Spatial genetic structure of the nine-banded armadillo in western Mississippi

A Thesis submitted
To the Graduate School
Valdosta State University

In partial fulfillment of requirements
For the degree of

MASTER OF SCIENCE

In Biology

In the Department of Biology
Of the College of Arts & Sciences

May 2018

Loren A. Binns

BS, Valdosta State University, 2013

This thesis, "Spatial genetic structure of the nine-banded armadillo in western Mississippi" by Loren A. Binns, is approved by:

Dissertation Committee Chair

Corey D. Anderson, Ph.D.
Associate Professor of Biology

Committee Member

William J. Loughry, Ph.D.
Professor of Biology

Colleen M. McDonough, Ph.D.
Professor of Biology

Dean of the Graduate School

James T. LaPlant
Professor of Political Science

Defense Date 6 April 2018

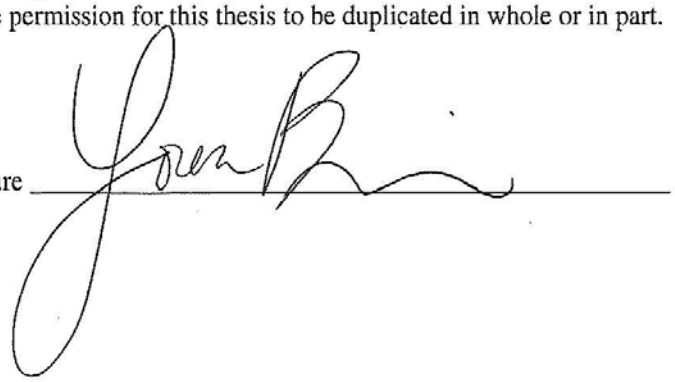
FAIR USE

DUPLICATION

I authorize the Head of Interlibrary Loan or the Head of Archives at the Odum Library at Valdosta State University to arrange for duplication of this thesis for educational or scholarly purposes when so requested by a library user. The duplication shall be at the user's expense.

Signature 

I refuse permission for this thesis to be duplicated in whole or in part.

Signature 

ABSTRACT

The nine-banded armadillo is unique for producing multiple embryos derived from a single fertilized egg (polyembryony), resulting in the production of four genetically identical offspring. Juvenile littermates are known to maintain close proximity to one another during their first summer above ground, but it is uncertain if they remain together as adults. Typically in armadillo populations adults disperse, which may be due to inbreeding avoidance and/or resource competition. There have been multiple studies that have examined sex-biased dispersal in armadillos, but with conflicting results as to which sex disperses. The goal of the present study was to determine whether spatial genetic structure diminishes at a certain life stage (juvenile, adult) within a population and if there are any detectable differences in the degree of genetic structure between the sexes. Genomic DNA from a total of 402 individual armadillos was isolated from ear clip tissue from a wild population of armadillos inhabiting the Yazoo National Wildlife Refuge, Mississippi. Individuals were grouped based on age (juvenile, adult) and further subdivided between the sexes (male, female) for each age group. Genetic measures of spatial autocorrelation based on eight microsatellite loci were used in correlograms to resolve patterns of spatial genetic structure within this population. My results show significant spatial genetic structure in juveniles (both males, females) and adult males, but not adult females. These results support either female-biased dispersal or high variance in male reproductive success (via polygyny). However, further work is required to discriminate between these two possibilities.

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Restricted dispersal can result in increased relatedness between neighboring individuals, a phenomenon that has been referred to as viscous population structure (in the context of the evolution of altruism (Queller, 1992; Wilson et al., 1992) and as isolation by distance (in the context of the balance between gene flow and drift; Wright, 1943). Studies of spatial genetic structure use genetic markers (e.g., allozymes, mtDNA, microsatellites, and SNPs) to make inferences about the ecological and microevolutionary processes that shape the distribution of genetic variation over geographic space.

For many organisms, dispersal estimates are often difficult to obtain from direct field observations and differences in dispersal tendencies of different sex and age classes can be even more difficult to ascertain (Greenwood, 1980; Koenig et al., 1996). Ideally, a combination of both field and genetic methods are required to obtain an understanding of dispersal patterns and to make inferences about sex-biased dispersal (Lawson Handley & Perrin, 2007). The power to detect dispersal and sex-biased dispersal patterns can be improved by increasing the number of polymorphic loci, but there have been instances where it is better to invest in the number of individuals sampled rather than the number of loci (Goudet et al., 2002; Lawson Handley & Perrin, 2007; Banks & Peakall, 2012). For example, Goudet et al. (2002) found intensive sampling (large sample size) to be the most effective strategy to resolve sex-biased dispersal patterns and that sex biases would have to be intense to provide valid detectable differences using spatial genetic methods.

Dispersal in Mammals

Dispersal can occur at different stages of a mammal's life. As juveniles, they may associate with their parents and siblings until they reach sexual maturity (Greenwood, 1980; Lawson Handley & Perrin, 2007). When sexually mature (i.e., as adults), they may engage in dispersal for mates, inbreeding avoidance, and territory or resources (Wolff, 1994). Dispersal, however, can differ between males and females.

Sex-biased dispersal involves individuals of one sex staying or returning to their home site to breed (known as philopatry) while individuals of the other sex disperse. Greenwood (1980) proposed that dispersal bias was driven by the mating system of the population. For example, in most polygynous and promiscuous populations of mammals, juvenile males are the predominant dispersers while in monogamous species both male and female juveniles disperse equally often and equally far (Dobson & Jones, 1985). Even though social systems play a key role in understanding the intensity and direction of sex-biased dispersal, other factors may also play a role, such as inbreeding avoidance, kin competition, and mortality (Gaines & McClenaghan, 1980; Wolff, 1994; Lawson Handley & Perrin, 2007). Understanding the factors that generate sex-biased dispersal also provides insight into the genetic structure of populations. When one sex or age group is philopatric, genetic similarity and spatial genetic structure is established within the population, while the opposite is true for the sex that disperses.

Not all mammalian populations have a detectable dispersal difference between age groups or sexes. However, in most species, dispersal probability is male biased and males tend to travel greater distances than do females (Bowman et al., 2002). Generally, because the costs of reproduction are higher for females, they may benefit more from

remaining in the natal area, which has proven to have sufficient resources to support reproduction due to the fact that the females were born there (Wolff, 1994; Gauffre et al., 2009). Because females remain within their natal area, they are often in proximity to other females that are genetically similar to them (Nussey et al., 2005). This creates genetic structure within the population and can potentially generate further benefits to females via kin selection. In contrast, because males are the dispersing sex, one expects decreased spatial genetic structure amongst them.

Juvenile nine-banded armadillos (*Dasypus novemcinctus*) are born in litters of genetically identical quadruplets (Taulmage & Buchanan, 1954; Prodöhl et al., 1996). This form of reproduction, known as polyembryony, is unique among mammals (Prodöhl et al., 1996; Loughry et al., 1998). After birth in late spring, littermates typically maintain close proximity to one another during their first summer above ground (Taber, 1945). Subsequent dispersal away from natal areas in the fall appears to dilute adult spatial genetic structure and the potential for kin selection (Prodöhl et al., 1996). Nonetheless, certain findings from capture-recapture studies suggest a viscous population structure could exist that might promote the evolution of kin-selected interactions. For example, the mean distance between successive capture locations of an individual is usually less than 200 m, with no significant differences between sex and age classes (Loughry & McDonough, 1998, 2013). This suggests that population viscosity could result from high site fidelity, coupled with high variance in reproductive success (Prodöhl et al., 1998). On the other hand, viscous population structure could be compromised by occasional long-distance dispersal, although the frequency of this phenomenon is generally difficult to estimate (Nathan et al., 2003). This could be a

serious issue in the nine-banded armadillo because the ongoing range expansion of this species in the United States (Taulman & Robbins, 1996) suggests long-distance dispersal events do occur. Even so, the majority of existing behavioral and population genetic data suggest fine-scale spatial genetic structure among juveniles (due to associations among littermates), with the potential for genetic viscosity in adult populations.

In the present study I used genetic measures of spatial autocorrelation (based on μDNA loci) to resolve patterns of fine-scale spatial genetic structure in a wild population of the nine-banded armadillo at the Yazoo National Wildlife Refuge in western Mississippi. The large number of animals sampled at this location allowed me to evaluate spatial genetic structure over continuous distances and to compare these patterns between different sex/age subgroups. Based on previous studies of this species, I expected to find enhanced spatial genetic structure in juveniles relative to adults, and no differences in spatial genetic structure between males and females (both as juveniles and adults).

METHODS

Field Sampling

Armadillo samples were collected at Yazoo National Wildlife Refuge in Mississippi from May 2005-July 2010. Captures included road kills as well as live animals. Live armadillos were captured using long dip nets (Loughry & McDonough, 1996). Each live captured armadillo was marked permanently with a PIT tag injected under the dorsal area of the front carapace. They were also marked temporarily by attaching different shapes and colors of reflective tape to their carapace. A veterinary ear notcher was used to obtain a small piece of ear tissue from each sampled animal as the source of DNA for the genetic analyses (preserved in vials containing 100 percent ethanol until screened). Individuals were then sexed and divided into three age groups based on weight measurements: juvenile, yearling, and adult (Loughry & McDonough, 1996; McDonough & Loughry, 2005). The location of each armadillo capture (or recapture) was recorded using a Global Positioning System (Trimble GeoExplorer 3).

DNA Extraction and PCR

Eighteen microsatellite primer sets have been developed for nine-banded armadillos (Prodöhl et al., 1996; Chinchilla, 2011). Of the eighteen available primer sets, eight were used in the spatial genetic analysis. These included five described by Prodöhl et al. (1996) and three developed by Chinchilla (2011; see Appendix A). The four remaining microsatellite primers of Prodöhl et al. (1996) (i.e., Dnov 2, Dnov 3, Dnov 16, Dnov 65) were not included in this study because they produced anomalous bands that could not be scored. Of the six remaining primer sets developed by Chinchilla (2011), Dnov 2433 was only partially genotyped because of evidence of a

severe heterozygote deficiency at this locus that may have been indicative of a high frequency of null alleles. Dnov 4035 and 2426 produced weak bands and were also excluded. Other primer sets developed by Chinchilla (2011) (i.e., Dnov CTLA4, Dnov 4724, Dnov VDR) were linked to known leprosy resistance genes and were not included due to potential linkage disequilibrium.

Genomic DNA from a total of 480 individual armadillos was isolated from ear clip tissue samples using DNeasy Blood & Tissue kits (Qiagen). To increase yields, soak times in lysis buffer at 56° C were extended from ten minutes to a minimum of twelve hours, with occasional vortexing to help break down the tissue. The concentration of nucleic acids in each genomic extraction was measured using a NanoDrop 2000 (Thermo Scientific) spectrophotometer and each sample was then standardized to a working stock of 100 µg/ml (if yields were lower than 100 µg/ml, they were not diluted further). Labeled and unlabeled oligonucleotide primer sets were custom ordered from Integrated DNA Technologies (IDT). For the Prodöhl et al. (1996) primers, 5' ends were labeled with IRDye 700 or 800. Primer sets for the Chinchilla (2011) primers were synthesized with a 19-mer (5'-CACGACGTTGTAAAACGAC -3') M13 primer on the 5' end, and the complementary IRDye-labeled (700 or 800) primer was included in the PCR reaction at concentrations specified by the author. IRDye 700 and 800 M13 dye labels used in PCR protocols were purchased from LI-COR Biosciences. Isolated DNA was amplified using Polymerase Chain Reaction (PCR). For Prodöhl et al. (1996) primers, Qiagen 2X PCR Master Mix was used following manufacturer protocols to give a final 1X concentration. Each 15 µl PCR tube contained 1µl of genomic DNA, 7.5 µl 2X Master mix, and a final forward and

reverse primer concentration of 0.3µM, with a 3:1 concentration of unlabeled forward primer to labeled forward primer. Promega GoTaq 2X PCR Master Mix was used for Chinchilla (2011) primers following the manufacturer's protocol to give a final concentration of 1X. Each 10µl PCR reaction tube contained 1.5 µl of genomic DNA, 5 µl of Master Mix, and a final forward and reverse primer concentration of 0.45 µM, with a 3:1 concentration of unlabeled forward primer to labeled forward primer.

The PCR protocol included two minutes of activation at 94 °C, one minute denaturation at 94° C, followed by a one minute annealing period with temperatures specified by Prodöhl et al. (1996) and Chinchilla (2011) and one minute extension at 72° C. PCR reactions included 37 cycles and were extended for ten minutes at 72° C at the end of the final cycle.

Gel Electrophoresis and Genotyping

Fragments were sized using the LI-COR 4300 DNA analyzer and SAGA automated microsatellite software. Six percent acrylamide gels were used for genotyping following LI-COR protocols for microsatellite DNA loci. PCR products were diluted between six and eight times before loading samples into the gels. To each diluted PCR reaction tube, 1.5 to 2 µl of sucrose loading dye was added. Tubes were then placed into a centrifuge chamber for ten seconds at 4000 rpm to spin down contents. When loading samples into the gel wells, 0.25 µl was used in each lane. PCR products of the same locus were run in every other lane to avoid leakage across lanes. Lanes 1, 16, 32, 48, and 64 were used for the size standard (0.2µl) of either IRDye 700 or 800. Each gel ran for two hours in LI-COR and produced gel images in either 700 or 800 spectra (depending on the dye label used). Individual genotypes that were difficult

to interpret, produced weak bands, or did not amplify were re-run following the same protocol. Alleles were scored for each individual for all primer sets with the assistance of the SAGA automated microsatellite software. Genotypes were amplified twice to validate readings.

Population Genetic Analysis

GenAlEx 6.5 (Peakall and Smouse 2012) was used to conduct standard population genetic analyses, including calculation of observed and expected heterozygosities and testing for Hardy-Weinberg equilibrium and linkage disequilibrium (Appendix A). A significance level (α) of 0.05 was used for all statistical tests.

Spatial Genetic Analysis

Fine-scale spatial genetic structure was evaluated for the entire sample, as well as among different sex/age subgroups. Originally, there were 480 individuals used for DNA analysis, but 59 individuals lacked genotyping scores and/or GPS coordinates, so they were removed from the data set. Yearlings were removed as well due to their small sample size (n = 19), resulting in a final sample size of 402 animals. Age groups included adults (n = 327) and juveniles (n = 75). The data were further partitioned by sex to include adult males (n = 161), adult females (n = 166), male juveniles (n = 40), and female juveniles (n = 35).

For each group, correlograms were generated in GenAlEx 6.5 (Peakall and Smouse 2006) based on the autocorrelation coefficient (*r*) proposed by Smouse and Peakall (1999). The *r* coefficient is bounded by an interval [-1, +1]. Geographic distance matrices were generated from GPS coordinates (UTM zone 17N; datum = WGS 1984) for each captured individual. For individuals that were captured more than

one time, the first capture location was used. For each correlogram, pairs of individuals were binned into ten even distance classes (= 200 m per class). This distance lag (= 200 m) was chosen based on previous work by Loughry and McDonough (1998) and Perez-Heydrich et al. (2016), which showed that recaptures were, on average, less than 200 m from the previous capture location. For comparison, correlograms with distance classes that contained even sample sizes were generated. These correlograms did not yield different inferences from those that used even distance classes; hence, only data from even distance class correlograms are presented here. The significance of spatial correlation in each distance class was assessed via Monte Carlo analysis with 999 permutations, which is the minimum number of permutations required for a test at significance level of 0.1 percent. Bootstrap resampling was used to build upper and lower 95 percent confidence intervals around the estimated autocorrelation coefficient for each class (Smouse et al., 2008).

RESULTS

Spatial Genetic Structure

For the pooled data (all samples combined), significant positive autocorrelation was detected in the first (0 to 200 m; $r = 0.051$, $P = 0.001$), second (200 to 400 m; $r = 0.014$, $P = 0.001$) and third (400 to 600 m: $r = 0.007$, $P = 0.008$) distance classes (Appendix B, Figure 1A). For males, significant positive autocorrelation was detected in the first (0 to 200 m; $r = 0.110$, $P = 0.001$), second (200 to 400 m; $r = 0.042$, $P = 0.001$) and third (400 to 600 m; $r = 0.017$, $P = 0.003$) distance classes as well (Appendix B, Figure 1B). The correlogram line for males suggested a patchy pattern; it began to decline at 800 m and showed significant negative autocorrelation at distance classes five (800 m to 1000 m; $r = -0.020$, $P = 0.001$) and six (1000 m to 1200 m; $r = -0.016$, $P = 0.002$; Appendix B, Figure 1B). Spatial autocorrelation values then overlapped zero in a positive direction and later declined again with significant negative autocorrelation at distance class nine (1600 m to 1800 m; $r = -0.018$, $P = 0.002$) (Appendix B, Figure 1B). Females exhibited significant positive autocorrelation in the first distance class only (0 to 200 m; $r = 0.034$, $P = 0.001$; Appendix B, Figure 1C).

When juveniles were removed from the analysis, pooled adult data displayed significant positive autocorrelation in the first (0 to 200 m; $r = 0.033$, $P = 0.001$), second (200 m to 400 m; $r = 0.016$, $P = 0.001$) and third (400 m to 600 m; $r = 0.006$, $P = 0.028$) distance classes (Appendix B, Figure 2A). Likewise, when each sex was examined separately, positive spatial autocorrelation was detected for adult males in the first two distance classes (0 to 200 m; $r = 0.091$, $P = 0.001$; 200 to 400 m; $r = 0.046$, $P = 0.001$; Appendix B, Figure 2B). Based on the correlogram line (Appendix B, Figure 2B), a

patchy pattern of spatial genetic structure was suggested for males, with spatial autocorrelation steadily declining and exhibiting significant negative autocorrelation at distance class five (800 m to 1000 m; r = -0.018, P = 0.004; Appendix B, Figure 2B). Autocorrelation values became positive for the next two distance classes but at distance class eight values began to decline again, remaining negative to the last distance class (1400 m to 1600 m, r = -0.016, P = 0.009; 1600 m to 1800 m; r = -0.012, P = 0.045; 1800 m to 2000 m; r = -0.013, P = 0.039; Appendix B, Figure 2B). In contrast, adult females did not exhibit any distance classes with significant autocorrelation coefficients (Appendix B, Figure 2C).

When all juveniles were pooled and analyzed separately, significant positive autocorrelation was detected in the first distance class (0 to 200 m, r = 0.251, P = 0.001; Appendix B, Figure 3A). Spatial autocorrelation quickly declined after the first distance class and reached significant negative autocorrelation at distance class three (200 m to 400 m; r = -0.034, P = 0.046; Appendix B, 3A). The correlogram line ascended in a positive direction at 600 m, but then at a distance class of 600 m to 800 m, negative significant autocorrelation occurred again (r = -0.05, P = 0.040; Appendix B, Figure 3A). Male juveniles also exhibited positive autocorrelation at distance class one (0 to 200 m, r = 0.348, P = 0.001; Appendix B, Figure 3B). At distance class four spatial autocorrelation became significantly negative (r = -0.102, P = 0.003) and again at 1200 m (r = -0.084, P = 0.006; Appendix B, Figure 3B). The correlogram line (Appendix B, Figure 3B) suggested a uniform patchy pattern for juvenile males due to frequent fluctuations in positive and negative values. Female juveniles exhibited significant positive autocorrelation in distance class one (0 to 200 m; r = 0.265, P =

0.001; Appendix B, Figure 3C). Beginning at a distance class of 600 m to 800 m negative spatial autocorrelation was found (r = -0.065, P = 0.038; Appendix B, Figure 3C). The line of the correlogram for juvenile females (Appendix B, Figure 3C) suggested an irregular patchy pattern, with a sudden decline at 800 m, as previously stated, which remained until 1200 m and then declined again steadily at 1400 m.

DISCUSSION

Prior population genetic (Prodöhl et al., 1996, 1998) and behavioral (Loughry & McDonough, 1998) studies have established that juvenile armadillos maintain close proximity to their littermates prior to fall dispersal, but it is unknown whether they remain together subsequently. The results of the present study support prior inferences of clustering of clonal siblings prior to dispersal (Prodöhl et al., 1996, 1998), while also providing novel evidence of spatial genetic structure in adult male armadillos. Because female-biased dispersal is rare in mammals (Lawson Handley & Perrin, 2007), my results raise interesting questions about ecological and population genetic processes that could result in increased genetic similarity between geographically proximate male armadillos.

Even though there were a limited number of loci used in this study (n = 8), it has been demonstrated that larger sample sizes are more effective when estimating dispersal (Goudet et al., 2002; Lawson Handley & Perrin, 2007; Banks & Peakall, 2012). According to Goudet et al. (2002), the power to detect sex-biased dispersal is positively correlated with bias intensity, sampling intensity and genetic variation per locus. Thus, the results of the present study suggest that the sample size of armadillos and level of polymorphism at each locus were sufficient to detect even the weak differences in spatial genetic structure between males and females that are reported here.

A previous population genetic study of this species by Prodöhl et al. (1996) used seven microsatellite DNA loci to identify presumptive siblings and found that average distances between juvenile siblings were significantly less than distances between adult siblings. The results of the present study, based on measurement of spatial genetic

autocorrelation between pairs of individuals over different distance lags (i.e., correlogram analysis) indicated significant, positive autocorrelation between juveniles in the first distance class (≤ 200 m), at a magnitude that was two to three times higher than what was observed for adult males. This high level of spatial autocorrelation at relatively short distances likely reflects close proximity between clonal siblings prior to dispersal, thus supporting the inferences of Prodöhl et al. (1996). Such positive spatial autocorrelation could also reflect patches of related individuals that have different mothers but share the same father (i.e., polygyny), albeit additional analytical work would be required to test this possibility.

The shapes of the correlograms can be used to provide additional information about the patterns of genetic similarity within the Yazoo population. Most correlograms indicated irregular patches of genetically similar individuals; significant positive autocorrelation quickly diminished beyond the first several distance classes and then oscillated above and below the null expectation. While most correlograms were consistent with irregular patchiness, some subtle differences are worth noting for the sake of further investigation. For juveniles, the spatial autocorrelation coefficient was lower for females in the first distance class and seemed to decline steadily, indicative of a steady decline in genetic distance with increasing geographic distance. Although inconclusive, due to the fact that the confidence intervals for the autocorrelation coefficients overlap for males and females in each distance class, it seems unlikely that the steady decline in the autocorrelation coefficient for juvenile females was due to chance alone. This may indicate reduced spatial cohesion in juvenile females, perhaps

indicative of female-biased dispersal at an earlier stage than had been previously recognized.

The correlogram pattern for adults was also consistent with irregular patches of genetically similar individuals, but with substantially lower autocorrelation than observed for juveniles over the same distances. For adults, however, the significant autocorrelation over short distances appears to be driven entirely by positive autocorrelation between males; significant autocorrelation was not observed in any distance class for adult females. The gradual decline in the autocorrelation coefficient for males suggests that genetic similarity was less patchy than what was observed for juvenile males.

Sex-biased differences in spatial genetic structure were unexpected for the nine-banded armadillo because behavioral data (based on long term capture/recapture data from this and other populations) have failed to detect significant sex differences in adult capture/recapture distances (Loughry & McDonough, 2001). The results of the present study suggest that there could be differences in long-distance dispersal tendencies between males and females after leaving natal areas and/or that there could be high variance in male reproductive success associated with a polygynous mating system (resulting in higher genetically similarity between males as compared to females). Observational data provide some evidence of polygyny in armadillos (McDonough, 1997), which may support this latter hypothesis.

Mating systems play a key role in understanding the intensity and direction of sex-biased dispersal, along with inbreeding avoidance, kin competition, and mortality (Gaines & McClenaghan, 1980; Wolff, 1994; Lawson Handley & Perrin, 2007). Two of

these factors may play a role in explaining the patterns of spatial genetic structure found in this study. First, populations of nine-banded armadillos exhibit overlap between generations; hence, to avoid inbreeding, some mechanism must exist to avoid possible mother-son or father-daughter mating. If males are philopatric, female-biased dispersal could occur as a mechanism of inbreeding avoidance. Second, Greenwood's (1980) resource-competition hypothesis predicts female-biased dispersal if males benefit more than females from philopatry, perhaps because of increased familiarity with an area and the resources it provides. In the case of nine-banded armadillos, breeding males might also benefit from philopatry because of increased access to female mates. Breeding male armadillos defend their home ranges, thereby generating minimal space overlap with other breeding males, and are then able to pair (and presumably mate) with the adult females whose home ranges overlap theirs (McDonough, 1994, 2000). If establishing a home range elsewhere that will provide sufficient mating opportunities is more costly (or less likely) than remaining in the natal area, then males might be selected to be philopatric, thus generating the enhanced spatial genetic structure in adult males that was detected. In contrast, females do not have to defend home ranges in order to gain multiple breeding opportunities. Dispersing females may easily find a home range that has food resources and areas for burrow construction, and may typically pair with the male that overlaps their home range the most (McDonough, 2000). Thus, females might be better able to "afford" dispersal than males because they can settle anywhere and still have the opportunity to reproduce. In any case, while there are plausible hypotheses to explain the patterns of genetic structure I have found, they

remain somewhat speculative and consequently, no firm conclusions can be made at this time.

Even though female dispersal is rare in most mammalian species there have been studies that document its occurrence. For example, the greater white toothed shrew, *Crocidura russula,* showed strong female bias in post-natal dispersal, as revealed by direct evidence through marking of immigrants and emigrants (Favre et al., 1997). The Hamadryas baboon, *Papio hamadryas hymadryas*, showed evidence for female-biased gene flow as population genetic structure was roughly four times higher for paternally inherited versus maternally inherited DNA (Hammond et al., 2006). Another study involving the sac-winged bat, *Saccopteryx bilineata*, found increased levels of female-biased dispersal based on the facts that all female juveniles had dispersed prior to reproduction, and that new reproductive females in a population were immigrants (Nagy et al., 2007). Previous population genetic studies of the nine-banded armadillo have produced conflicting results regarding sex-biased dispersal. Frutos and Van den Bussche (2002) found multiple female exchanges among populations per generation in Paraguay, which they interpreted as reflecting high levels of female-biased dispersal. However, analyses of populations in Mexico indicated that male-biased dispersal was more prevalent and females were philopatric (Arteaga et al., 2012). My results support the findings of Frutos and Van den Bussche (2002), but it remains unclear how widespread female-biased dispersal is in populations of armadillos, or what is different between populations that exhibit male- versus female-biased dispersal.

While male-biased spatial genetic structure could result from female-biased dispersal, observed spatial genetic structure depends on both the pattern of dispersal and

the mating system. For example, in a polygynous mating system, males may mate with groups of females that occupy an area, resulting in high levels of relatedness between offspring of different females. Nevertheless, spatial genetic structuring associated with cohorts of harem offspring may or may not be incorporated into the gene pool of adults depending on the dispersal patterns of juveniles; if both males and females disperse, spatial genetic structure associated with polygyny may be diluted (McCracken & Bradbury, 1977; Storz, 1999). In the present study of the nine-banded armadillo, the male-biased spatial genetic structure observed in adults probably reflects some combination of the mating system and dispersal, but, due to a lack of clear information about the mating system and long-distance dispersal tendencies in this species, the mechanisms underlying the observed pattern remain unclear. With that said, the fact that the observed spatial genetic structure was weak suggests that it probably does not reflect both high variance in male reproductive success and strongly female-biased dispersal. Further population genetic analyses and simulation-based modeling would be required to clarify this issue.

In summary, my results demonstrate differences in spatial genetic structure between different sex/age subgroups within the Yazoo population of armadillos driven primarily by adult and juvenile males. Although only eight polymorphic microsatellite markers were examined, 402 individuals provided enough power to determine spatial genetic structure between sex/age subgroups.

BIBLIOGRAPHY

Arteaga, M.C., D. Pinero, L.E. Eguiarte, J. Gasca and R.A. Medellin. "Genetic structure and diversity of the nine-banded armadillo in Mexico." Journal of Mammalogy 93(2012): 547-559.

Banks, S.C. and R. Peakall. "Genetic spatial autocorrelation can readily detect sex-biased dispersal." Molecular Ecology 21(2012): 2092-2105.

Bowman, J., J.A.G. Jaeger and L. Fahrig. "Dispersal distance of mammals is proportional to home range size." Ecology 83(2002): 2049-2055.

Chinchilla, L. S. "Genetic variation and leprosy distribution in nine-banded armadillos." MS thesis, Mississippi State University, Starkville (2011).

Dobson, F.S. and W.T. Jones. "Multiple causes of dispersal. The American Naturalist 126(1985): 855-858.

Favre, L., F. Balloux, J. Goudet and N. Perrin. "Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns." Proceedings of the Royal Society of London B: Biological Sciences 264(1997): 127-132.

Frutos, S.D. and R.A. Van Den Bussche. "Genetic diversity and gene flow in nine-banded armadillos in Paraguay." Journal of Mammalogy 83(2002): 815-823.

Gaines, M.S. and L.R. McClenaghan Jr. "Dispersal in small mammals." Annual Review of Ecology and Systematics 11(1980): 163-196.

Gauffre, B., E. Petit, S. Brodier, V. Bretagnolle and J.F. Cosson "Sex-biased dispersal patterns depend on the spatial scale in a social rodent." Proceedings of the Royal Society of London B: Biological Sciences 276(2009): 3487-3494.

Goudet, J., N. Perrin and P. Waser. "Tests for sex-biased dispersal using bi-parentally inherited genetic markers." Molecular Ecology 11(2002): 1103-1114.

Greenwood, P.J. "Mating systems, philopatry and dispersal in birds and mammals." Animal Behaviour 28(1980): 1140-1162.

Hammond, R.L., L.J. Lawson Handley, B.J. Winney, M.W. Bruford and N. Perrin. "Genetic evidence for female-biased dispersal and gene flow in a polygynous primate." Proceedings of the Royal Society of London B: Biological Sciences 273(2006): 479-484.

Koenig, W.D., D. Van Vuren and P.N. Hooge. "Detectability, philopatry, and the distribution of dispersal distances in vertebrates." Trends in Ecology and Evolution 11(1996): 514-517.

Lawson Handley, L.J. and N. Perrin. "Advances in our understanding of mammalian sex-biased dispersal." Molecular Ecology 16(2007): 1559-1578.

Loughry, W.J. and C.M. McDonough. "Are road kills valid indicators of armadillo population structure?" The American Midland Naturalist 135(1996): 53-59.

Loughry, W.J. and C.M. McDonough. "Spatial patterns in a population of nine-banded armadillos (*Dasypus novemcinctus*)." The American Midland Naturalist 140(1998): 161-169.

Loughry, W.J. and C.M. McDonough. "Natal recruitment and adult retention in a population of nine-banded armadillos." Acta Theriologica 46(2001): 393-406.

Loughry, W.J. and C.M. McDonough. The nine-banded armadillo: a natural history. University of Oklahoma Press (2013).

Loughry, W.J., P.A. Prodöhl, C.M. McDonough, W.S Nelson and J.C. Avise. "Correlates of reproductive success in a population of nine-banded armadillos." Canadian Journal of Zoology 76(1998): 1815-1821.

McCracken, G.F. and J.W. Bradbury. "Paternity and genetic heterogeneity in the polygynous bat, *Phyllostomus hastatus*." Science 198(1977): 303-306.

McDonough, C.M. "Determinants of aggression in nine-banded armadillos." Journal of Mammalogy 75(1994): 189-198.

McDonough, C.M. "Pairing behavior of the nine-banded armadillo (*Dasypus novemcinctus*)." The American Midland Naturalist 138(1997): 290-298.

McDonough, C.M. "Social organization of nine-banded armadillos (*Dasypus novemcinctus*) in a riparian habitat." The American Midland Naturalist 144(2000): 139-151.

McDonough, C.M. and W.J. Loughry. "Impacts of land management practices on a population of nine-banded armadillos in northern Florida." Wildlife Society Bulletin 33(2005): 1198-1209.

Nagy, M., G. Heckel, C.C. Voigt and F. Mayer. "Female-biased dispersal and patrilocal kin groups in a mammal with resource-defence polygyny." Proceedings of the Royal Society of London B: Biological Sciences 274(2007): 3019-3025.

Nathan, R., G. Perry, J.T. Cronin, A.E. Strand and M.C. Cain. "Methods for estimating long-distance dispersal." Oikos 103(2003): 261-273.

Nussey, D.H., D.W Coltman, T. Coulson, L.E.B. Kruuk, A. Donald, S.J. Morris, T.H. Clutton-Brock and J. Pemberton. "Rapidly declining fine-scale spatial genetic structure in female red deer." Molecular Ecology 14(2005): 3395-3405.

Peakall, R. and P.E. Smouse. "GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update." Bioinformatics 28(2012): 2537-2539.

Prodöhl, P.A., W.J. Loughry, C.M. McDonough, W.S. Nelson and J.C. Avise. "Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine banded armadillo *Dasypus novemcinctus*." Proceedings of the Royal Society of London B: Biological Sciences 263(1996): 1643-1649.

Prodöhl, P.A., W.J. Loughry, C.M. McDonough, W.S. Nelson, E.A. Thompson and J.C. Avise. "Genetic maternity and paternity in a local population of armadillos assessed by microsatellite DNA markers and field data." The American Naturalist 151(1998): 7-19.

Queller, D.C. "Quantitative genetics, inclusive fitness, and group selection." The American Naturalist 139(1992): 540-558.

Smouse, P.E. and R. Peakall. "Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure." Heredity 82(1999): 561-573.

Storz, J.F. "Genetic consequences of mammalian social structure." Journal of Mammalogy 80(1999): 553-569.

Taber, F.W. "Contribution on the life history and ecology of the nine-banded armadillo." Journal of Mammalogy 26(1945): 211-226.

Talmage, R.V., and G. D. Buchanan. The armadillo: a review of its natural history, ecology, anatomy and reproductive physiology. Rice University, 1954.

Taulman, J.F. and L.W. Robbins. “Recent range expansion and distributional limits of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States.” Journal of Biogeography 23(1996): 635-648

Wilson, D.S., G.B. Pollock and L.A. Dugatkin. “Can altruism evolve in purely viscous populations?.” Evolutionary Ecology 6(1992): 331-341.

Wolff, J.O. “More on juvenile dispersal in mammals.” Oikos 71(1994): 349-352.

Wright, S. “Isolation by distance.” Genetics 28(1943): 114-138.

APPENDIX A:

Genetic Diversity Measures of Eight Genetic Markers

Table 1: Genetic diversity measures of each locus using GenAlEx 6.5 (Peakall and Smouse 2012) software. The number of alleles is given for each locus as well as Expected Heterozygosity (H_E) and Observed Heterozygosity (H_O). $P > 0.05$ = no significance, $P \leq 0.05$* = significant.

Locus	H_E	H_O	*P*-value	# of alleles
Dnov 1	0.708	0.738	0.905	7
Dnov 5	0.661	0.624	0.070	7
Dnov 7	0.528	0.511	0.991	5
Dnov 24	0.659	0.646	0.187	5
Dnov 51	0.197	0.195	0.962	3
Dnov 2092	0.254	0.254	0.995	4
Dnov 2179	0.586	0.536	0.017*	7
Dnov 3824	0.506	0.513	0.008*	4

APPENDIX B:

Spatial Correlograms of Nine-Banded Armadillos

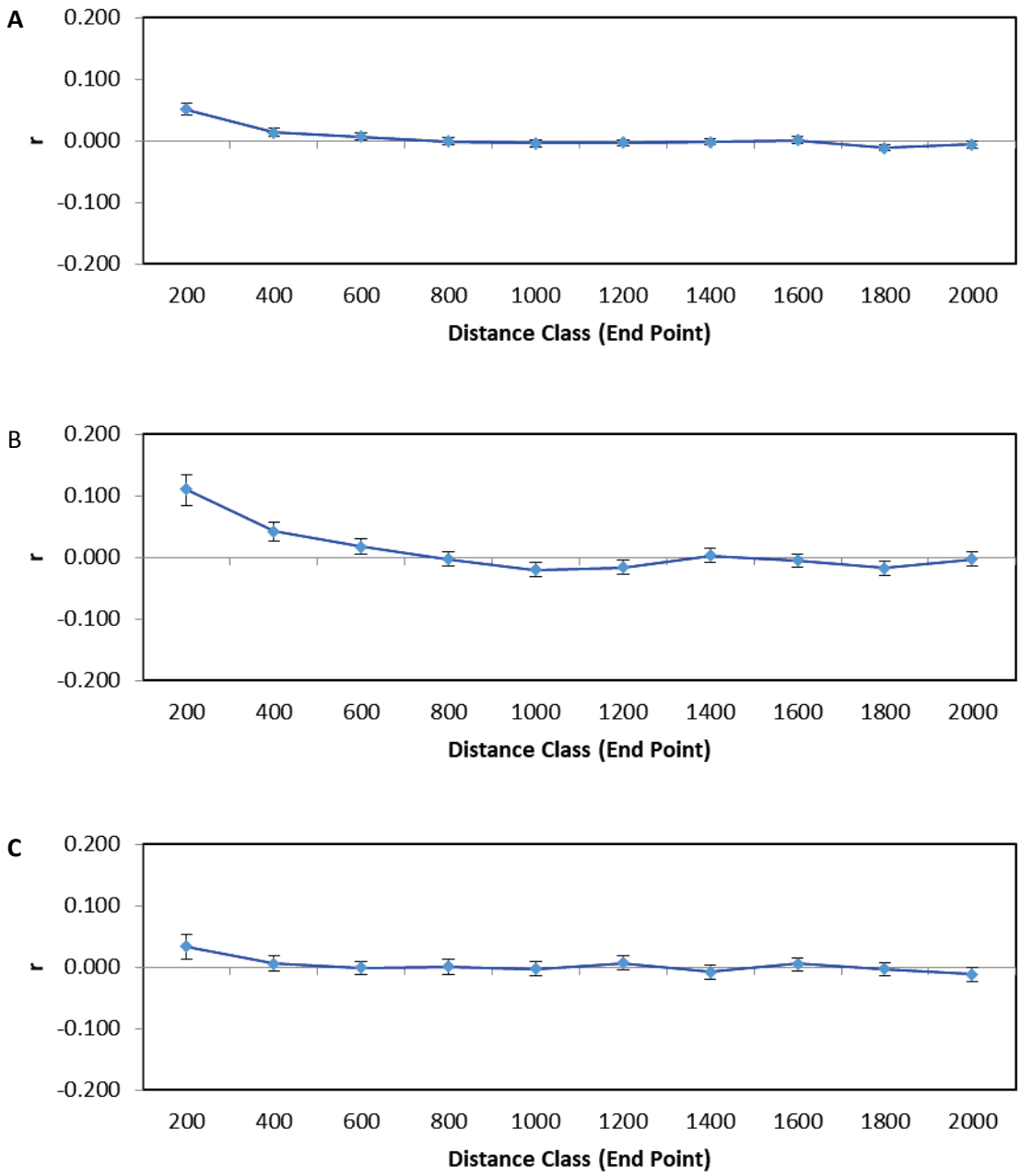


Figure 1: Correlograms of A) All armadillos, B) All males, C) All females. The correlogram line represents spatial autocorrelation values of the *r* coefficient. Upper and lower error bars represent 95 percent confidence intervals as determined by bootstrap resampling.

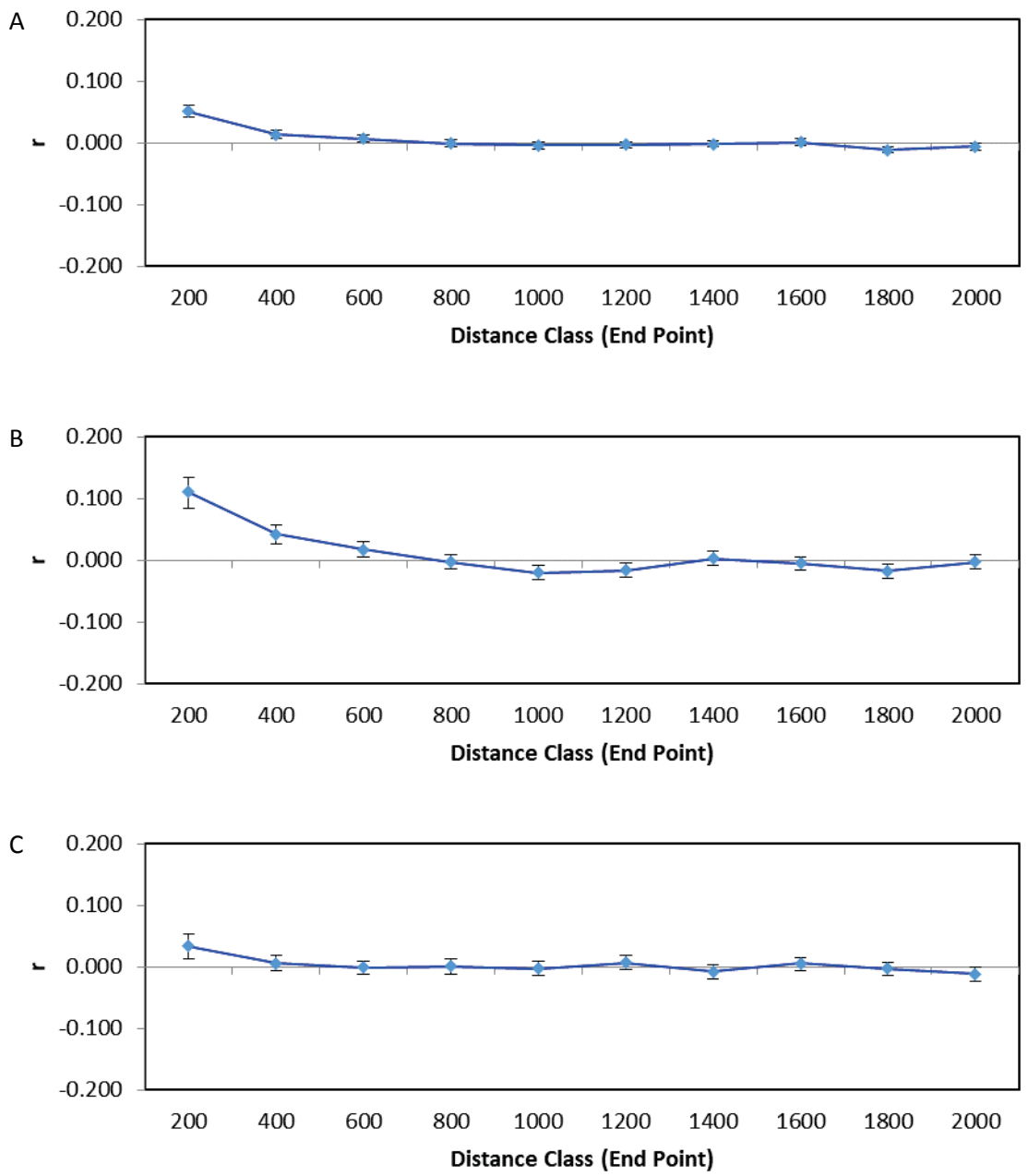


Figure 2: Correlograms of A) All adult armadillos, B) Adult males, C) Adult females. The correlogram line represents spatial autocorrelation values of the *r* coefficient. Upper and lower error bars represent 95 percent confidence intervals as determined by bootstrap resampling.

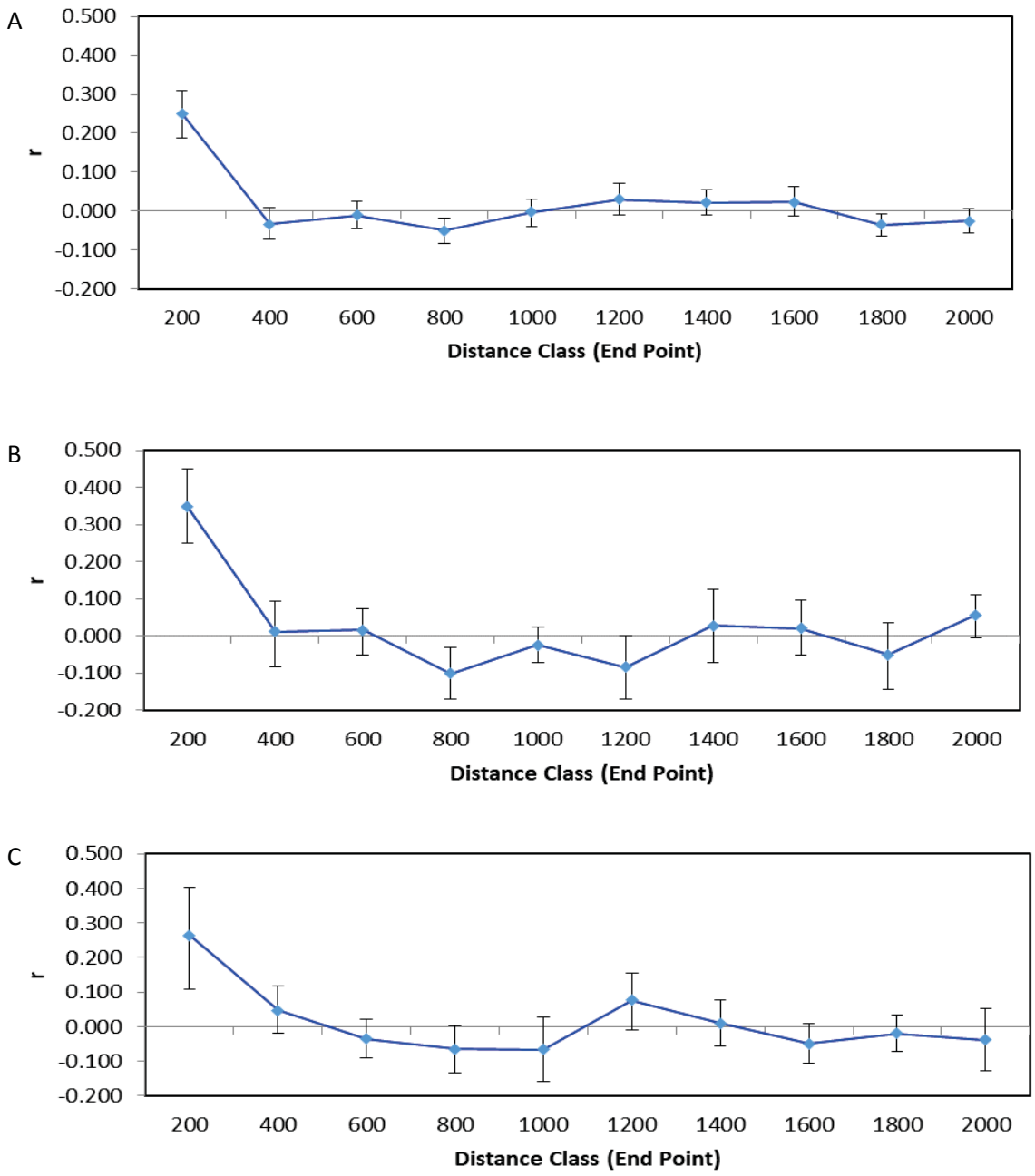


Figure 3: Correlograms of A) All Juvenile armadillos, B) Juvenile males, C) Juvenile females. The correlogram line represents spatial autocorrelation values of the *r* coefficient. Upper and lower error bars represent 95 percent confidence intervals as determined by bootstrap resampling.