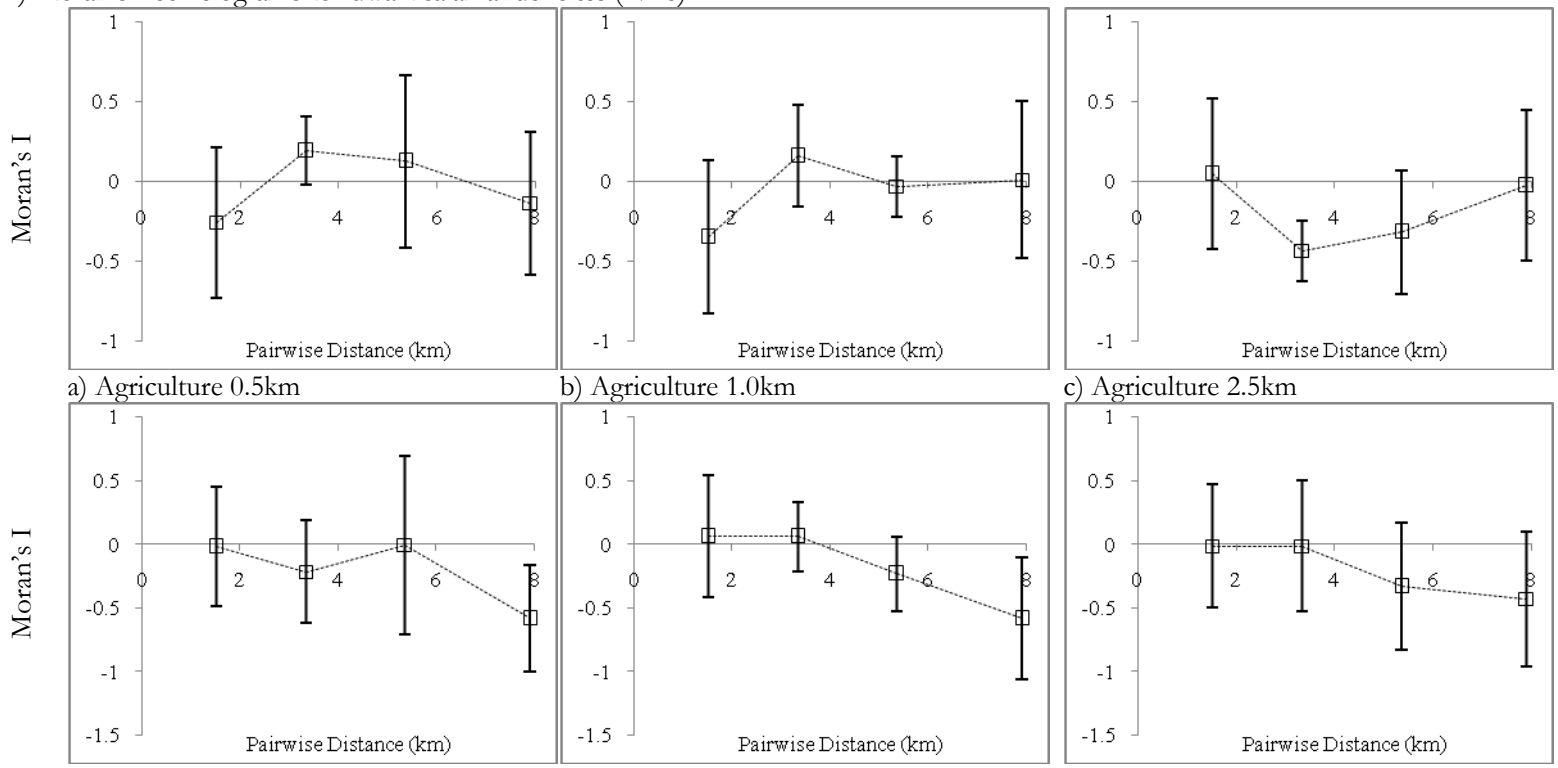
Model averaged estimate directional associations of dwarf salamander allelic richness without P58, southern leopard frog allelic richness without P53, and species richness estimates without P53. The sample sizes for dwarf salamander r_g, southern leopard frog r_g, and species richness estimates (S_{obs}, S_{chao}, and S_{jack}), were N=7, 8, and 14, respectively. S_{obs} refers to the number of observed species, S_{chao} and S_{jack} represent the Chao2 and Jackknife2 species richness estimates. --- indicates that this parameter had too small a sample size to compute model averaged models of all four land cover features at once, therefore the variable in the single variable model with the least support (highest AICc value) was dropped from analysis.

	Local			Land cover			
Parameter/ Scale	AREA	HYDRO	ISO	DEVELOP	FOREST	AG	WTLND
Dwarf salamander r_g							
Local							
0.5km				---	+	-	+
1.0km				---	+	-	+
2.5km				---	+	-	+
Southern leopard frog r_g							
Local	+		+				
0.5km					-	+	-
1.0km				+	-	+	-
2.5km				-		+	-
Species richness estimates							
S_{obs}							
Local			+				
0.5km							
1.0km				-		-	+
2.5km					+		
S_{chao}							
Local	+		+				
0.5km				-			
1.0km				-		-	+
2.5km				-	+		
S_{jack}							
Local	+		+				
0.5km				-			
1.0km				-		-	+
2.5km				-	+		

APPENDIX H

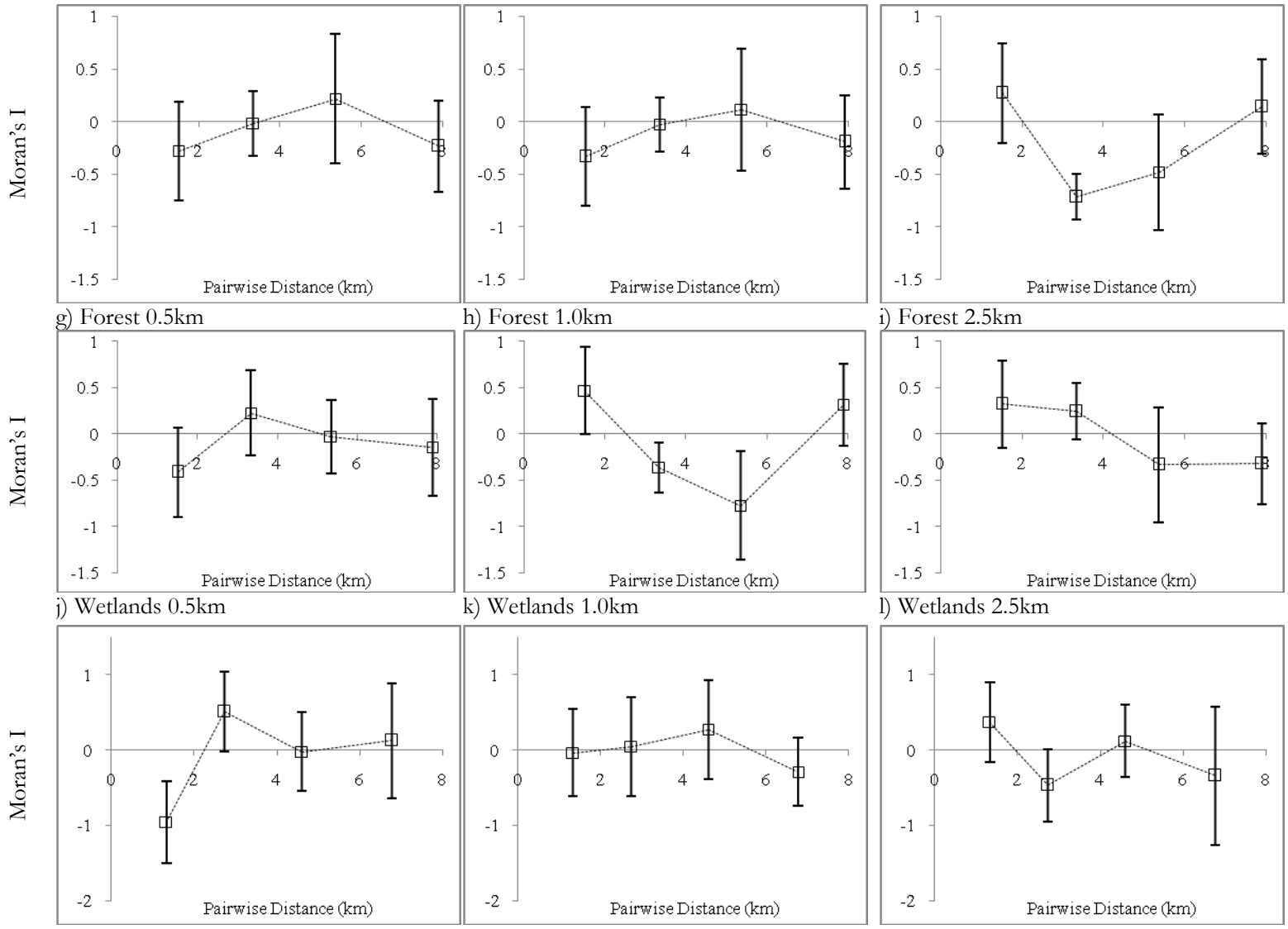
Moran's I correlograms of predictor and response variables. Error bars represent 95% CI. Note the differences in y-axis scales among variable correlograms.

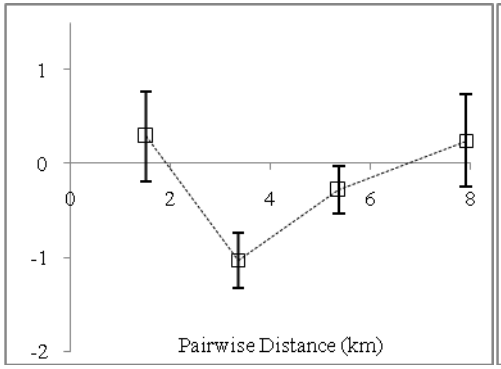
1) Moran's I correlograms for dwarf salamander sites (N=8).



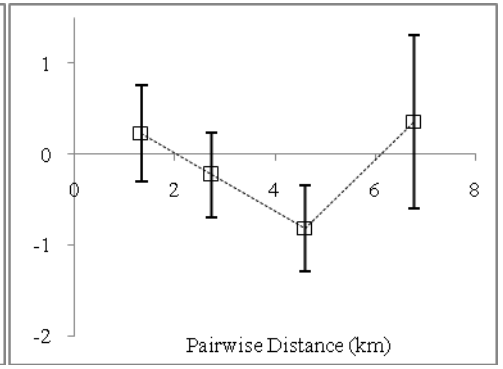
a) Agriculture 0.5km b) Agriculture 1.0km c) Agriculture 2.5km

d) Development 0.5km e) Development 1.0km f) Development 2.5km

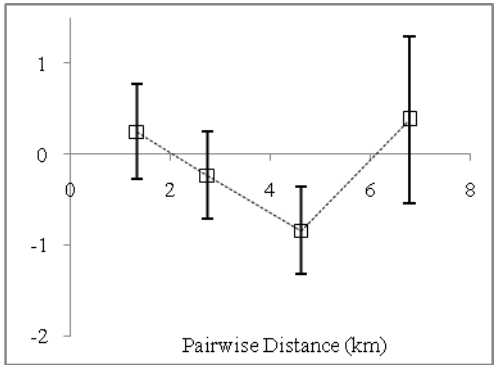

Moran's I
Pairwise Distance (km)
g) Forest 0.5km
h) Forest 1.0km
i) Forest 2.5km
j) Wetlands 0.5km
k) Wetlands 1.0km
l) Wetlands 2.5km
m) Area
n) Hydroperiod
o) Isolation



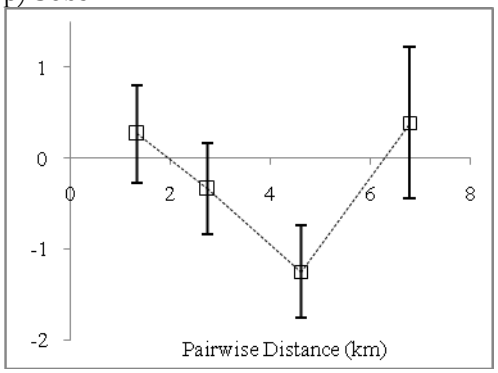
p) Sobs



q) Chao2

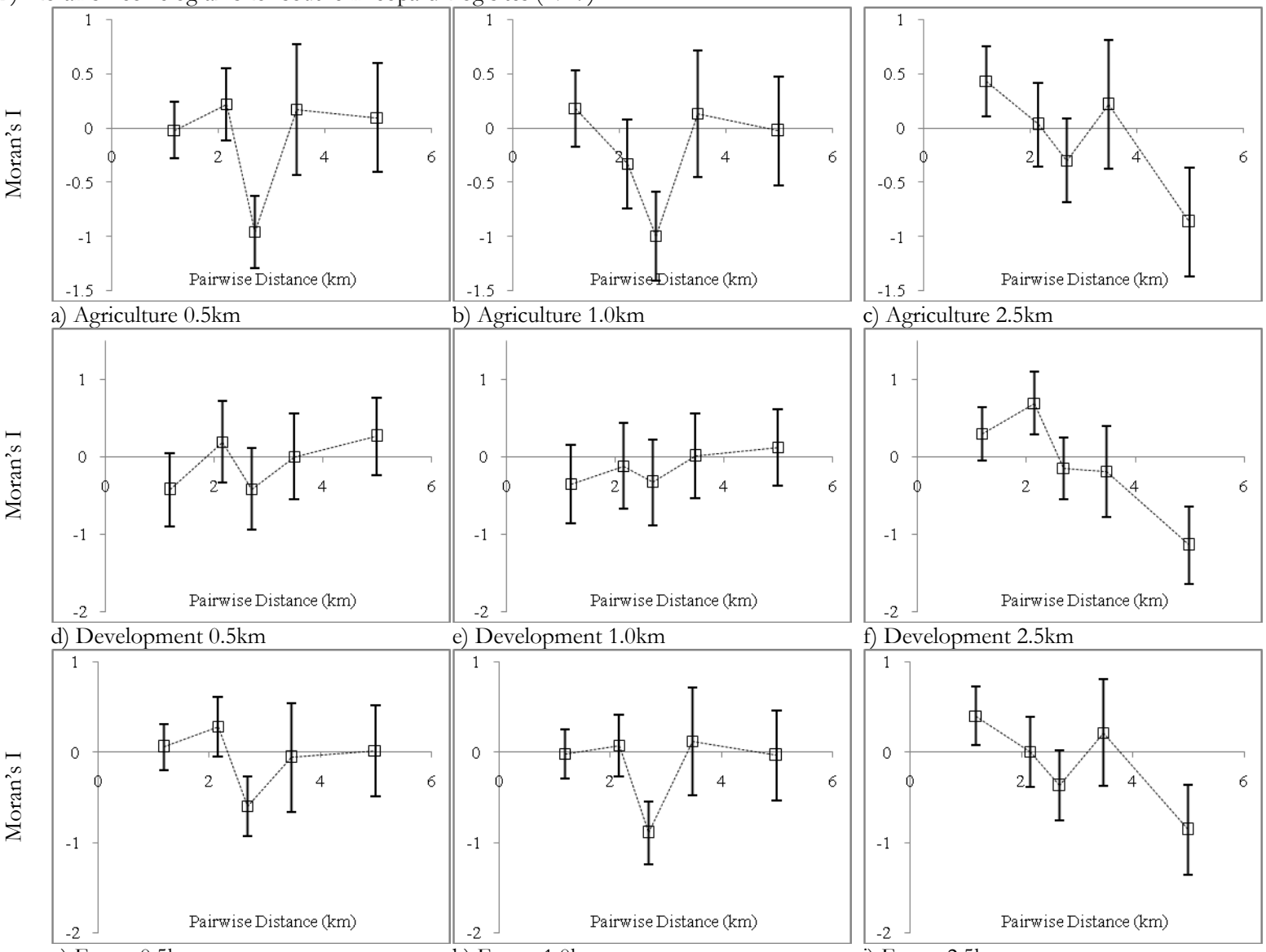


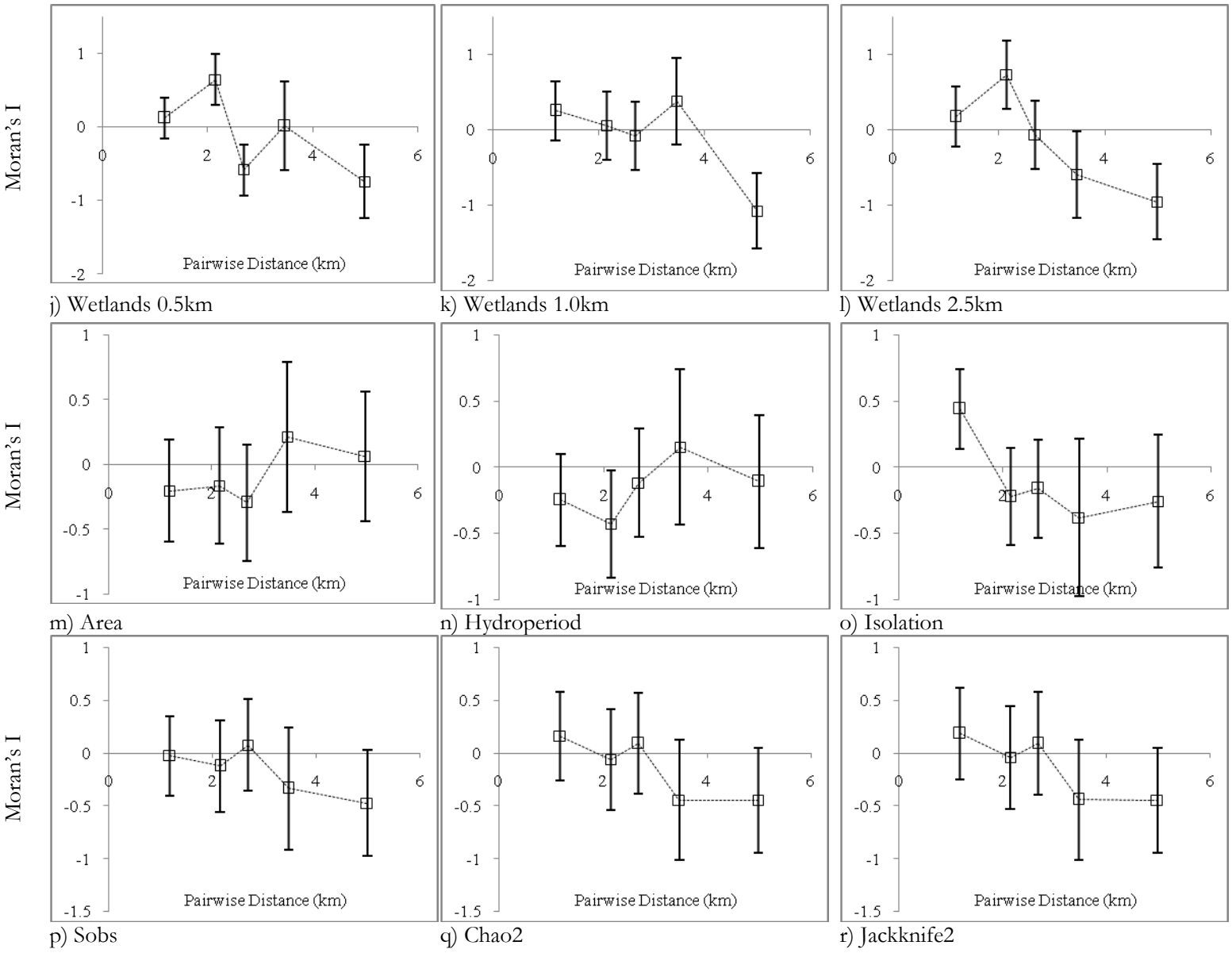
r) Jackknife2

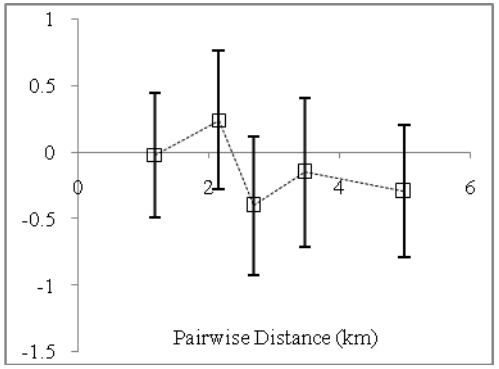


s)Allelic Richness

2) Moran's I correlograms for southern leopard frog sites (N=9).

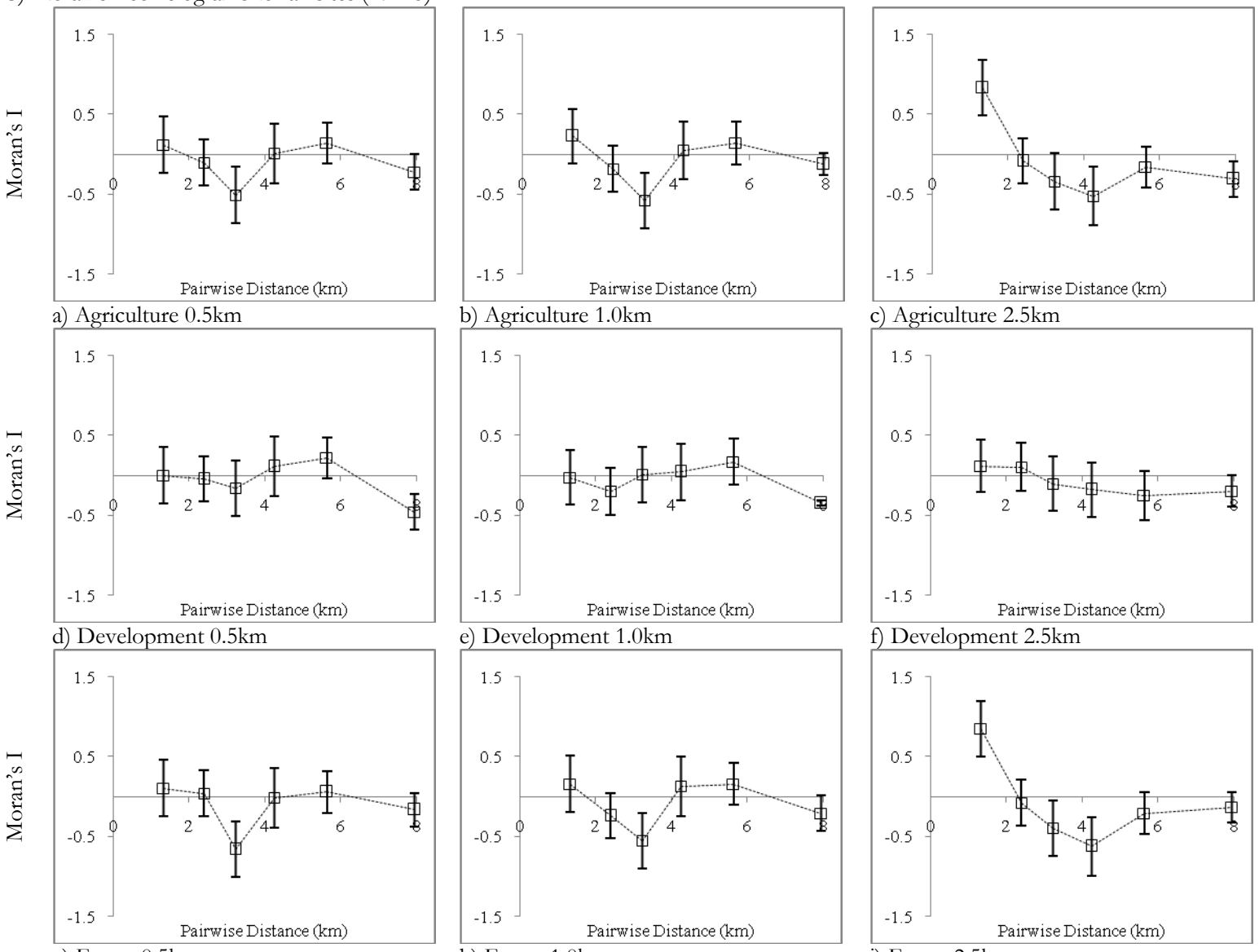



Moran's I
Pairwise Distance (km)
j) Wetlands 0.5km
k) Wetlands 1.0km
l) Wetlands 2.5km
m) Area
n) Hydroperiod
o) Isolation
p) Sobs
q) Chao2
r) Jackknife2



s) Allelic Richness

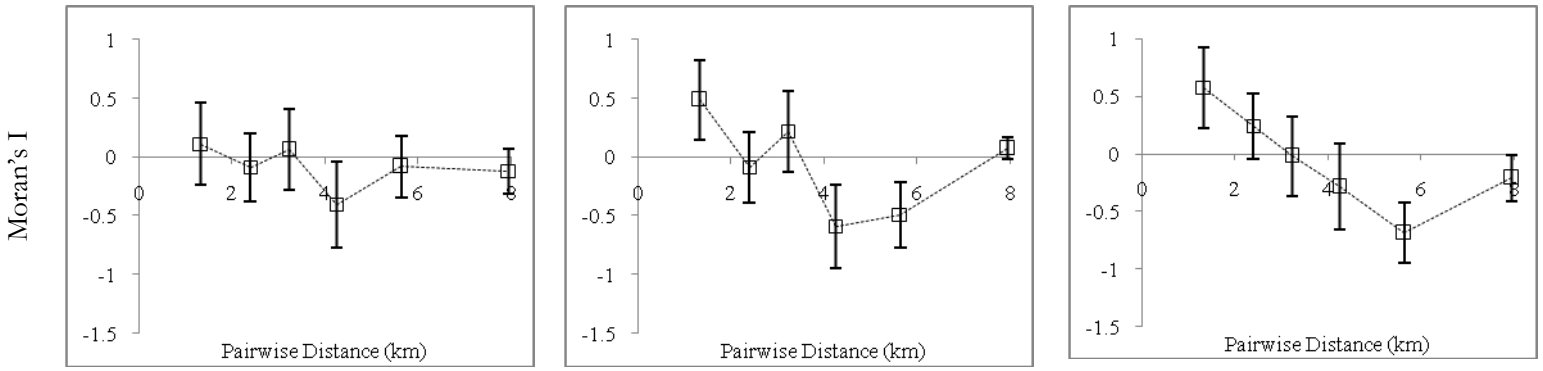
3) Moran's I correlograms for all sites (N=15).



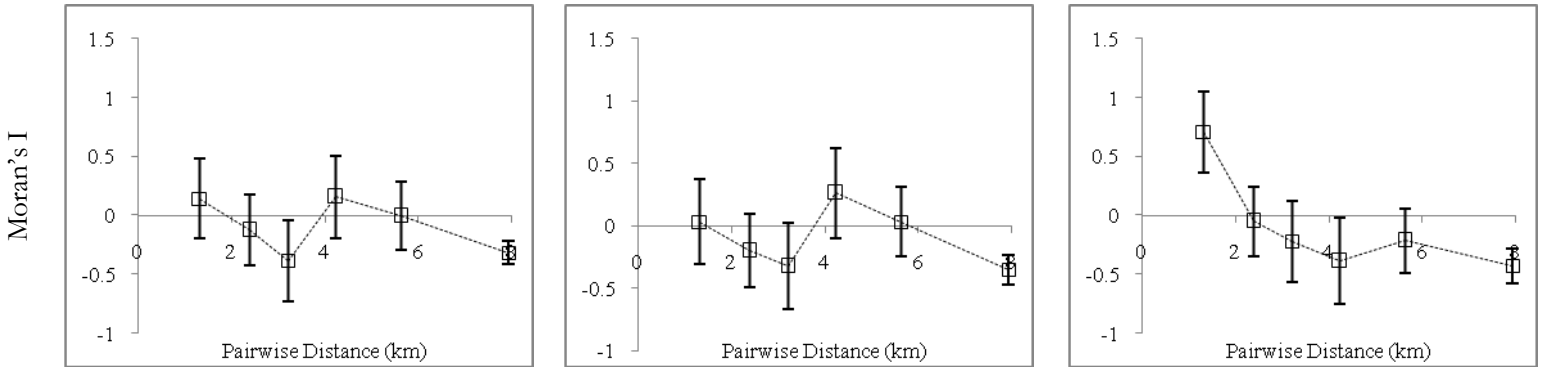
a) Agriculture 0.5km b) Agriculture 1.0km c) Agriculture 2.5km

d) Development 0.5km e) Development 1.0km f) Development 2.5km

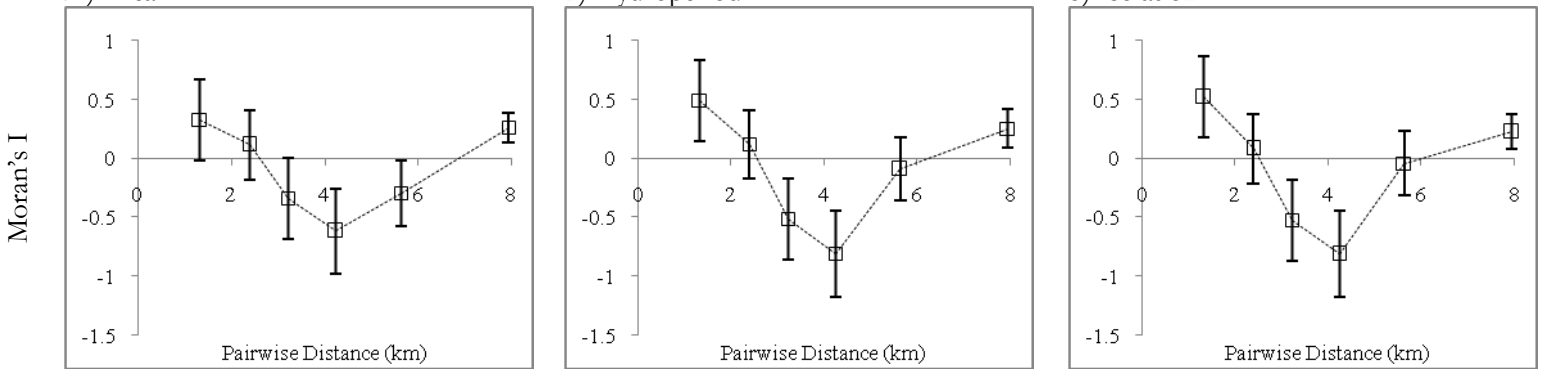
g) Forest 0.5km h) Forest 1.0km i) Forest 2.5km



j) Wetlands 0.5km k) Wetlands 1.0km l) Wetlands 2.5km



m) Area n) Hydroperiod o) Isolation



p) Sobs q) Chao2 r) Jackknife 2

APPENDIX I.

Top models of species richness estimates at dwarf salamander and southern leopard frog subsets of sites. $N_{salamander}$ = 8, N_{frog} = 9, S_{obs} refers to the number of observed species, S_{chao} and S_{jack} represent the Chao2 and Jackknife2 species richness estimates. Condition number (CN) is the degree of multicollinearity in the model, when CN < 2, multicollinearity is not an issue in the model. AICcWi is the model weight relative to all other models for the same diversity measure at the same spatial scale. * Indicates the top model for a given parameter and number of populations. ‡ Indicates the 95% confidence interval of the variable does not cross 0.

Parameter/ Scale	Variable	Coeff.	SE	t	95% CI	r^2	CN	AICc	AICc Wi
Dwarf salamander sites									
S_{obs}									
Local	Constant‡	9.01	2.10	4.29	4.89 - 13.13	0.39	1.00	37.44	0.72
	ISO‡	0.61	0.31	1.98	0105 - 1.21				
0.5km	Constant	7.91	4.84	1.64	-1.57- 17.39	0.06	1.00	40.97	0.29
	WTLND	-7.80	12.74	-0.61	-32.77- 17.16				
1.0km	Constant‡	6.23	2.25	2.77	1.82- 10.63	0.05	1.00	41.02	0.28
	DEVEL	-5.58	9.61	-0.58	-24.41- 13.25				
2.5km*	Constant‡	-8.88	4.35	-2.04	-17.39 - -0.36	0.63	1.00	33.50	0.65
	FOREST‡	15.95	4.99	3.20	6.17- 25.74				
S_{chao}									
Local	Constant‡	10.31	3.44	3.00	3.57 - 17.05	0.18	1.00	45.32	0.51
	ISO	0.58	0.50	1.15	-0.41 - 1.56				
0.5km	Constant‡	7.12	1.41	5.04	4.35 - 9.89	0.05	1.00	46.47	0.28
	DEVEL	-3.75	6.45	-0.58	-16.40 - 8.90				
1.0km	Constant	2.91	4.65	0.63	-6.20 - 12.01	0.09	1.00	46.14	0.28
	WTLND	11.14	14.32	0.78	-16.91 - 39.20				
2.5km*	Constant	-12.38	6.44	-1.92	-24.99 - 0.24	0.59	1.00	39.78	0.72
	FOREST‡	21.71	7.39	2.94	7.23 - 36.19				
S_{jack}									
Local	Constant‡	10.38	3.11	3.34	4.30 - 16.47	0.19	1.00	43.69	0.51
	ISO‡	0.54	0.45	1.19	-0.35 - 1.43				
0.5km	Constant‡	7.28	1.30	5.62	4.74 - 9.82	0.04	1.00	45.09	0.27
	DEVEL	-2.89	5.92	-0.49	-14.49 - 8.70				
1.0km	Constant	3.53	4.23	0.84	-4.75 - 11.81	0.09	1.00	44.63	0.29
	WTLND	10.17	13.02	0.78	-15.35 - 35.69				
2.5km*	Constant	-10.08	5.99	-1.68	-21.83 - 1.66	0.57	1.00	38.63	0.70
	FOREST‡	19.43	6.88	2.83	5.95 - 32.91				
Southern leopard frog sites									
S_{obs}									
Local*	Constant‡	9.23	1.95	4.73	5.40 - 13.05	0.42	1.00	44.53	0.78
	ISO‡	0.47	0.21	2.23	0.06 - 0.88				
0.5km	Constant‡	6.59	1.74	3.79	3.18 - 9.99	0.12	1.00	48.26	0.29
	WTLND	-5.83	6.10	-0.96	-17.79 - 6.13				
1km	Constant‡	6.64	2.66	2.50	1.43 - 11.84	0.05	1.00	48.89	0.28
	DEVEL	-6.83	11.17	-0.61	-28.72 - 15.07				
2.5km	Constant‡	11.32	3.12	3.63	5.20 - 17.43	0.37	1.00	45.16	0.35
	AG‡	-11.28	5.53	-2.04	-22.11 - -0.44				
S_{chao}									
Local*	Constant‡	10.92	2.13	5.12	6.74 - 15.10	0.45	1.00	46.12	0.82
	ISO‡	0.55	0.23	2.41	0.10 – 1.00				
0.5km	Constant‡	7.39	2.01	3.68	3.46 - 11.33	0.08	1.00	50.87	0.28
	WTLND	-5.31	7.05	-0.75	-19.13 - 8.51				
1.0km	Constant‡	8.10	2.97	2.73	2.28 - 13.93	0.07	1.00	50.90	0.28
	DEVEL	-9.14	12.49	-0.73	-33.63 - 15.34				
2.5km	Constant‡	13.59	3.36	4.04	7.00 - 20.18	0.43	1.00	46.51	0.44

		AG‡	-13.69	5.96	-2.30	-25.37 - -2.01				
S_{jack}										
	Local*	Constant‡	11.22	1.98	5.66	7.34 - 15.10	0.48	1.00	44.79	0.84
		ISO‡	0.54	0.21	2.57	0.13 - 0.96				
	0.5km	Constant‡	7.73	1.91	4.04	3.98 - 11.48	0.08	1.00	50.01	0.28
		WTLND	-5.24	6.72	-0.78	-18.41 - 7.940				
	1.0km	Constant‡	8.17	2.86	2.86	2.57 - 13.78	0.06	1.00	50.22	0.27
		DEVEL	-7.87	12.03	-0.65	-31.44 - 15.71				
	2.5km	Constant‡	13.72	3.18	4.32	7.49 - 19.95	0.44	1.00	45.50	0.44
		AG‡	-13.27	5.63	-2.36	-24.31 - -2.23				

APPENDIX J.

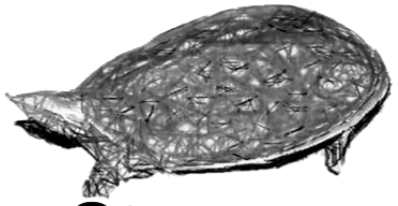
Model averaged estimate directional associations between local and land cover features, and species richness at dwarf salamander and southern leopard frog subsets of sites. $N_{salamander}$ = 8, N_{frog} = 9, S_{obs} refers to the number of observed species, S_{chao} and S_{jack} represent the Chao2 and Jackknife2 species richness estimates.

	Local			Land Cover			
Parameter/ Scale	AREA	HYDRO	ISO	DEVELOP	FOREST	AG	WTLND
Dwarf salamander sites							
S_{obs}							
local	-	+	+				
0.5km					+	-	
1.0km					+	-	+
2.5km				-	+	-	+
S_{chao}							
local			+				
0.5km				-			
1.0km				-		-	+
2.5km				-	+	-	+
S_{jack}							
local			+				
0.5km							
1.0km				-			+
2.5km				-	+	-	+
Southern leopard frog sites							
S_{obs}							
local	-		+				
0.5km							-
1.0km				-	+	-	
2.5km				-		-	
S_{chao}							
local			+				
0.5km							-
1.0km				-		-	
2.5km				-		-	+
S_{jack}							
local			+				
0.5km					-	+	-
1.0km				-			
2.5km				-		-	+

APPENDIX K.
Animal picture sheets for the biodiversity activity.

ALLIGATOR

HORNED FROG



SOFTSHELL TURTLE

BOX TURTLE

POISON DART FROG

SOUTHERN TOAD

GECKO

HELLBENDER

SALAMANDER

TUATARA

APPENDIX L.

Species richness data sheet, allelic richness data sheet, and island characteristic sheet.

Island #_____ Island size_____ Island Isolation_____

Species Richness Datasheet

Round	Alligator	Box Turtle	Gecko	Hellbender	Horned Frog	Poison Dart Frog	Salamander	Softshell Turtle	Southern Toad	Tuatara	Species Rich
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											

Island #_____ Island size_____ Island Isolation_____

Species Richness Datasheet

Species	Round	Orange Starburst	Pink Starburst	Red Starburst	Yellow Starburst	Apple Jolly Rancher	Blue Raspberry Jolly Rancher	Cherry Jolly Rancher	Grape Jolly Rancher	Watermelon Jolly Rancher	Allelic Rich
Alligator	1										
Box Turtle	1										
Gecko	1										
Hellbender	1										
Horned Frog	1										
Poison Dart Frog	1										
Salamander	1										
Softshell Turtle	1										
Southern Toad	1										
Tuatara	1										

Island #_____ Island size_____ Island Isolation_____

Species Richness Datasheet

Species	Round	Orange Starburst	Pink Starburst	Red Starburst	Yellow Starburst	Apple Jolly Rancher	Blue Raspberry Jolly Rancher	Cherry Jolly Rancher	Grape Jolly Rancher	Watermelon Jolly Rancher	Allelic Rich

Island #_____ Island size_____ Island Isolation_____

Species Richness Datasheet

Species	Round	Orange Starburst	Pink Starburst	Red Starburst	Yellow Starburst	Apple Jolly Rancher	Blue Raspberry Jolly Rancher	Cherry Jolly Rancher	Grape Jolly Rancher	Watermelon Jolly Rancher	Allelic Rich

Island #_____ Island size_____ Island Isolation_____

Species Richness Datasheet

APPENDIX M.

Sample exam questions and biodiversity survey.

Name:

Questions 1-18: True or False		**Using a scale of unconfident to confident, please circle how confident you are with your answer**	
Example: (T/F)	The majority of the earth is covered with water. True	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
Questions 1-14 test students understanding of the different constituents of biodiversity: Genetic, Species, and Ecosystems			
1. (T/F)	The various coniferous trees in the Colorado Rockies are an example of biodiversity. **T, SPECIES**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
2. (T/F)	A population of lizards, some of which have blue eyes and some of which have green eyes is an example of biodiversity. **T, GENETIC**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
3. (T/F)	Two male bighorn sheep that are fighting over a female is an example of biodiversity. **F**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
4. (T/F)	The variation in temperature over the winter is an example of biodiversity. **F**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
5. (T/F)	The composition of species that occur on the Hawaiian Islands is an example of biodiversity. **T, SPECIES**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
6. (T/F)	A plant species that has diploid (2 copies of each chromosome) and triploid (3 copies of each chromosome) variants is an example of biodiversity. **T, GENETIC**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
7. (T/F)	The predation of a metamorphic toad by a wolf spider is an example of biodiversity. **F**	i. ii. iii. iv. v.	Unconfident Somewhat unconfident Neutral Somewhat confident Confident
8. (T/F)	Coral reef ecosystems are examples of biodiversity.	i. ii.	Unconfident Somewhat unconfident

T, ECOSYSTEM

iii. Neutral
iv. Somewhat confident
v. Confident

9. (T/F) A community of pond-breeding amphibians is an example of biodiversity.

T, SPECIES

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

10. (T/F) Variation in water chemistry across two streams is an example of biodiversity.

F

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

11. (T/F) Longleaf pine and tidal marsh ecosystems in Georgia are examples of biodiversity.

T, ECOSYSTEM

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

12. (T/F) Different DNA sequences among three individuals from the same species are an example of biodiversity.

T, GENETIC

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat unconfident
v. Unconfident

13. (T/F) Igneous, sedimentary, and metamorphic rock types are examples of biodiversity.

F

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

14. (T/F) Rainforests, cloud forests, and dry pacific forests in Costa Rica are examples of biodiversity.

T, ECOSYSTEM

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Unconfident

Questions 15 – 18 Test students understanding of the effects of dispersal and drift on species and genetic diversity. In these questions, island size is a surrogate for population/community size; so larger islands are expected to have more species and greater genetic diversity.

---

15. (T/F) Given the same number of species on two islands, the extinction rate of the larger islands will tend to be higher than the extinction rate on the smaller island.

F, DRIFT, SPECIES

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

16. (T/F) Immigration decreases species richness over time.

F, DISPERSAL, SPECIES

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

17. (T/F) Immigration into a population helps maintain genetic diversity in that population.

i. Unconfident
ii. Somewhat unconfident
iii. Neutral

T, DISPERSAL, GENETIC

iv. Somewhat confident
v. Confident

18. (T/F) Larger populations tend to have more genetic diversity than small populations.

T, DRIFT, GENETIC

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

QUESTIONS 19 – 23: Circle the most appropriate answer to fill in the blank(s). ASSUME EQUAL HABITAT QUALITY AND NO SELECTION

Questions 19-23 Test students understanding of the effects of dispersal and drift on species and genetic diversity. In these questions, island size is a surrogate for population/community size; so larger islands are expected to have more species and greater genetic diversity.

---

19. Species richness in communities without immigration will _____ over time.

i. increase
ii. remain unchanged
iii. decrease

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

3, DISPERSAL, SPECIES

20. Immigration tends to have a _____ effect on the species richness of an island

i. positive
ii. neutral
iii. negative

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

1, DISPERSAL, SPECIES

21. Genetic diversity within populations tends to _____ with a (n) _____ in immigration rates.

i. increase, decrease
ii. decrease, increase
iii. increase, increase
iv. remain the same, increase or decrease

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

3, DISPERSAL, GENETIC

22. Large islands will generally have _____ species and _____ extinction rates compared to small islands.

i. more, higher
ii. more, lower
iii. less, higher
iv. less, lower
v. none of the above

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

2, DRIFT, SPECIES

23. Small populations lose genetic diversity _____ larger populations.

i. faster than
ii. slower than
iii. at the same rate as

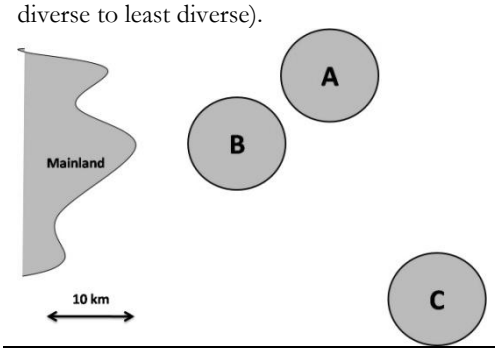
i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

1, DRIFT GENETIC

QUESTIONS 24-29: Circle the most appropriate answer. ASSUME EQUAL HABITAT QUALITY AND NO SELECTION

Questions 24, 25, and 29 Pertain effect of drift (island size) and dispersal (island connectivity) on species and genetic diversity. Questions 26 – 28 pertain to habitat reserve design, with the idea that larger, more well connected islands would maximize species and genetic diversity over smaller and more isolated islands

24. Rank the following islands in terms of expected genetic diversity (most diverse to least diverse).

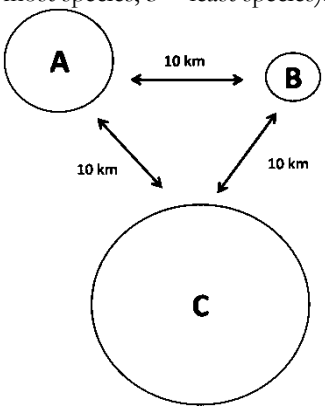


i. A, B, C
ii. A, C, B
iii. B, A, C
iv. B, C, A
v. C, A, B
vi. None of the above

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

3, DISPERSAL, GENETIC

25. Rank the following islands in terms of expected species richness (1 = most species, 3 = least species).



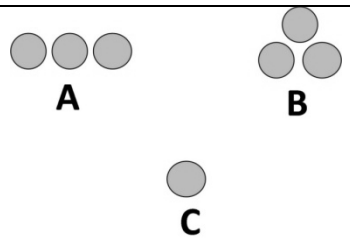
i. A, B, C
ii. A, C, B
iii. B, A, C
iv. B, C, A
v. C, A, B
vi. None of the above

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

5, DRIFT, SPECIES

26. Which of the following reserve designs would most likely maximize species and genetic diversity?

i. Unconfident
ii. Somewhat unconfident
iii. Neutral

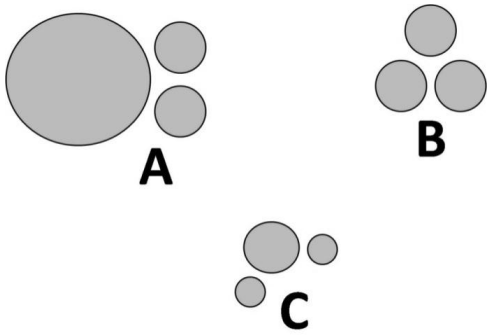


i. A
ii. B
iii. C
iv. A and B
v. None of the above

iv. Somewhat confident
v. Confident

2, RESERVE DESIGN, SPECIES AND GENETIC

27. Which reserve design would maximize genetic diversity?

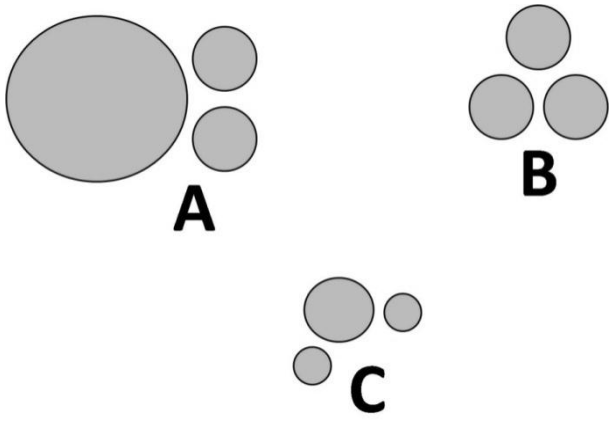


i. A
ii. B
iii. C
iv. A and B
v. None of the above

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

1, RESERVE DESIGN, GENETIC

28. Which reserve design would minimize the risk of species extinction?



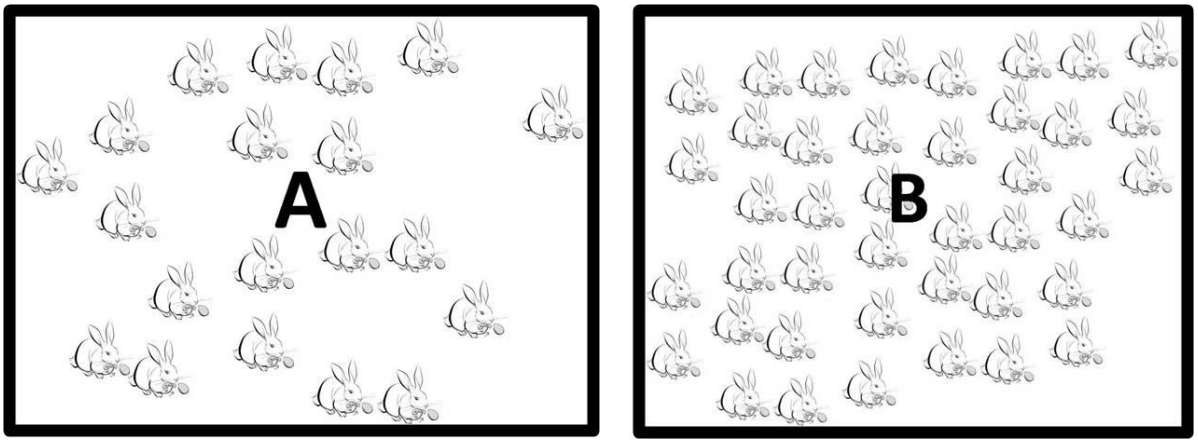
i. A

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

ii. B
iii. C
iv. A and B
v. None of the above

1, RESERVE DESIGN, SPECIES

29. Which population of Easter bunnies would you predict would lose genetic diversity faster?



i. A
ii. B
iii. They would likely lose genetic diversity at the same rate.

1, DRIFT, GENETIC

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

QUESTIONS 30–37: Using a scale of unconfident to confident, please rank how confident you would be in the following scenarios:

30. How confident are you that you could explain the meaning of the term "biodiversity" to another person?

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

31. How confident are you that you could write a short essay, without using notes, on the different components of biodiversity?

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat unconfident
v. Confident

32. How confident would you be in giving a short presentation on biodiversity in class?

i. Unconfident
ii. Somewhat unconfident
iii. Neutral
iv. Somewhat confident
v. Confident

33. How confident would you be discussing the effects of immigration on genetic diversity in populations to another person?

    i. Unconfident
    ii. Somewhat unconfident
    iii. Neutral
    iv. Somewhat confident
    v. Confident

34. How confident would you be discussing the effects of immigration on species diversity in populations to another person?

    i. Unconfident
    ii. Somewhat unconfident
    iii. Neutral
    iv. Somewhat confident
    v. Confident

35. How confident would you be explaining the general effects of population size on genetic diversity to another person?

    i. Unconfident
    ii. Somewhat unconfident
    iii. Neutral
    iv. Somewhat confident
    v. Confident

36. How confident would you be explaining the general effects of island size/habitat patch size on species diversity to another person?

    i. Unconfident
    ii. Somewhat unconfident
    iii. Neutral
    iv. Somewhat confident
    v. Confident

37. How confident are you that you could explain three or more reasons why biodiversity is important to another person?

    i. Unconfident
    ii. Somewhat unconfident
    iii. Neutral
    iv. Somewhat confident
    v. Confident

Age:

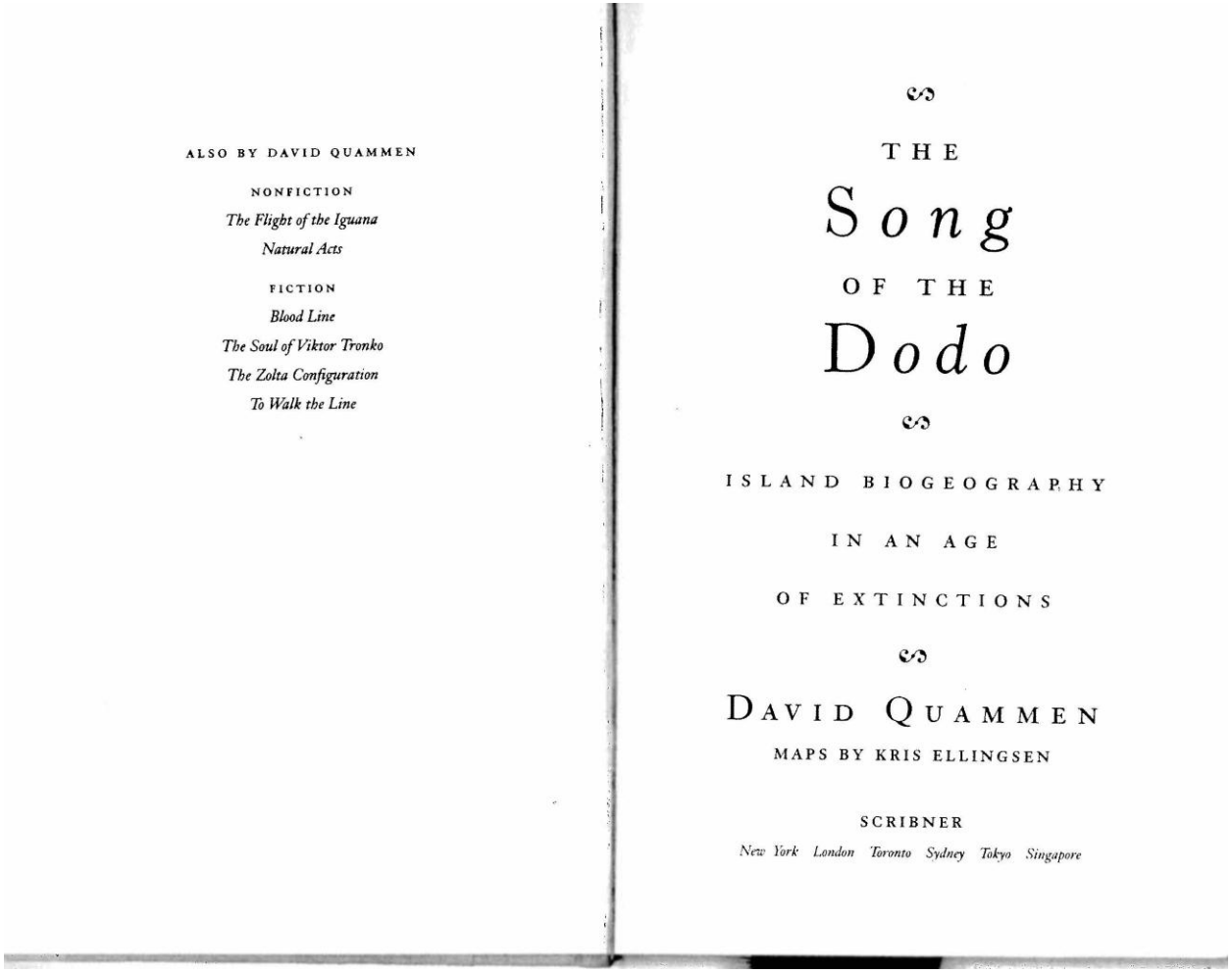
Gender:

Major:

Year of expected graduation and expected degree (PhD, MS, MFR, BS)

Come from rural or urban county (if you're not sure, write down the name of the county and the state):

APPENDIX N.
Song of the Dodo excerpt.



ALSO BY DAVID QUAMMEN

NONFICTION

The Flight of the Iguana

Natural Acts

FICTION

Blood Line

The Soul of Viktor Tronko

The Zolta Configuration

To Walk the Line

THE

Song

OF THE

Dodo

ISLAND BIOGEOGRAPHY

IN AN AGE

OF EXTINCTIONS

DAVID QUAMMEN

MAPS BY KRIS ELLINGSEN

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THE EQUILIBRIUM theory of island biogeography is not a piece of conceptual art. It's a tool. MacArthur and Wilson developed it for two reasons: to explain and to predict.

Although its mathematical details are egregiously complicated, its essence is simple. Think of it as analogous to your digital watch. You don't need to comprehend the circuitry on a silicon chip in order to read the time. Probably you have even mastered the task (after a trial-and-error struggle, if you're like me) of setting the little alarm tweeter and using the stopwatch function. Meanwhile you've preserved your happy ignorance of the chip. Yes? The deal with this equilibrium theory is similar. MacArthur and Wilson's 1963 paper, in which the theory was first presented, contains a moderate amount of arcane

mathematical circuitry. Their book, from 1967, contains an immoderate amount. You and I are gonna ignore it. We're interested in telling time and in using the tweeter, not in the how-to of electronic digital engineering.

Two patterns of real-world data served as the starting points for the theory, which was devised to account for them jointly.

First pattern: the species-area relationship. The ants that Ed Wilson knew so well, on islands of the western Pacific, showed a nicely regular version of that relationship. There were more ant species on the bigger islands, fewer ant species on the smaller islands. The carabid beetles and the reptiles and amphibians of the Antilles showed other neat versions of the species-area relationship, as Darlington had reported. The land birds of certain Indonesian islands showed still another. In each case, larger islands contained more species than smaller islands, and when the numbers of species were graphed against the sizes of the respective islands, the graph points arrayed themselves (with a little logarithmic jiggering) as a straight line. Despite its straightness, as you'll recall, the line was what scientists call a curve. The slope of each species-area curve could be expressed as a decimal number, which varied from one group of islands to another.

To restate the slope business in plain English: Among some island groups, the big islands contained *many* more species than the small islands, while in other island groups, the big islands contained only marginally more species than the small islands. Frank Preston had said as much in his 1962 paper, as MacArthur and Wilson acknowledged (though Preston had been talking mainly about sample areas, not isolates). The equation offered by MacArthur and Wilson for the species-area relationship was the same one that Preston had credited to Arrhenius: $S = cA^z$. The exponent z represented the slope of the curve—that is, the extremeness of the correlation between species number and island size within each group of islands. That exponent took one value for Antillean beetles, another value for Indonesian birds, still another for ants of the western Pacific. Every set of islands was different, though not *too* different; there did appear to be some inherent consistency in the degree to which big islands contained more species than small islands. The average slope value among the cases presented by MacArthur and Wilson was roughly 0.3—just another number to you and me, but a benchmark to anyone studying the interplay of species diversity and area.

The second pattern underlying MacArthur and Wilson's theory,

like the first, had long been familiar to biogeographers: Remote islands support fewer species than less remote islands.

This pattern shows itself in several different ways. An island of some given size, if located near a mainland, generally supports more species than a similar-sized island far offshore. Also, a small island near a large island (for instance, one of the satellite islands around New Guinea) generally supports more species than a small island with no big neighbor. And finally, an island that is part of a small-island archipelago, though far from the nearest mainland or big island, generally supports more species than a solitary island equally far from major neighbors. In each case, the species richness correlates inversely with the degree of isolation.

MacArthur and Wilson's predecessors had commonly explained that pattern in historical terms. Remoteness was an impediment that only eons could overcome. Impoverishment together with remoteness suggested that an island's history had been relatively brief. Colonization of any new oceanic island took time—vast sweeps of time, if the island was remote—and remote islands were generally not ancient enough to have acquired great richness of species. So said the historical hypothesis.

But MacArthur and Wilson suspected that history wasn't the answer. Time was the limiting factor only during the earliest period on a new oceanic island, they believed, and most of the world's island ecosystems had long since come to maturity, to a state of balance, to equilibrium, with the number of species on each a reflection of ongoing processes, not historical circumstances. The ongoing processes that most shaped the balance, they argued, were immigration and extinction.

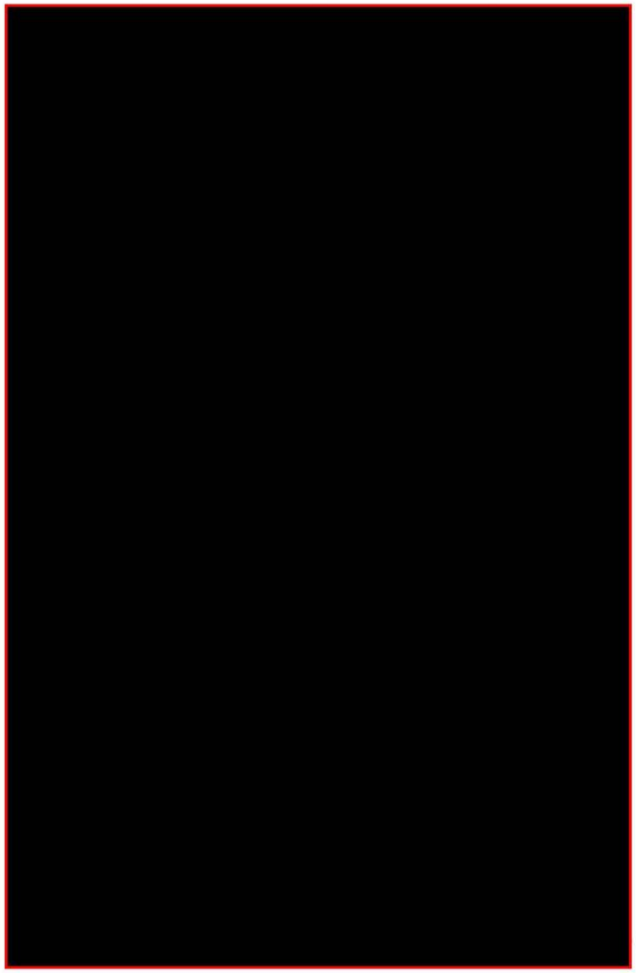
On page 21 of their book MacArthur and Wilson reprinted the sagging-X graph to illustrate this ahistorical theory. The immigration curve slopes downward from the left. The extinction curve slopes upward to the right. The decrease in immigration rate and the increase in extinction rate are graphed not against elapsed time but against the number of species present on a given island. As an island fills up with species, immigration declines and extinction increases, until they offset each other at an equilibrium level. At that level, the rate of continuing immigration is just canceled by the rate of continuing extinction, and there is no net gain or loss of species. The phenomenon of offsetting increase and decrease—the change of identities on the roster of species—is known as *turnover*. One species of butterfly arrives, another species of butterfly dies out, and in the aftermath the island has the same number of butterfly species as before. Equilibrium with turnover.

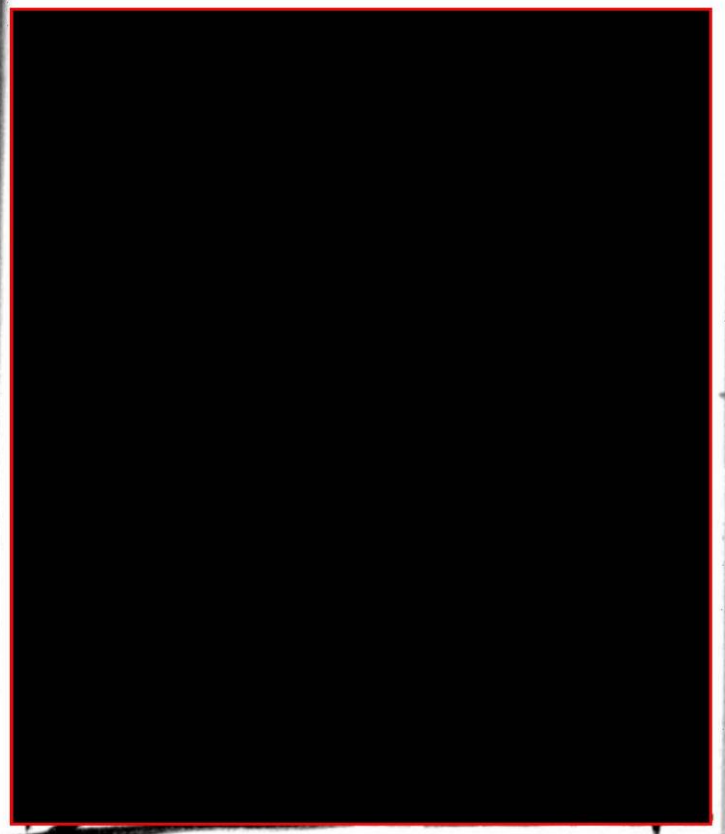
This sagging X of crossed curves became the most renowned and provocative graphic image in the ecological literature. It or its variants would eventually show up in countless books and papers. It would be fervently saluted by many ecologists and fervently denounced by quite a few others. If Preston's canonical curve was the bell heralding change, MacArthur and Wilson's equilibrium figure was the flag of revolt.

Their conceptual model does explain the two patterns of real-world data, and its explanatory power is what made it forceful. Small islands harbor fewer species than large islands—why?—because small islands receive fewer immigrants and suffer more extinctions. In MacArthur and Wilson's schema, that's known as the area effect. Remote islands harbor fewer species than near islands—why?—because remote islands receive fewer immigrants and suffer just as many extinctions. That's the distance effect. Area and distance combine their effects to regulate the balance between immigration and extinction. It all fits together ingeniously.

The sagging-X figure as it appeared in the book was just a generalized version of specific equilibrium graphs that can be drawn for specific islands. The curves will be steeper for some islands than for others, because of particular localized circumstances. As the steepness varies, the crossing point shifts downward or up (indicating a lower or higher turnover rate, at equilibrium) and leftward or right (indicating a lower or higher number of species, at equilibrium). When either curve is especially steep—reflecting the fact that immigration decreases especially sharply or extinction increases especially sharply—their crossing point shifts leftward, toward zero. The shift means that, at equilibrium, in this particular set of circumstances, there will be relatively few resident species.

In other words, high extinction and low immigration yield an impoverished ecosystem. To you and me it's just a dot in Cartesian space, but to an island it represents destiny.





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In 1974, the upheaval initiated by MacArthur and Wilson entered its next phase. The equilibrium theory by then had been published, widely noticed, and empirically tested. It had been projected onto a variety of natural situations—Janzen's plant islands, Culver's cave islands, Vuilleumier's páramo islands—and had helped to illuminate them. Intellectually, it was a success. The next phase was more practi-

cal: applied biogeography. Could the theory be used for problem solving in the real world?

A small handful of scientists, speaking up individually, suggested that it could. In their view, it was pertinent to the fate of natural landscape and wild creatures all over the planet. At a time when humanity was cutting forests and plowing savannas at a rapid pace, when habitat everywhere was becoming fragmented and insularized, the equilibrium theory embodied minatory truths. It was not just an interesting set of ideas—it was goddamned important. If heeded and applied, it might help save species from extinction. Conspicuous amid this chorus of opinion was Dan Simberloff.

Simberloff declared in print that the work of MacArthur and Wilson, along with the obscure papers by Frank Preston, had "revolutionized" biogeography with the suggestion of dynamic equilibrium. "Island biogeography has changed in a decade," Simberloff wrote, "from an idiographic discipline with few organizing principles to a nomothetic science with predictive general laws." He was a bright young man with a recondite vocabulary. "Idiographic" to "nomothetic": He meant that MacArthur and Wilson had succeeded just as they had hoped to, transforming biogeography from a descriptive endeavor into one that could articulate some of nature's governing rules. Yes, the theory brought fresh insight to the ecology of islands; but still more significant, Simberloff added, was that it also addressed insularized habitats on the mainlands. "We can therefore use island biogeographic theory to further our understanding of a variety of evolutionary and ecological phenomena and even to aid in the preservation of the earth's biotic diversity in the face of man's ecological despoliation."

Simberloff's statements appeared in 1974. Jared Diamond was saying much the same thing. That agreement, as you'll see, was a notable and fleeting convergence.

The following year Diamond published a journal article titled "The Island Dilemma: Lessons of Modern Biogeographic Studies for the Design of Natural Reserves." It would become one of his best-known papers. Because it represented a culmination in the development of these ideas, and because it triggered such vehement reaction, "The Island Dilemma" is worth looking at in detail.

Like Simberloff, Diamond believed that MacArthur and Wilson had touched off a "scientific revolution." One aspect of the revolution was a heightened awareness that insularity can occur under natural conditions on the mainlands: a mountaintop, a lake, a tract of woodland surrounded by meadow. As humanity chops the world's landscape into pieces, those pieces become islands too. A nature reserve, by definition, is an island of protection and relative stability in an ocean of jeopardy and change. So the dynamics of parks and reserves can be described—and predicted—by equilibrium theory. The theory, according to Diamond, yields a handful of serious implications.

On his way toward examining the implications, Diamond revisited some of the theory's logical and empirical underpinnings: the species-area equation and Darlington's tenfold-to-twofold ratio, among others. He invoked Vuilleumier's páramo islands in the Andes, as well as Brown's work on mountaintop mammals of the Great Basin. He nodded in the direction of Krakatau. He described the mangrove experiment by Simberloff and Wilson. He also drew on his own study of "relaxation times" for the satellite islands around New Guinea. He noted the high rate of extinction on Barro Colorado. This body of evidence and theory, as Diamond viewed it, led to certain conclusions about the survival prospects of species in isolated reserves, such as:

- A reserve newly isolated will *temporarily* hold more species than its equilibrium number—but that surplus of species will eventually disappear, as relaxation to equilibrium occurs.
- The rate at which relaxation occurs will be faster for small reserves than for large ones.
- Different species require different minimum areas to support an enduring population.

At the end of the paper he offered a set of "design principles" for a system of nature reserves, including:

- A large reserve can hold more species at equilibrium than a small reserve.
- A reserve located close to other reserves can hold more species than a remote reserve.
- A group of reserves that are tenuously connected to—or at least clustered near—each other will support more species than a group of reserves that are disjunct or arrayed in a line.
- A round reserve will hold more species than an elongated one.

Besides stating them verbally, Diamond presented his design principles graphically, in a figure suggesting tiddlywinks on parade. Circles of various sizes and in various spatial arrangements were shown as a menu of dichotomous options. Each pair was labeled with a letter: principle A, principle B, and on up to principle F. The clear visual message was that some patterns of insularity, in the abstract and in reality, are more damaging than others.

Diamond made two claims about his design principles: that they were applicable to conservation planning and that they derived from MacArthur and Wilson's theory. Although both claims were controversial, the first met especially strong challenges. Some ecologists rejected the whole notion of imposing abstract solutions on such various situations; and some who accepted a few of the principles were unwilling to swallow the whole group. There were quibbles about principle F, reservations about E, qualifications that should be added to C—but none of the others provoked such heated and lasting disagreement as the one Diamond listed as principle B.

Four little tiddlywinks were "worse" than one big tiddlywink, according to Diamond's graphic figure. But were four small reserves, in the real world, necessarily worse than a single large one? That question would be argued for a decade. The argument would grow bitter and personal. It would acquire a handy label: SLOSS. The acronym stood for "single large or several small." During the late 1970s it would become ecology's own genteel version of trench warfare.

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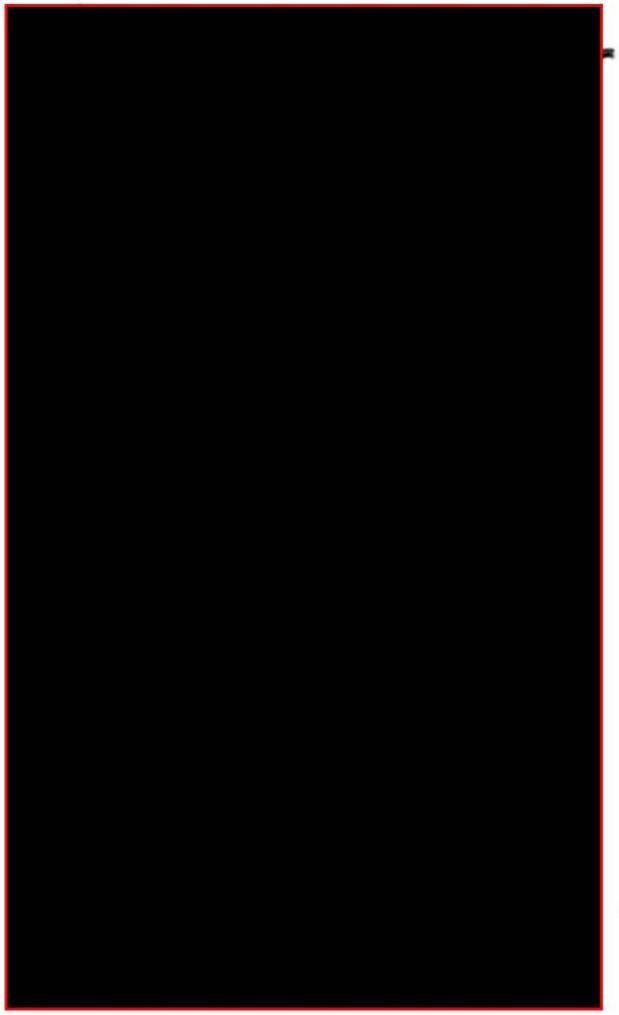

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THE SLOSS debate went public in 1976, when Dan Simberloff and a colleague named Lawrence Abele published a short paper in *Science*, voicing their concern over the recent vogue of applied biogeography. What made them uneasy was this business of deriving neat principles of reserve design from MacArthur and Wilson's theory. They cited Diamond's paper "The Island Dilemma" and pointed also to the brief

but prominent essay by Robert May, "Island Biogeography and the Design of Wildlife Preserves," which had run in *Nature*. They quoted May's statement that several small reserves "will tend to support a smaller species total" than will a single large reserve of equal area. Not so fast, said Simberloff and Abele.

The theory itself hadn't been broadly enough proven, they warned, to justify such confident application. And the most basic principle being offered by May, Diamond, and others—that nature reserves should always consist of the largest possible continuous area—might not be correct. It didn't follow necessarily from the theory. Some of the same evidence cited on behalf of the single-large option could also be adduced to support the several-small option. It was all premature, according to Simberloff and Abele. The complex decisions involved in reserve design weren't reducible to a half dozen sleek principles. Furthermore, given the cost and the irreversibility of ambitious conservation programs, any half-baked application of a three-quarters-baked theory could conceivably do more harm than good.

Simberloff's own stance here is an interesting matter. Back in the late 1960s, as Ed Wilson's graduate student, he had virtually been present at the creation of the equilibrium theory. Sweating his way through the Florida mangroves, he had helped gather experimental data that lent support to that theory. He seemed at the time to believe in the theory's predictive (as well as its descriptive) value. Was he contradicting himself, then, when he criticized its application in 1976? Not necessarily. But if his scientific convictions hadn't changed, his emphasis had. His attitude was subtly but firmly different from just two years earlier, when he had stated in print that island biogeographic theory might indeed "aid in the preservation of the earth's biotic diversity." He wasn't now repudiating the equilibrium theory. He was just cautioning that it might not fit all island ecosystems quite as well as it fit the Florida mangroves, and that it didn't provide any simplistic guidelines for conservation.

To some degree he was reacting against what he saw as the others' careless haste. Another factor was new data. Simberloff and his coauthor Abele had each been involved with recent field studies casting doubt on the notion that a bigger reserve is invariably better.

Simberloff had gone back to the mangrove islands—not just to the general area, but to the same individual clumps that he and Wilson had fumigated. In late 1971, with the original experiment finished, he had altered a few of those islands, cutting channels through the root

tangle and the canopy. His cuts reduced the total area only slightly, but a new level of fragmentation was introduced. Each mangrove island was now a tiny archipelago of islets. How would that fragmentation affect the total number of arthropod species supported on a given archipelago? Simberloff had waited three more years, allowing the islets again to equilibrate; then in the spring of 1975 he had returned for another census. His results were equivocal; a single large mangrove island did not always support more species than several small ones. He found a case in which four severed fragments combined for a higher total of species than the original island. In another case, two severed fragments supported fewer species than they had as a single intact island. Simberloff announced these mixed results in his 1976 paper with Lawrence Abele.

Abele's own fieldwork on marine ecosystems had turned up a similar situation. His "islands" were heads of coral, and the resident species were marine arthropods dependent for habitat on one coral head or another. Abele had found a consistent pattern: Two small coral heads harbored more arthropod species than one large coral head of equal total area. Abele's field results were intricate and equivocal, like Simberloff's, but their net significance was to challenge the categorical postulate that a big island is always richer than two small ones.

Given the long history of species-area studies and the recent excitement over the equilibrium theory, it seemed heretical. Simberloff and Abele might as well have announced that the pope *isn't* Catholic and the bear *doesn't* always shit in the woods.

What could account for the reversal against expectations? Simberloff and Abele mentioned some factors: (1) size of the mainland pool of species, (2) lack of differences in dispersal ability among the mainland species, and (3) competition between species. Each of those factors could contribute, for complicated reasons, toward a situation in which two small islands would contain more different species than a single large one.

Another potential factor—not mentioned by Simberloff and Abele in their 1976 paper but destined to figure eventually in the SLOSS debate—was habitat diversity. If two small islands offer three different types of habitat between them, and one large island offers only two types of habitat, the pair of small islands might conceivably harbor more species.

Or possibly not. And possibly a single large island might contain

more habitat types, not fewer, than two small islands. Possibly the habitat factor and others might determine that, in a given situation, large size and lack of fragmentation do indeed support greater diversity. Simberloff and Abele granted such possibilities. In the real world, islands are various. The principles of reserve design as enunciated by Diamond, on the other hand, assume a certain uniformity. The point that Simberloff and Abele wanted to make, they explained, was not that several small reserves are necessarily preferable to a single large reserve. Their point was that the species-area relationship (as subsumed into MacArthur and Wilson's theory) gives no guidance as to what is preferable, and that each situation should be judged on its own details.

"In sum," Simberloff and Abele concluded, "the broad generalizations that have been reported are based on limited and insufficiently validated theory and on field studies of taxa which may be idiosyncratic." Even within a context of formalized scientific discourse, those words don't sound especially harsh. But the paper's effect was incendiary.

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