SIZE, SIRING SUCCESS, AND MULTIPLE PATERNITY IN THE GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*)

by

KIMBERLY NICOLE WHITE

(Under the Direction of Tracey D. Tuberville and Jeff A. Hepinstall-Cymerman)

ABSTRACT

I evaluated the rate of multiple paternity and the effects of male size on likelihood of siring offspring and how many offspring a sire in a population of gopher tortoises at Archbold Biological Station in Florida. Additionally, I assessed how the ratio of male:female size influenced multiple paternity patterns. I collected nests in 2015 and 2016. Using microsatellite markers, I genotyped all hatchlings and most potential dams and sires in the population. I observed multiple paternity in 24% of clutches, within range of previously reported rates. Larger males were significantly more likely to sire offspring than smaller males. Additionally, size was positively correlated (though not significant) with number of offspring sired. I found no effect of male:female size ratios on clutch paternity. Understanding factors influencing reproduction of this declining species may be important for developing management strategies that maximize evenness in reproductive success.

INDEX WORDS: *Gopherus polyphemus*, Siring success, Mating system, Reproduction

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B.S., Birmingham-Southern College, 2011

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2017

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August 2017

DEDICATION

I would like to dedicate my thesis to my parents, Brad White and Barbara Saurer. They have been a never-ending source of support and inspiration during this project and I would not be in the position for success that I am now without them.

ACKNOWLEDGEMENTS

First and foremost, I extend my sincerest gratitude to my co-advisors, Dr. Tracey Tuberville and Dr. Jeff Hepinstall-Cymerman, and my committee members, Dr. Betsie Rothermel and Dr. Brian Shamblin. Throughout my thesis, they provided essential support without which this project would not have been possible.

There have been many hands and backs that supported this project. I would like to thank Michael White, Rebecca Tucker, Kurt Buhlmann, Ethan Noel, Laura Elston, Alessandro Molina, Susan Deans, Jessica Dryer, Samantha Apgar, and Amanda Chang for field help and moral support throughout my fieldwork. I greatly appreciate the support of Archbold Biological Station for allowing me to reside in field housing during my first season of fieldwork. Special thanks go to Scott Rothermel and Betsie Rothermel for providing housing during my second field season.

I would like to acknowledge Dr. Clint Moore for statistics advice and support, and John Yeiser for figure and model coding help. From Cornell University, I would like to thank Miranda Gray for essential lab instruction and assistance and Dr. Kelly Zamudio for advice and funding support.

This research was conducted using approved protocols under University of Georgia Animal Care and Use Committee (# A2014 05-024-Y3-A3) and under Florida Fish and Wildlife State Collecting Permit # LSSC-10-00043D. This project was funded by the Department of Energy under award number DE-FC09-07SR22506 to the University of Georgia Research

Foundation. Additional funding was provided by Disney World Wildlife Conservation Fund, the Warnell School of Forestry and Natural Resources at the University of Georgia, Archbold Biological Station, the Riverbanks Zoo Conservation Foundation, the American Wildlife Conservation Foundation, the Chicago Herpetological Society, the North Carolina Herpetological Society, the American Society of Ichthyologists and Herpetologists, and the Gopher Tortoise Council.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Literature Review

Body size effects on life history traits have been relatively well documented across taxa (Blueweiss *et al.* 1978; Blanchfield *et al.* 2003; Hughes 2016). Though size effects on fitness are not always positively correlated, larger size can provide advantages for reproduction in many species. Larger body sizes can confer advantages to both males and females and are often associated with increased mating opportunities (Schuett 1997; Kovach & Powell 2003) and increased fecundity (Congdon & Gibbons 1985; however, see Ashton et al. 2007). Most reptile mating systems are polygynous which typically results in large skew in the distribution of reproductive success among males (Emlen & Oring 1977). While a variety of traits have been correlated with increases in reptile reproductive success, including residence time (Massot *et al.* 1994; Tuberville *et al.* 2011; Mulder *et al.* 2017), courtship intensity (Sacchi *et al.* 2003), and dominance status (Schuett 1997), body size effects on reproductive success of males has been one of the most widely documented. Body size advantages in reproductive success (number of mates) have been reported in red-sided garter snakes (*Thamnophis sirtalis parietalis;* Shine *et al.* 2000), copperheads (*Agkistrodon contortrix;* Schuett 1997), and several species of turtles including desert tortoises (*Gopherus agassizii*; Schuett 1997), Blanding's turtles (*Emydoidea blandingii;* McGuire *et al.* 2015), and painted turtles (*Chrysemys picta marginata*; McGuire *et al.* 2014).

Multiple paternity (MP), the distribution of paternity of a single clutch to more than one male, has been observed in many reptile species (Schuett & Gillingham 1986; Valenzuela 2000; Roques *et al.* 2006; Refsnider 2009). Direct benefits of multiple mating, such as increased parental care or nuptial gifts, are uncommon in reptiles (Emlen & Oring 1977; Uller & Olsson 2008). Thus, indirect benefits of MP are more likely driving the frequency of MP in reptiles (Pearse & Avise 2001). Indirect benefits can include insurance against infertile partners, potential reduction in likelihood of inbreeding (Tregenza & Wedell 2002; Bretman *et al.* 2004), increased genetic diversity (Calsbeek *et al.* 2007), and further assessment of male quality via sperm competition (Fedorka & Mousseau 2002). The characteristics of multiple paternity have been poorly studied in many species, due to difficulty in obtaining sufficient proportions of offspring and adults to fully assess parentage patterns.

Rates of multiple paternity in turtles range widely (0-100%) and vary by population and species (reviewed by Davy *et al.* 2011). Mating order, not size, accounts for patterns of MP in *Chrysemys picta* (Pearse *et al.* 2001, 2002), suggesting a last-in-first-out scenario of last male precedence in siring offspring. In tortoises, no effects of male size on MP have been reported, though in *Testudo hermanii hermanii*, male size is correlated with increased mounting success (Sacchi *et al.* 2003). Assessing drivers of MP patterns has proven difficult in most testudines.

The gopher tortoise (*Gopherus polyphemus*) is native to the southeastern United States, requiring habitats with open canopies and diverse understories (Diemer 1986) characteristic of the longleaf pine savannahs that once dominated the southeast. The gopher tortoise is designated as a keystone species (Catano & Stout 2015) and considered an ecosystem engineer (Kinlaw & Grasmueck 2012). The gopher tortoise burrow plays a valuable role for more than

390 species (Speake 1981; Jackson & Milstrey 1989) by providing shelter, nesting sites (White *et al.* 2017a; b), and foraging opportunities (White & Tuberville *in press*). However, reductions in available suitable habitat, habitat degradation, and habitat fragmentation have produced corresponding declines in gopher tortoise populations in the last century. In the western portion of its range, the gopher tortoise is federally threated Endangered Species Act in 2012, though it remains a candidate species for listing in the remaining portion of its range (US Fish and Wildlife Service 2011).

Much of the current literature on social behaviors and mate selection in the gopher tortoise has primarily been assessed in the gopher tortoise via behavioral observations. Gopher tortoises form relatively complex, stable social groups, with males being most central to the social network (Guyer *et al.* 2014). Male home ranges are typically larger than those of females, driven by frequent and sometimes long-distance courtship visits to females (McRae *et al.* 1981; Diemer 1992). Although molecular parentage assignment has revealed sires tend to be larger than non-sires (Tuberville *et al.* 2011), male size does not appear to be under intense behavioral selection as large males are neither central to social groups (Guyer *et al.* 2014) nor do they have any apparent advantage over smaller males in terms of mounting success (Boglioli *et al.* 2003). Molecular evaluations of gopher tortoise parentage results reveal population-level differences in rates of multiple paternity (28%, Moon *et al.* 2006; 57%, Tuberville *et al.* 2011). While Moon *et al.* (2006) suggest that multiple paternity is driven by large male domination of mating opportunities with smaller females, their small sample size (n = 7) limits the strength of this conclusion.

The gopher tortoise mating system has been described as both harem defense polygyny (Douglass 1986) and, more recently, scramble competition polygyny (Boglioli *et al.* 2003; Johnson *et al.* 2009). Both assessments accurately describe the mating system from the male perspective in that males visit multiple females within a mating season. However, both descriptions fail to account for multiple mating by females. Females may receive courtship visits from up to 14 males each mating season (Boglioli *et al.* 2003). Additionally, male gopher tortoises visit the same females in multiple years (Douglass 1986). Incorporation of all life history and behavioral knowledge into descriptions of the mating system is critical to developing effective conservation and management strategies.

Purpose of the Study

The goal of this study is to provide further evaluation of the gopher tortoise (*Gopherus polyphemus*) mating system dynamics. Specifically, I evaluate how body size of males affects siring success (whether individuals sire offspring) and reproductive success (number of offspring produced). I also investigated whether male coercion (forcible matings) might contribute to multiple paternity in this species, by examining the effects of male:female body size ratio on whether or not a clutch is produced by one or multiple sires. My overall aim is to provide a more complete description of the gopher tortoise mating system using molecular techniques to explore parentage patterns.

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CHAPTER 2

SIZE EFFECTS ON SIRING SUCCESS AND MULTIPLE PATERNITY IN THE GOPHER TORTOISE (*GOPHERUS POLYPHEMUS*)[1]

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[1] White, K. N., B. B. Rothermel, K. R. Zamudio, and T. D. Tuberville. To be submitted to *Molecular Ecology*.

Abstract

In many vertebrates, male body size likely influences individual fitness; hence, it may be important to siring success. More fit individuals may be more likely to dominate mating opportunities, skew siring success rates, and lower effective population sizes and genetic diversity. The mating system and reproduction of the gopher tortoise (*Gopherus polyphemus*) have been explored primarily through behavioral observations while just two studies have used molecular approaches to investigate reproductive patterns. We used molecular techniques to investigate how body size influences patterns of paternity and reproductive success in the gopher tortoise, a long-lived terrestrial turtle native to the southeastern United States. In 2015 and 2016, we collected 31 nests from a gopher tortoise population at Archbold Biological Station, Florida, and incubated the eggs until hatching. Using 11 previously-developed microsatellite markers, we genotyped all hatchlings (n = 220) and most potential breeders in the population (n = 97) and used programs CERVUS and COLONY to assign each hatchling to the most likely dam and sire. We determined the frequency of multiple paternity and evaluated the effects of male body size on the probability of siring and the number of offspring sired using a zero-inflated Poisson mixture model. We observed multiple paternity in 24% of clutches. Larger males were significantly more likely to sire offspring than smaller males. Additionally, number of offspring sired increased with male size, although this trend was not significant. The likelihood of a clutch being multiply-sired was unrelated to female body size or male:female size ratio. Our findings provide further support for previous evidence of male body size effects on reproductive success and underscore the need for combining molecular techniques and behavioral observations for assessments of animal mating systems.

Introduction

Distribution of mating success within a population can vary with mating system (reviewed by Kokko & Rankin 2006). In monogamous mating systems, male mating success is generally more evenly distributed, while in polygamous systems, the success is often skewed toward a handful of males, driving sexual selection according to the traits of successful males. Which traits are important vary widely by species (Emlen & Oring 1977). Effects of body size of males and females on reproductive success have been investigated widely. In females, larger body size may result in increases in size or quality of clutches or offspring size (Hailey & Davies 1987; Rothermel & Castellón 2014). Increases in male body size results in concurrent increases in offspring sired (Blouin-Demers *et al.* 2005).

Reptile mating systems are typically polygynous. In some reptile species, male body size drives reproductive success (Olsson & Shine 1996; Schuett 1997; Shine et al. 2000; Hofmann & Henle 2006). Reproductive success may be driven directly through female selection for larger males (Hunt *et al.* 2009; Cutuli *et al.* 2014) or indirectly through competitive interactions between males that afford the winner increased access to females (Schuett 1997). That is, male traits may not be directly assessed by females but may influence male success by increasing or limiting opportunities to mates through male competition. Larger size advantage in male-male competition and dominance status has been observed in copperheads (*Agkistrodon contortrix;* Schuett 1997), red-sided garter snakes (*Thamnophis sirtalis parietalis;* Shine *et al.* 2000), and desert tortoises (*Gopherus agassizii*; Schuett 1997).

Although the effects of size on mate selection have been investigated, the factors influencing multiple paternity (i.e., the inclusion of more than one sire in a single clutch of eggs;

henceforth, MP) are not well understood, despite the fact that MP has been recorded in many species of reptiles (Pearse & Avise 2001; Roques et al. 2006; Uller & Olsson 2008; Lance et al. 2009; Farke et al. 2015; Duran et al. 2015). Direct benefits of multiple mating, such as increased parental care observed in other taxa (as reviewed by Fedorka & Mousseau 2002), are uncommon in reptiles. Thus, it is more likely females experience indirect fitness gains, such as increased clutch fertility (Pearse & Avise 2001) or increased hatching success (Blouin-Demers *et al.* 2005). The degree of MP varies greatly both among taxa and within species (Pearse & Avise 2001). However, the difficulty of obtaining sufficient sample sizes of nests is problematic in many studies (Valenzuela 2000; Roques et al. 2004; Moon et al. 2006; Cutuli et al. 2014; Guyer et al. 2014). Even when nest sample sizes are high, adequate adult sampling to evaluate effects of morphological traits influencing MP can be challenging if potential breeders are difficult to capture (Pearse et al. 2002). These factors complicate efforts to quantify reproductive success and siring success in reptiles.

The gopher tortoise (*Gopherus polyphemus*) is a long-lived, terrestrial turtle native to the southeastern United States. Gopher tortoises are typically aggregated in clusters of burrows referred to as colonies (McCoy & Mushinsky 2007). A recent examination of the gopher tortoise social system revealed complex social structure, including formation of restricted social groups or cliques (Guyer et al. 2014). Males typically have larger home ranges, conducting regular forays from their burrows in search of courtship and mating opportunities (McRae et al. 1981; Diemer 1992). Gopher tortoises exhibit female-biased sexual dimorphism (McRae *et al.* 1981). Females may receive courtship opportunities from multiple males (up to 26 visits by 6-12 males) within a mating season (Boglioli et al. 2003; Johnson et al. 2007). In addition, females are capable of storing sperm from multiple males for several years (Palmer & Guillette Jr. 1988).

Two mating system types have been reported for the gopher tortoise. Based on opportunistic observations of free-ranging tortoises as well as observations of assigned pairs in an experimental setting, Douglass (1986) first described the gopher tortoise mating system as a loose or incipient harem defense polygyny. Harem defense polygyny tends to arise when females are spatially clumped, making it feasible for males to defend multiple females (Emlen & Oring 1977). However, Johnson et al. (2009), based on remote-camera observations of tortoise behaviors, argued the mating system is best characterized as scramble competition polygyny, in which males primarily attempt to outcompete other males for access to females. In Johnson *et al.*'s study population, harem defense appeared to be prevented by the widely-spaced dispersion of females in their study populations. None the previous characterizations the mating system evaluated which males were ultimately successful in siring offspring. Therefore, the ultimate patterns of reproductive success and variation in male fitness remained unclear.

Molecular techniques, such as genotyping of individuals using highly variable microsatellite markers, can provide direct assessment of siring and reproductive success. However, genetic assessments of parentage in gopher tortoises are limited to two studies (Moon et al. 2006; Tuberville et al. 2011). Moon *et al* (2006) observed MP in two of seven (28%) clutches in a naturally occurring population on the University of South Florida Ecological Research Area near Tampa, Florida. In contrast, Tuberville *et al.* (2011) was able to collect a larger sample of gopher tortoise nests from a population of translocated gopher tortoises on St.

Catherines Island in Camden Co., Georgia. They observed MP in 57% of clutches (8/14 clutches). Like Moon *et al.* (2006), Tuberville *et al.* (2011) also found that larger males were more likely to successfully sire offspring than smaller counterparts. Tuberville *et al.* (2011) also found that male residence time exerted stronger influence on the number of offspring sired. However, because the Tuberville *et al.* study was based on a translocated population, it may not necessarily reflect patterns in unmanipulated natural populations. While Moon et al. (2006) reported a much lower rate of MP than Tuberville *et al.* (2011), this was perhaps due to differences in study design or characteristics of their study populations. Moon *et al.* (2006) also proposed that smaller females were more likely to produce MP clutches due to their inability to defend against nonconsensual mating attempts from larger males. Both studies were limited in the scope of inference due to small sample sizes and the proportion of females in each population whose clutches were sampled.

In this study, we assessed the influence of size on male reproductive success in the gopher tortoise by determining genetic patterns in siring success of males and rates of MP across two nesting seasons in a natural population in central Florida. We expect that in a scramble-competition polygynous mating system, morphological characteristics of a competing male should be less important than male effort expended per female. Conversely, male morphology (e.g., size) is more likely to determine reproductive success in female defense mating systems (FDMS; Emlen & Oring 1977). If our study system adhered to FDMS, we expect male body size would be less important in determining reproductive. Specifically, we hypothesized that 1) siring success (defined as whether a male sires offspring) increases with male body size and 2) reproductive success (defined as the number of offspring sired per male) also increases with male size. We also investigated whether male-to-female body size ratio influences the siring outcome of a clutch (i.e., whether it is sired by one male or multiple males). If smaller females are less able to defend themselves against larger males, a larger ratio of male to female body size should be more likely to result in a MP clutch as smaller females would likely be forced to mate with more males than larger females.

Methodology

*Study Site***:** Archbold Biological Station (ABS) is located at the southern end of the Lake Wales Ridge in Highlands County, Florida. This 2,102-ha site encompasses Florida scrub, southern ridge sandhill, and flatwood habitats (Abrahamson et al. 1984). Inland Florida scrub and southern ridge sandhills have well-drained soils and are restricted to remnant dunes formed during the Plio-Pleistocene epoch (Abrahamson et al. 1984). Southern ridge sandhill vegetation is dominated by turkey oak (*Quercus laevis*), scrub hickory (*Carya floridana*), and south Florida slash pine (*Pinus elliottii* var *densa*). The midstory shrub layer is dominated by oaks (*Quercus* spp.) and palmettos (*Serenoa repens* and *Sabal etonia*; Abrahamson et al. 1984). A diverse understory of grasses and forbs, including wiregrass (*Aristida stricta*) and gopher apple (*Licania michauxii*), provides abundant forage for tortoises (Abrahamson et al. 1984).

Our study population is centered on a site within ABS known as Hill Garden (hereafter, HG). HG is a 7-ha area of human-modified, old-field habitat surrounded by approximately 50 ha of southern ridge sandhill in varying stages of restoration following decades of fire suppression (Fig. 2.1). After a wildfire in 1927, fires were actively suppressed in the units surrounding HG (Ashton et al. 2008), which led to canopy closure and loss of the diverse ground-level herbaceous plant community. As a result, tortoises in this part of ABS were relegated to the remaining open areas such as HG and the edges of sand firebreaks and roads (Ashton et al. 2008). During 1985-2011, management of the HG area consisted of occasional prescribed fire and much of the area remained fire suppressed. Since 2012, the HG and adjacent units have been subjected to more intensive restoration efforts involving prescribed fire and mechanical thinning. The Archbold HG gopher tortoise population is the focus of a long-term mark-recapture study initiated in 1967 (Layne 1989), and was the focus of a previous observational study of mating behaviors (Douglass 1986).

Data Collection: Female tortoises often place their nests in the burrow apron (i.e., the mound of sand just outside the burrow), although they also select open sandy sites away from the burrow (Landers et al. 1980; Butler & Hull 1996). We searched the aprons of active, inactive, and collapsed burrows (1-10 times) during May – July 2015 and 2016 in and around HG, expending more search effort on burrows used by 37 radio-telemetered females. To prevent depredation, we covered nests with plastic hardware cloth (2 x 2-cm mesh size) staked to the soil before covering the hardware cloth to initial nest depth. We left all nests in situ until the 2nd week of July in 2015 and until the 1st week of July 2016 (when we found the first clutch of fully hatched eggs while nest searching). We then excavated all nests and placed them in incubators to complete incubation. We grouped eggs by clutch in Sterilite containers filled with 1:1 diH_20 and perlite substrate (by mass) and placed them in incubators maintained at 29 ± 1 ˚C with ~ 80% humidity.

Most adult tortoises (n = 101) residing in the HG had previously been captured for blood sample collection prior to 2014 (Yuan et al. 2015). During 2014 – 2016, as part of the mark-recapture study at ABS, we also hand-captured and collected blood from additional adults encountered within the study area. We recorded sex and straight carapace length (SCL; mm) for all individuals. We assigned sex based on plastral concavity, a secondary sex characteristic of males (Mushinsky et al. 1994).

For genotyping and parentage analyses, we collected <0.2-mL blood samples from the subcarapacial vein of each hatchling and 1-2 mL of blood from each adult (Hernandez-Divers et al. 2002), corresponding to less than 5% mass of each individual (Mader 2006). We stored blood samples in lysis buffer (100 mM Tris Base, 100 mM EDTA, 150 mM NaCl, 1% SDS) at -20 C until DNA extraction and extracted whole genomic DNA using QIAGEN DNeasy Blood and Tissue kits (QIAGEN N.V., Hilden, Germany).

We conducted all sampling under the approved Institutional Care and Use Committee though University of Georgia (A2014 05-024-Y3-A3), Archbold Biological Station approved protocol (ABS-AUP-014-R), and approved Florida Fish and Wildlife Commission scientific collecting permit (LSSC-10-00043).

Genotyping and Parentage Analyses: We initially screened 15 polymorphic microsatellite loci previously optimized for the tortoise population at ABS (see Yuan et al. 2015 for detailed methods) to genotype all hatchlings and adults (Schwartz et al. 2003; Tuberville et al. 2011; Kreiser et al. 2013). We calculated allele frequencies using CERVUS and included all potential dams and sires and two hatchlings with unique alleles to fully encompass the alleles present. We excluded loci with low polymorphic information content (PIC < 0.382), high frequency of null alleles (F(null) > 0.05), or failure to adhere to Hardy-Weinberg equilibrium. The 11 selected loci (Table 2.2) had low non-exclusion probabilities for both individual parents and combined parent pairs to increase likelihood of accurate parentage assignment (Table 2.3). We used

GeneMarker (v. 2.6.7) to assign genotypes to all individuals and reviewed and visually confirmed all calls made by GeneMarker.

From the assigned genotypes, we used two likelihood-based programs, CERVUS (Kalinowski et al. 2007) and COLONY (Jones & Wang 2010), to assign parentage of hatchlings. CERVUS uses a pair-wise likelihood approach, while COLONY operates on a pedigree-reconstruction approach with known sibship included. When combined, these programs provide a sound approach to assigning parentage to offspring (Karaket & Poompuang 2012).

In CERVUS, we simulated analyses of 10,000 hatchlings with 47 candidate dams and 50 candidate sires. Given the intensity of mark-recapture sampling at ABS, we estimated that 100% of potential dams and 90% of potential sires had been accounted for in the data. Individuals were only included in the simulation if at least 6 loci were successfully genotyped. With the simulation results, we used CERVUS to assign the top candidate dams and sires. We compared all delta scores for parent pairs to the 95% critical delta value calculated by CERVUS during the analysis. We used allele frequencies and null allele frequency rates calculated in CERVUS as input into COLONY. We included a flat error rate of 0.01 to account for other potential sources of error (including mutation; Hoffman & Amos 2004; Christie et al. 2014) in our data.

We compared all assignments from both programs to assess convergence of results. Exclusion-based approaches to parentage assignments, such as that employed by (Richard *et al.* 2005; Serbezov *et al.* 2010; Davy *et al.* 2011), can be sensitive to mismatches at even just one locus (Karaket & Poompuang 2012). With the addition of more loci, accuracy of CERVUS increases while accuracy of COLONY assignments may decrease (Karaket & Poompuang 2012). Given that we had sampled the majority of the HG adult population and used a relatively high number of loci to increase CERVUS accuracy, we used CERVUS as a conservative benchmark for assignment when reconciling assignments between the two programs. We reviewed genotypes of any hatchling that presented mismatches in parentage assignment between the two programs. For clutches for which multiple females were assigned to a single clutch, we compared genotypes of each hatchling to the assigned sire and majority dam. If there were fewer than two allele mismatches with the candidate dam for all hatchlings not assigned the majority dam, we assigned the majority dam to the full clutch. Any parent pairs assigned to offspring that did not meet the 95% critical delta value but were still assigned as the most likely parent pair were reviewed for allele mismatches. If fewer than three mismatches occurred across all loci, the parent pair was assigned. Assignment of additional sire(s) to the clutch by both programs was checked for number of mismatches between primary sire and offspring. Allele mismatches at >2 (18%) loci between offspring and primary clutch sire were required to merit addition of another sire (Lance et al. 2009). We excluded any additional sires assigned by COLONY if they did not meet these requirements and assigned the primary sire for the clutch.

Statistical Analyses: We selected a hierarchical zero-inflated Poisson (ZIP) mixture model to evaluate male size (SCL) effects on siring success (whether a male was a sire or non-sire) and reproductive success (count of offspring sired). We selected the zero-inflated Poisson after determining that the offspring count data were overdispersed and that a simple Poisson regression was not sufficient to assess the source of the high frequency of zeroes in the data associated with those males for which no offspring were assigned (Martin et al. 2005; Kéry 2010). The logistic component of the model evaluated the effect of sire size on whether a male was successful in siring any offspring, resulting in a binary outcome of sire or non-sire. The

Poisson regression component of the model assessed the effect of sire size on expected reproductive success (number of offspring), providing a total count of offspring. The combined effect provides an estimate of the number of offspring produced by a sire, evaluating that individual on the probability of producing any offspring multiplied by the expected number of offspring.

We included all candidate sires in our analyses. The logistic term models the phenomena that only some males are successful in siring any offspring, hence our data has many zeros (i.e., failure to reproduce). Whether an individual sired offspring ($sired_i$) is drawn from a Bernoulli distribution with the probability, $p.sire_i$, which is based on a linear model with mean-centered sire size (centered around the sample mean; *n* = 50) as the predictor variable (i.e., larger males are predicted to be more likely to successfully sire offspring) and is estimated by the logit:

$$sired_i \sim Bernoulli(p.sire_i)$$

$$logit(p.sire_i) = \alpha_o + \alpha_1 SCL_i$$

The effect of male body size on reproductive success (number of offspring sired by those males who were confirmed sires; number of offspring pooled from 2015-2016) is illustrated by the following equations where total offspring sired (t_i) is drawn from a Poisson distribution with probability zip_i. The parameter zip_i is a function of the combined effect of sire size (SCL) on λ_i from the linear model and the probability of siring any offspring ($sired_i$).

$$t_i \sim Poisson(zip_i)$$

$$zip_i = \lambda_i * sired_i$$

$$logit(\lambda_i) = \beta_0 + \beta_1 SCL_i$$

In addition, we evaluated the ratio of male-to-female (hereafter M:F) body size on the likelihood of a clutch being sired by only one male (single paternity, or SP) or by multiple males (MP) using a Bernoulli logistic regression. We used the M:F body size ratio for each single paternity clutch. For MP clutches, we calculated the average M:F ratio of contributing males for each clutch. We hypothesized that larger M:F ratios (>1; larger male(s) mated with smaller females) would be more likely to result in multiple sires per clutch than clutches produced by M:F ratios ≤1 (smaller male(s) mated with larger females). Paternity outcome (MP vs. SP) of a clutch (MP_i) is drawn from a Bernoulli distribution with probability $p.MP_i$, which is function of M:F body size ratio ($Ratio_i$).

$$MP_i \sim Bernoulli(p.MP_i)$$

$$logit(p.MP_i) = \ b_0 + b_1 Ratio_i$$

For all models, we lacked data or estimates on which to base our priors so we selected uninformative priors. We conducted all analyses in program R (R Development Core Team 2008) and OpenBugs (Lunn et al. 2009). We used packages 'R2WinBUGS' (Sturtz et al. 2005) and 'CODA' (Plummer et al. 2006).

Results

Nest searching: During May – July 2015, we searched the aprons of 149 tortoise burrows 1 – 10 times. During May – July 2016, we excavated 101 tortoise burrows 1 – 3 times. While we searched both occupied and unoccupied burrows in both seasons, in 2016, we focused our surveys on burrows used by telemetered females later in the season to minimize disturbance to potential nest sites during the egg-laying season. We collected 17 clutches in 2015 and 18 clutches in 2016 (Fig. 2.1). Clutch sizes were similar between years (mean: 8.73 ± 2.05; range: 6-

12 eggs in 2015, mean: 8.11 ± 2.54; range: 5-15 eggs in 2016; Table 2.1). Due to some egg damage during nest searching and collection, we did not incubate all eggs encountered in the field (Table 2.1). Hatching success varied among clutches but average hatching success (overall 77%) was similar between years (Table 2.1).

Genotyping: We sampled all successfully hatched offspring and all partially developed, unhatched eggs. In 2015, we collected 116 unique samples from 102 hatchlings and 14 unhatched eggs (15 clutches). In 2016, we collected 115 samples, including 21 from unhatched eggs or deceased hatchlings (16 clutches). We attempted to genotype all samples at the selected loci (see below). We excluded six samples from 2015 and five samples from 2016 due to poor amplification, resulting in 110 samples from each year for parentage analysis.

For parentage analysis, we selected 11 microsatellite loci (of 15 candidate loci; Table 2.1). Frequency of null alleles ranged from -0.0072 - 0.0518. Polymorphic information content (range 0.382 – 0.883) was greater in loci with more alleles (k; range: 3-15; Table 2.3). Combined non-exclusion probabilities were very low, indicating a low likelihood that we would be unable to exclude a non-parent based on the combination of selected loci.

Parentage Assignments: We detected 22 unique dams and 16 unique sires as contributing to the 2015-2016 clutches we sampled. For three clutches from 2016, we were unable to assign parentage to known (i.e., sampled) individuals in the population. One unsampled male and two unsampled females contributed to three clutches (Table 2.4). Most males (*n* = 32; Fig. 2.3) did not have any sampled offspring assigned to them. Of those males to which offspring were assigned, individual males contributed to 1 – 5 clutches and sired a total of 2-28 offspring from the 2015-2016 clutches sampled (Fig. 2.4). The average number of offspring attributed to successful males was similar between 2015 and 2016, although variation in number of assigned offspring among successful sires was greater in 2015 than 2016 (7.33 ± 5.73 and 8.75 ± 2.98, respectively). All females detected in our sample in both 2015 and 2016 (*n* = 9) produced offspring with the same male in both years. Most of these females produced offspring with just a single male (*n* = 8). One female produced MP clutches both years with the same two males, although the proportion of offspring attributed to each male differed between years (Table 2.3).

Male Body Size and Siring Success: We included 50 candidate males in our analyses. Male SCL ranged from 209-330 mm with an average of 275.2 ± 27.5 mm (mean ± 1 SD). Sires were on average 37 mm larger than non-sires (298.4 ± 17.1 and 261.1 ± 32.8, respectively). The results of the Bernoulli regression on probability of siring success (being a sire or non-sire) indicate that siring success increases with sire size (Table 2.4; Fig. 2.4). Males larger than 295.3 mm (14 males in this sample) had at least a 50% probability of siring offspring and males larger than 329.6 mm had a 90% probability of siring offspring. Among successful sires, the effect of size on expected number of offspring was slightly positive, indicating that larger sires are slightly more likely than smaller sires to sire more offspring, though 95% credible intervals narrowly overlap zero (Table 2.5; Fig. 2.6). However, the combined effect of the Bernoulli and Poisson regressions of size on reproductive success of male gopher tortoises is apparent (Fig. 2.7). At larger sizes, males are more likely to experience increased siring and reproductive success. We achieved model convergence on all parameters with 50,000 iterations of three chains with 1,000 iteration burn-in.

Multiple Paternity: For estimating frequency of MP, we excluded two clutches (one from 2015 and one from 2016) from female 1223 which were both too small to include (<3 offspring sampled). Thus, we calculated rates of MP in 2015 and 2016 based on 14 and 15 clutches, respectively. MP was slightly more common in 2015 (29% of clutches) than in 2016 (20% of clutches). The overall rate of MP across years was 24% (7/29 clutches). Most MP clutches were explained by assignment of two males (n = 6). However, one clutch (565-2015) required assignment of at least three males to explain offspring genotypes (Fig. 2.8).

Of males known to sire offspring, six individuals only contributed to MP clutches (mean SCL 299.5 ± 16.7 mm), while six males were assigned solely to singly-sired clutches (mean SCL 300.2 ± 22.5 mm). Three males contributed to both multiply-sired clutches and singly-sired clutches (mean SCL 292.7 ± 12.4 mm; Fig. 2.9). Proportional contributions of males to MP clutches were variable. The average contribution of the primary male was 64.5% (Fig. 2.7). Some clutches exhibited skewed distributions with one male siring at least 80% of the offspring in the clutch (Clutches 586-2015 and 620-2015), while five clutches had a more even distribution of within-clutch contribution among sires. Approximately 62.5% of the clutch with three sires (565-2015) was attributed to one male with the two remaining males contributing 25% and 12.5%.

For the analysis of M:F size ratio, we excluded five clutches (one from 2015 and four from 2016) for either insufficient sampling of clutch due to low hatching success (*n* = 2) or assignment to unsampled parents (*n* = 3). We used the remaining 7 MP clutches (3 from 2015 and 4 from 2016) and 19 SP clutches (11 from 2015 and 8 from 2016) in our analyses. We found no significant effect of M:F body size ratio on likelihood of producing MP clutches (Fig. 2.10). The model reached convergence on all parameters across three chains of 100,000 iterations with a 1,000 iteration burn-in period. Mean ratios of M:F body size were nearly identical between MP and SP clutches (0.95, 0.97 respectively; range = 0.86 – 1.11). Mean size of females that sired SP clutches (315.2 ± 17.4 mm SCL) was similar to mean size of females that produced MP clutches (319.1 ± 11.8 mm SCL).

Discussion

The goal of this study was to evaluate the role of male body size in siring and reproductive success and to assess whether the ratio of male: female body size influences patterns of MP in the gopher tortoise. Based on the offspring we sampled, siring success was heavily skewed towards a subset of the candidate males. We found that male body size (SCL) was a significant predictor of the probability of siring offspring and also contributed to reproductive success of males in terms of number of offspring sired. As expected, we detected both singly and multiply sired clutches, but we found no significant relationship between the ratio of M:F body size and the likelihood of a clutch being sired by multiple males.

Male body size was a significant predictor of siring success, which corroborates previous findings for gopher tortoises by Tuberville *et al.* (2011) and Moon *et al.* (2006). Sires in our study were on average 37 mm longer than non-sires, which was an even greater size discrepancy than that previously observed in the gopher tortoise (~10 mm; Tuberville *et al.* (2011). As in similar studies, we were unable to find and sample all offspring produced; however, we genotyped clutches of at least half the females in the HG population, resulting in a large and presumably representative sample. The effect of size on siring success could be less of a factor of female choice or signal of male quality but rather an indirect result of competition among males. Larger males are more likely to be successful in competitive interactions with other males, as observed in the desert tortoise mating system (Niblick et al. 1994), a closely related *Gopherus* species in the western United States. If larger males preclude smaller males from maintaining burrows or home ranges near females, as observed by McRae et al. (1981), then smaller males may have reduced opportunities for breeding. Sire and non-sires sizes overlapped in range (262 – 330 mm, 131-304 mm respectively) and the Hill Garden and surrounding areas are small enough that males should be able to access most females over the breeding season, providing a wider range of males sizes from which females might choose. Thus, we suspect either sexual selection by females or male-male competition may be limiting mating opportunities for some males.

Although male body size predicted which males sired offspring relatively well, significant unexplained variation remained in predicting reproductive success. Behavioral observations may provide some insight into why certain males have greater reproductive success after accounting for differences in body size. Based on behavioral observations, larger males do not necessarily experience increased mounting success compared to smaller males (Boglioli et al. 2003). In male Blanding's Turtles (*Emydoidea blandingii*), reproductive success increases with number of partners or number of clutches sired (McGuire *et al.* 2015) while in Hermann's tortoise (*Testudo hermanii hermanii*) success increases with courtship intensity (Cutuli *et al.* 2014). Further observations of courtship and mounting behaviors between males and females in this population and others is necessary to determine how behavior influences reproductive success.

Interestingly, clutches produced by individual females in consecutive years in were sired by the same males as in the previous year. Tuberville *et al.* (2011) observed a single female producing clutches sired by the same male in consecutive years. Our study is the first to report paternity patterns in consecutive clutches from multiple female gopher tortoises, and that all females exhibited this pattern is striking. It is unclear as to whether this pattern is the result of remating or repeated use of stored sperm. Within a nesting season, painted turtles (*Chrysemys picta*) produce multiple clutches utilizing sperm from one male, suggesting that females only copulate with one male and store sperm throughout the year. Between years, however, 22% of females used sperm from the same male (Pearse et al 2001). Pearse *et al.* (2002) concluded that sperm storage, rather than remating is the mechanism for siring of consecutive clutches by the same male. In other reptile species without sperm storage capacity, mate fidelity (i.e., remating) appears to be the mechanism for repeated clutches sired by a single male (Rawlinson 1974; Stow & Sunnucks 2004; Lance et al. 2009). Given the number of social interactions and extended mating season of gopher tortoises observed using wildlife cameras, there is ample opportunity for males at ABS to remate with females. Gopher tortoises appear to limit their social interactions to a limited set of individuals within a colony, forming social cliques (Guyer et al. 2014). Additionally, Douglass (1986) observed males visiting the same females in subsequent years, reinforcing the idea that there may be mate preference or mate fidelity in this species. The formation of longer-term partners may provide further advantage to males in securing access to particular females and result in corresponding increases in male reproductive success. Concurrent evaluation of mating behavior and molecular techniques to assign parentage in the gopher tortoise will be needed to determine which mechanism(s) – sperm storage or mate fidelity—is driving the repeat siring of clutches in our study population.

The frequency of MP we observed in our study (24%) is similar to the frequency reported by Moon *et al.* 2002 (28%) and half that reported by Tuberville *et al.* 2011 (57%) for the gopher tortoise. Because detection of multiple sires increases with clutch size (Pearse et al. 2002), we excluded several clutches due to insufficient sampling of hatchlings, which may have led us to underestimate rates of MP. We were also conservative in our approach of attributing additional sires to clutches. Most multiply-sired clutches in our population required just two males to explain paternity patterns – similar to both the Moon et al (2002) and Tuberville et al (2011) studies. However, one clutch in our study required at least three males, which has not previously been reported in the gopher tortoise. Tertiary male contributions have been reported in American alligators (*Alligator mississippiensis*; Lance *et al.* 2009) and squamates (Friesen & Mason 2013), though their prevalence appears to be much lower in testudines (Uller & Olsson 2008).

As reported in previous studies, reproductive success varied greatly among candidate sires (Moon *et al.* 2006; Tuberville *et al.* 2011). In our study, 86.8% of offspring were attributed to just nine of the 51 candidate sires in the population (including an unsampled male). Of the nine males with the greatest reproductive success, five sired only SP clutches; three produced both MP and SP clutches, and one produced offspring in solely MP clutches. Our sample may indicate a strategy in which siring SP clutches results in higher reproductive success of individual males, but this remains to be critically evaluated. There has been considerable debate over the function and utility of MP and polyandry in reptiles. While there are likely genetic benefits (Uller & Olsson 2008) via increased hatching success (*Crysemys picta*; McTaggart 2000), increased effective population size (*Crysemys picta*; Pearse & Anderson 2009), and increased genetic diversity (*Anolis sagrei*; Calsbeek et al. 2007), these are primarily benefits to females. The potential benefits of polyandry and MP to males is less clear. However, considering females may be visited by as many as 14 males in one mating season (Boglioli et al. 2003), female choice may be the driving factor in determining the outcome of matings in this species, particularly if she is unreceptive to attempted matings or copulates with multiple males.

The physiological and behavioral drivers of MP are more costly and difficult to assess, although one study coarsely evaluated the effects of size on MP (Moon *et al.* 2006) and found no significant difference in male body size between sires that contributed to MP versus SP clutches. They suggested, however, that the size disparity between males and females was an important factor, such that smaller females were more likely to be inseminated by multiple, larger males, leading to multiply-sired clutches. Our findings do not support this claim. Male tortoises are on average smaller than their female counterparts in this sexually dimorphic species (McRae et al. 1981). Though potential sires ranged widely in size (209-330 mm), we found that the M:F size ratios of parental pairs hovered near 1, which may be suggestive of size-assortative mating, though males were typically slightly smaller than their female mates. The effect of M:F size ratio on mounting and reproductive success has been show to vary among turtle species. For the marginated tortoise, *Testudo marginata*, a larger M:F size ratio is correlated with increased mounting success (Sacchi et al. 2003); whereas in the Hermann's tortoise, *Testudo hermanni hermanni*, there is no effect of M:F body size ratio on reproductive success (Cutuli et al. 2014). In painted turtles (*Chrysemys picta*), female body size was not a determinant of MP of a clutch but female turtles that laid at least one MP clutch were on average larger than those that produced no MP clutches, suggesting male preference for larger females (Pearse *et al.* 2002). Increases in frequency of MP with increases in female body size have also been observed in Blanding's turtles (*Emydoidea blandingii;* Refsnider 2009). However, female body size in our sample was similar for both MP and SP clutches.

Despite intensive monitoring and concurrent mark-recapture efforts within our study area, we detected two unsampled females and one unsampled male that contributed to the sampled offpsring, presumably due to movement of breeding adults in and out of the study area. We suggest that male movement out of the study area resulted in the single instance of clutch paternity being assigned to an unsampled male. Female desert tortoises (*Gopherus agassizii*) can store sperm for at least three years (Palmer et al. 1998). Thus, female gopher tortoises in HG could have produced clutches fertilized with sperm stored from previous matings with males that have since moved out of the focal study area. In support of this, we detected a male (1477) that sired offspring (clutch 753-2016) but that had not been physically recaptured in the study area since 2013 – prior to our study. Additionally, there was a gap in mark-recapture efforts during 2003 – 2011. Depending on how long stored sperm remains viable, males present during that period may have mated with females and left the area but still sired some offspring in 2015-2016. In contrast, the unsampled females were detected from clutches collected at burrows located at the perimeter of the study area. The dams of clutches found at these burrows may reside in peripheral units where there has been little mark-recapture effort during recent years.

Our study represents that largest study of parentage for an *in situ* population of gopher tortoises and the most complete sampling of a single population to date. We provide further evidence of polygyny and polyandry in the gopher tortoise. Previous characterizations of the gopher tortoise mating system by Johnson *et al.* (2009) and Boglioli *et al.* (2003) as scramble competition polygyny and by Douglass (1986) as harem defense polygyny fall short of fully describing the mating patterns we observed. Previous characterizations were based on behavioral observations whereas our study provided insight into the genetic patterns resulting from mating behaviors. A combined approach to evaluate courtship and mating interactions will likely provide the most comprehensive information on mate preference and mating strategies of males and females in this species by quantifying the success of different strategies employed. Thus, rather than come to opposing conclusions of the type of mating system, future studies would do well to combine molecular and behavioral approaches to provide a greater understanding of the mechanisms influencing reproductive success and the mating system in gopher tortoises and other reptile species.

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Tables:

Table 2.1. Summary of clutch data from gopher tortoise nests collected during 2015 and 2016 at Archbold Biological Station, Highlands Co., FL. Total samples include blood samples from successfully hatched hatchlings and tissue and blood samples from deceased embryos when feasible.

	2015	**2016**
Total nests	17	18
Average clutch size (± 1SD)	8.73 ± 2.05	8.11 ± 2.54
Total eggs collected	131	146
Eggs incubated	126	129
Mean hatching success (% ± 1SD)	73.3 ± 34.5	75.4 ± 34.9
Total samples	110	110

Table 2.2. Basic properties of 11 microsatellite loci for gopher tortoises used in our study, including locus name, length (size in base pairs), forward and reverse primer sequences, touchdown temperature (C), and the original primer source.

Locus	Size (bp)	5'-3'F	5'-3'R	Touchdown Temperature (˚C)
*Gopo*B011[1]	243-261	CAGTGGTGGGTAAGTTTCTATC	CTTGTCTGTGCCTCTGTCTC	63
*Gopo*B104[1]	272-287	CGCTGGATGCTGAGACTT	ATGTCCCTTTCACCTTATGGA	60
*Gopo*B118[1]	192-201	TCTTGGGAAATCTTCAGAGG	CACCGATGATGGACACAG	63
*Gopo*D004[1]	165-209	AGTGCCTACTCAGTTTCTACCA	GATTGACGCCCAGTTCTAC	58
*Gopo*D006[1]	264-313	GCGAGGATTATTACACATTGG	CTTGATGGGCACAACAGTT	58
*Gopo*D011[1]	150-197	TGCCTTTTGCCAGATGTC	GGGATGTGTTCATTAGAAGAGC	60
*Gopo*D107[1]	280-303	CCCAGATAAGAGGGAGGAA	GGCTGTGTGAGGGTAACTG	63
Gopo-12[2]	348-368	CAGTCGGGCGTCATCACTTTGGAAGCCATTGTAATA	GTTTCATTTGCACCAGTTTAACTA	60
Gopo-02[2]	244-340	CAGTCGGGCGTCATCAGGCAGCAGAGAATAGAT	GTTTTATCAGCTATCCCGTGTA	64
GP61[3]	197-245	GCATTAAACCATTGTGCCTCA	AGTGGTGGTCGAAGTGGAAC	60
GP81[3]	397-415	TCACACAAACCCCATCCATA	TCCATTGAATTGCCATCTGA	60

[1] Kreiser *et al.* 2013

[2] Tuberville *et al.* 2011

[3] Schwartz *et al.* 2003

Table 2.3. Properties of loci used in parentage assignment of hatchling gopher tortoises collected at Archbold Biological Station, Highlands Co., FL, including number of alleles (k), frequency of null alleles (F(Null)), observed heterozygosity (HObs), expected heterozygosity (HExp), polymorphic information content (PIC), non-exclusion probabilities for parent 1 (NE-1P), parent 2 (NE-2P) and parent pair (Parent Pair). Combined non-exclusion probabilities across all loci are also provided. All statistics were calculated in CERVUS. No loci deviated significantly from Hardy-Weinberg equilibrium.

Locus	**k**	**F(Null)**	**HObs**	**HExp**	**PIC**	**NE-1P**	**NE-2P**	**Parent Pair**
*Gopo*B011	3	0.0499	0.513	0.564	0.467	0.842	0.733	0.601
*Gopo*B104	5	-0.0101	0.684	0.678	0.618	0.749	0.586	0.413
*Gopo*B118	4	-0.0423	0.446	0.41	0.382	0.912	0.771	0.619
*Gopo*D004	14	-0.0072	0.878	0.87	0.852	0.426	0.269	0.108
*Gopo*D006	15	0.019	0.861	0.896	0.883	0.357	0.216	0.073
*Gopo*D011	13	0.0021	0.823	0.837	0.816	0.487	0.319	0.141
*Gopo*D107	12	-0.0069	0.842	0.836	0.811	0.505	0.334	0.159
Gopo-12	6	-0.0327	0.478	0.468	0.439	0.882	0.724	0.553
Gopo-02	14	0.0184	0.724	0.744	0.718	0.627	0.441	0.232
GP61	3	-0.0518	0.56	0.508	0.386	0.872	0.802	0.702
GP81	4	-0.0419	0.649	0.605	0.531	0.815	0.673	0.52
					Combined	**8.448E-03**	**3.5319E-04**	**1.28E-06**

Table 2.4. Gopher tortoise (*Gopherus polyphemus*) clutch data from nests collected during May – July 2015 and 2016 at Archbold Biological Station, Highlands Co., FL. Clutch information includes the clutch size (number of eggs), number of hatched offspring, number of offspring successfully genotyped (including samples taken from deceased hatchlings and unhatched eggs when possible), assigned dam and sire and their respective straight carapace lengths (SCL). For clutches with multiple sires (MP), the number of offspring attributed to sires is denoted in parentheses under Sire ID.

Clutch ID	Clutch Size	Offspring Hatched	Offspring Genotyped	Dam ID	Dam SCL (mm)	Sire ID	Sire SCL (mm)
743 – 2015	10	9	10	216	312	625	285
663 – 2016	8	7	7	216	312	625	285
556 – 2016	6	5	5	256	327	273 (3), 780 (2)	307, 330
533 – 2016	8	6	7	332	323	158	304
699 – 2015	7	7	7	663	307	158	304
575 – 2016	8	8	8	663	307	158	304
747 – 2015	7	7	7	683	304	868	262
589 – 2016	7	4	7	683	304	868	262
564 – 2015	7+	4	7	711	324	780 (4), 1483 (3)	330, 294
824 – 2016	10	7	9	711	324	780 (4), 1483 (5)	330, 294
745 – 2015	6	6	6	718	288	158	304
738 – 2015	11	10	10	1141	344	273	307
741 – 2015	11	9	9	1223	327	1251	315
549 – 2016	15	7	8	1223	327	1251	315
586 – 2015	7	3	6	1234	292	1024 (1), 1464 (5)	279, 297
603 – 2016	5	4	5	1242	263	Unknown Male	---
541 – 2015	11	2	2	1245	338	1251	315

Clutch ID	Clutch Size	Offspring Hatched	Offspring Genotyped	Dam ID	Dam SCL (mm)	Sire ID	Sire SCL (mm)
552 – 2016	6	0	1	1245	338	1251	315
707 – 2015	12	10	10	1250	333	1422	320
542 – 2016	11		10	1250	333	1422	320
775 – 2016	8	4	4	1456	299	273	307
620 – 2015	6	6	6	1467	319	625 (5), 1235 (1)	285, 286
812 – 2016	6	6	6	1467	319	1235	286
715 – 2015	9	6	6	1468	327	1251	315
549 – 2015	10	10	10	1469	327	1228	315
639 – 2016	10	9	9	1469	327	1228	315
764 – 2015	6	6	6	1512	269	625	285
565 – 2015	10	8	8	1680	329	217 (1), 780 (2), 1464 (5)	304, 330, 297
761 – 2016	10	8	8	1922	317	625 (4), 1473 (4)	285, 300
753 – 2016	11	9	11	Unk. Dam 1	---	1477	285
798 – 2016	5	4	5	Unk. Dam 2	---	1235	286

Table 2.5. Parameter estimates with 95% credible intervals converted to the original scale from the ZIP model evaluating effects of male size on siring and reproductive success in gopher tortoises at Archbold Biological Station, Highlands Co., FL. Asterisks (*) indicate significant effect with a 95% credible interval that does not overlap zero.

	Mean	**SD**	**2.5%**	**97.5%**
α_0	-20.709*	5.890	-33.350	-10.460
α_1	0.07*	0.02	0.03	0.11
β_0	0.609	1.221	-1.772	2.963
β_1	0.007	0.004	-0.001	0.015

Figures

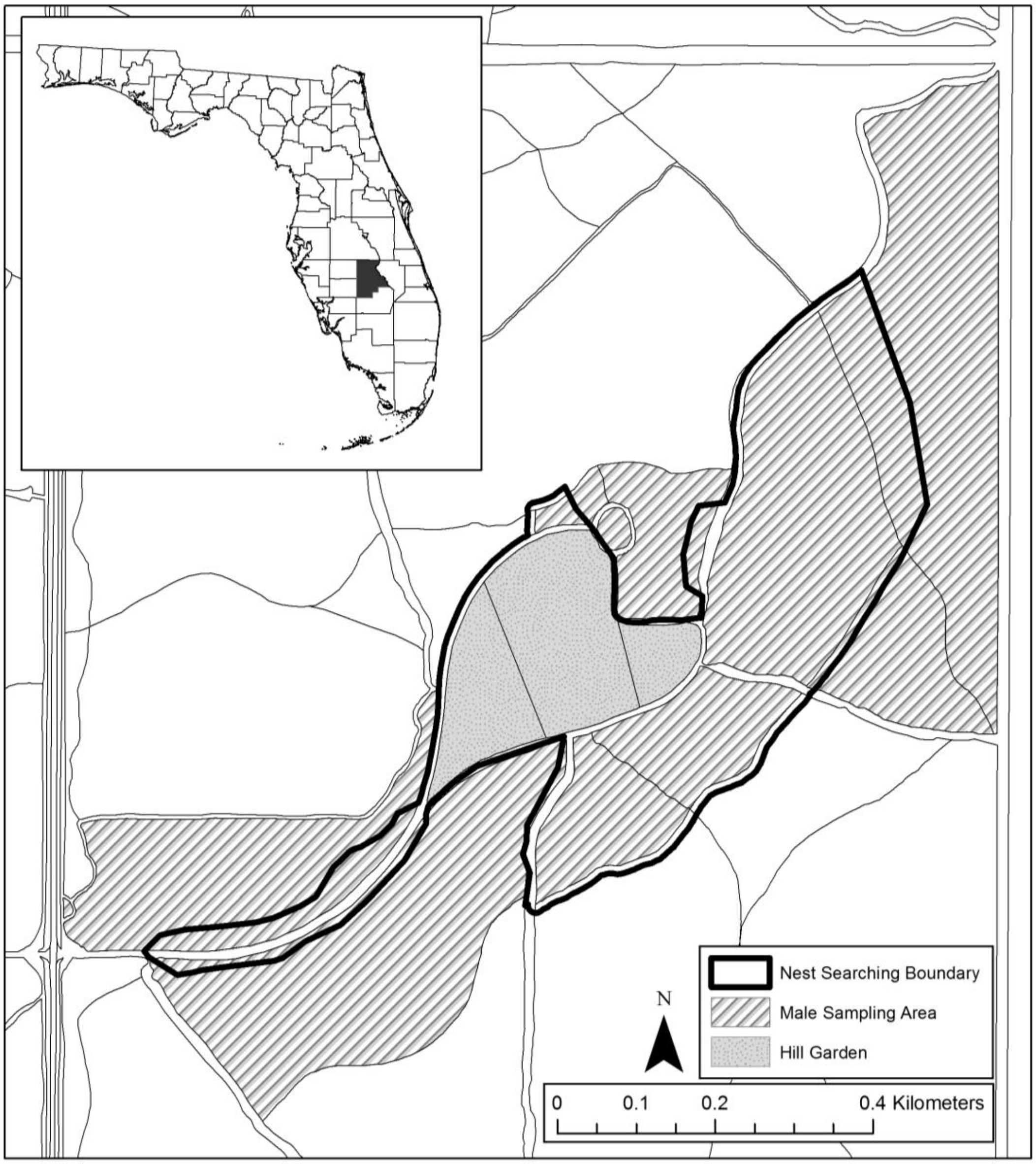

Figure 2.1. The Hill Garden and effective sampling areas of Archbold Biological Station, Highlands Co., FL (Inset: Florida counties; Highlands Co. filled in black). Males were sampled from both the "Male Sampling Area" and the "Hill Garden."

Figure 2.2. Locations of gopher tortoise nests in 2015 and 2016 within the nest searching boundary of Archbold Biological Station, Highlands Co., FL.

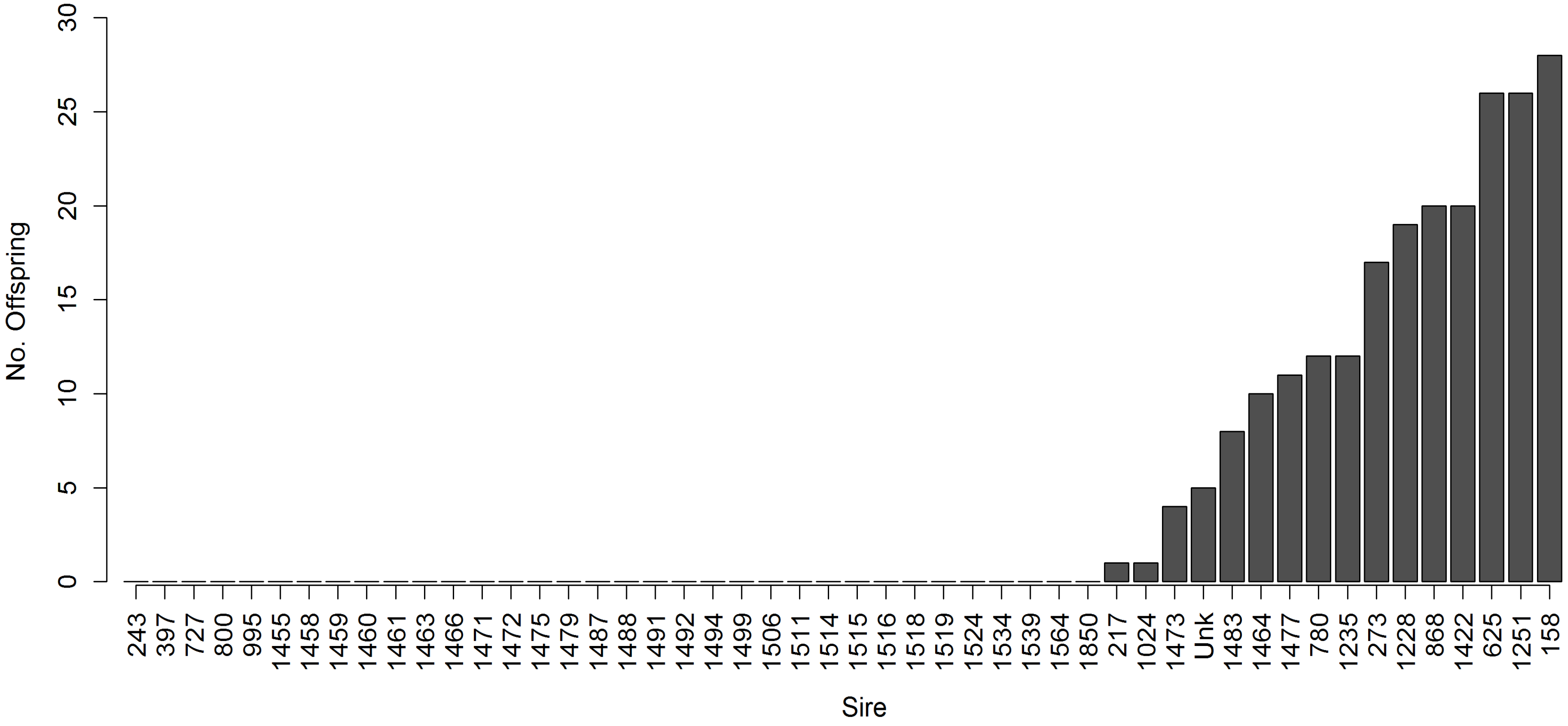

Figure 2.3. Reproductive skew in number of offspring produced by candidate males (including the unidentified male – here "Unk") from nests (n = 33) collected at Archbold Biological Station, Highlands Co., Florida during 2015-2016.

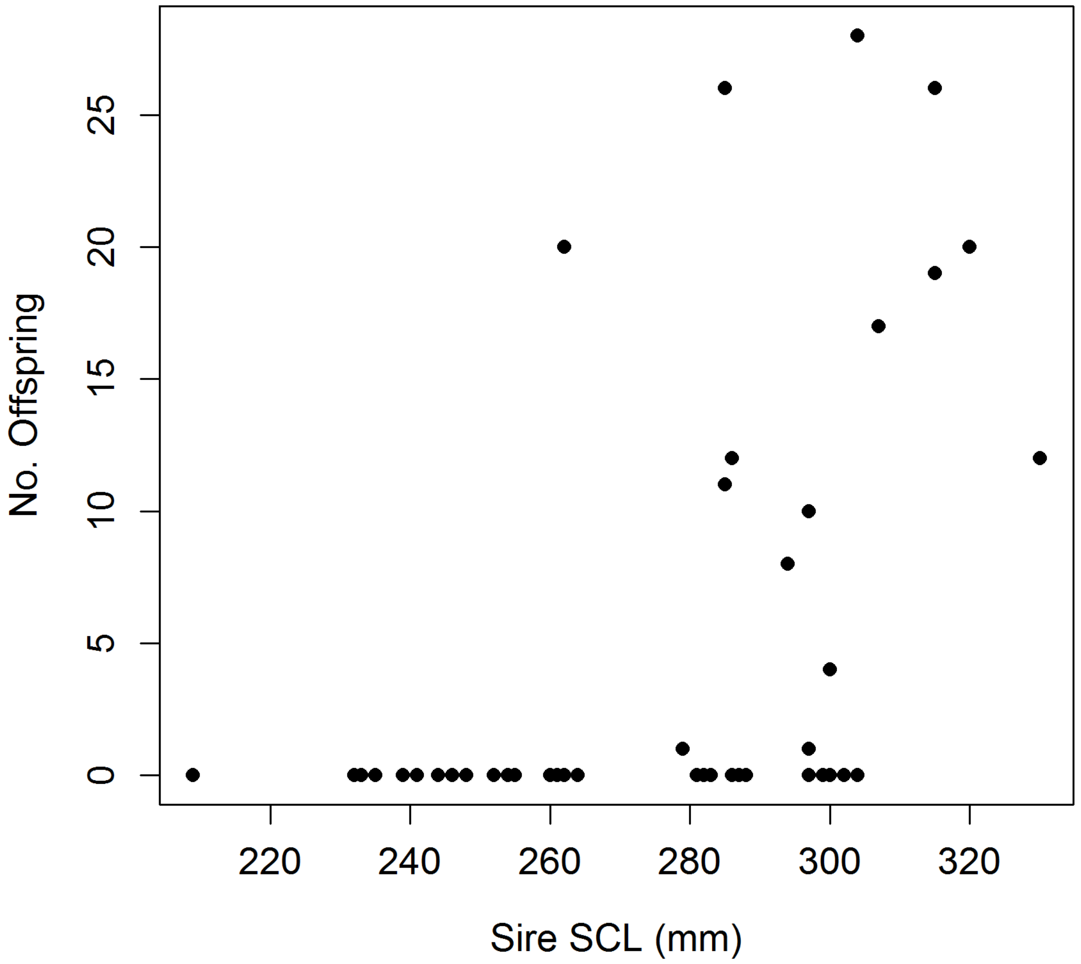

Figure 2.4. Total number of offspring produced by males during 2015 -2016 from gopher tortoise nests collected at Archbold Biological Station, Highlands Co., FL during 2015-2016 as a function of male size (SCL).

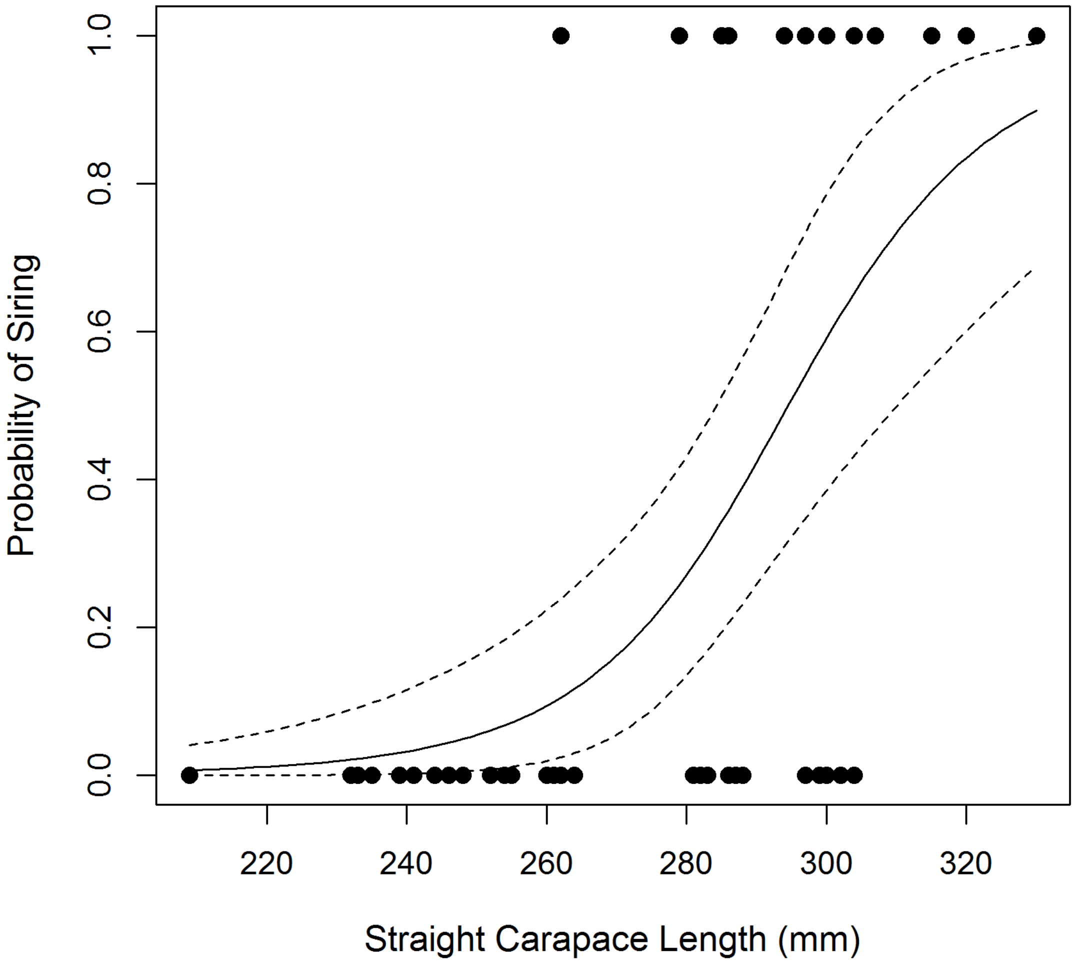

Figure 2.5. Mean probability of siring success (solid line) as a function of sire carapace length with 95% credible intervals (dashed lines) bounding the regression line from gopher tortoise nests collected at Archbold Biological Station, Highlands Co., FL during 2015-2016.

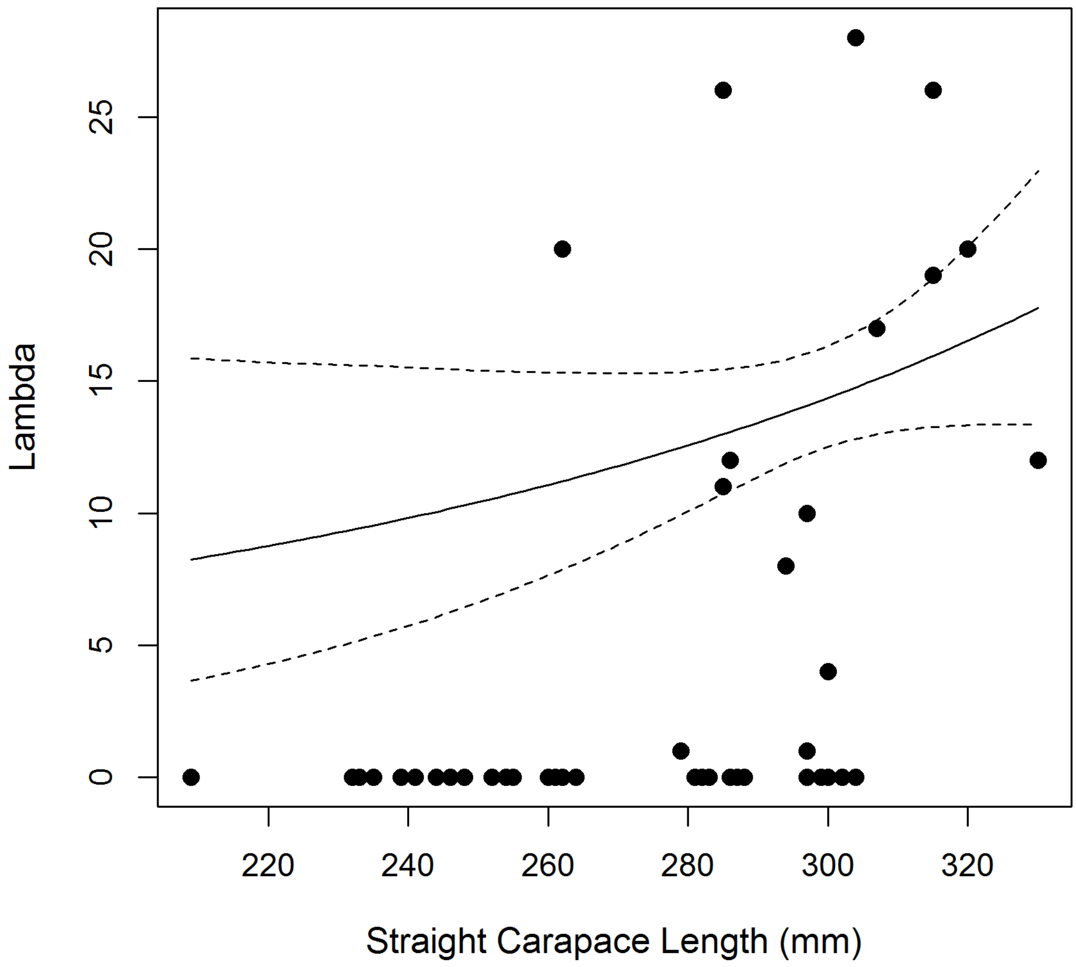

Figure 2.6. Mean effect of sire carapace length on mean lambda values (solid line) with 95% credible intervals (dashed lines) bounding the regression line from gopher tortoise nests collected at Archbold Biological Station, Highlands Co., FL during 2015-2016.

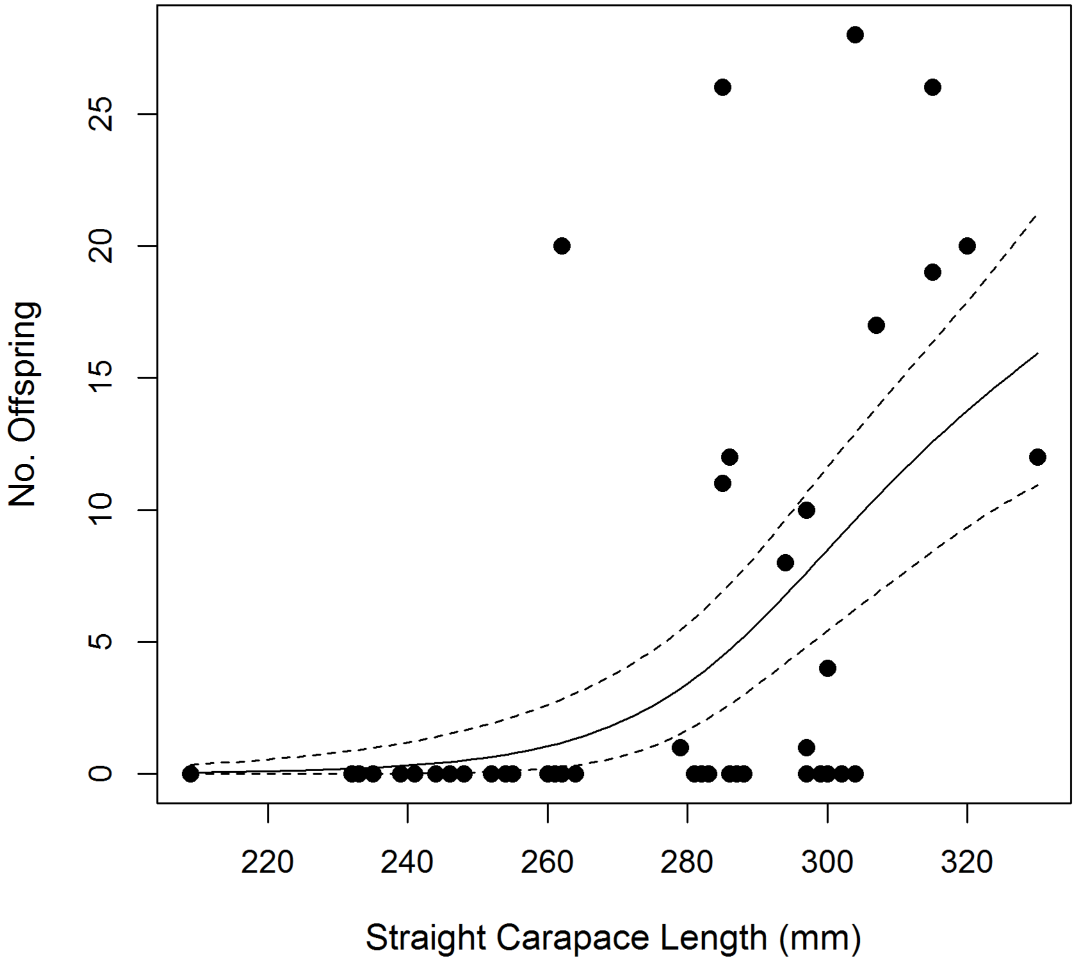

Figure 2.7. Mean effect of sire body size (solid line) on number of offspring sired with 95% credible intervals (dashed lines) from the zero-inflated Poisson mixture model from gopher tortoise nests collected at Archbold Biological Station, Highlands Co., FL during 2015-2016.

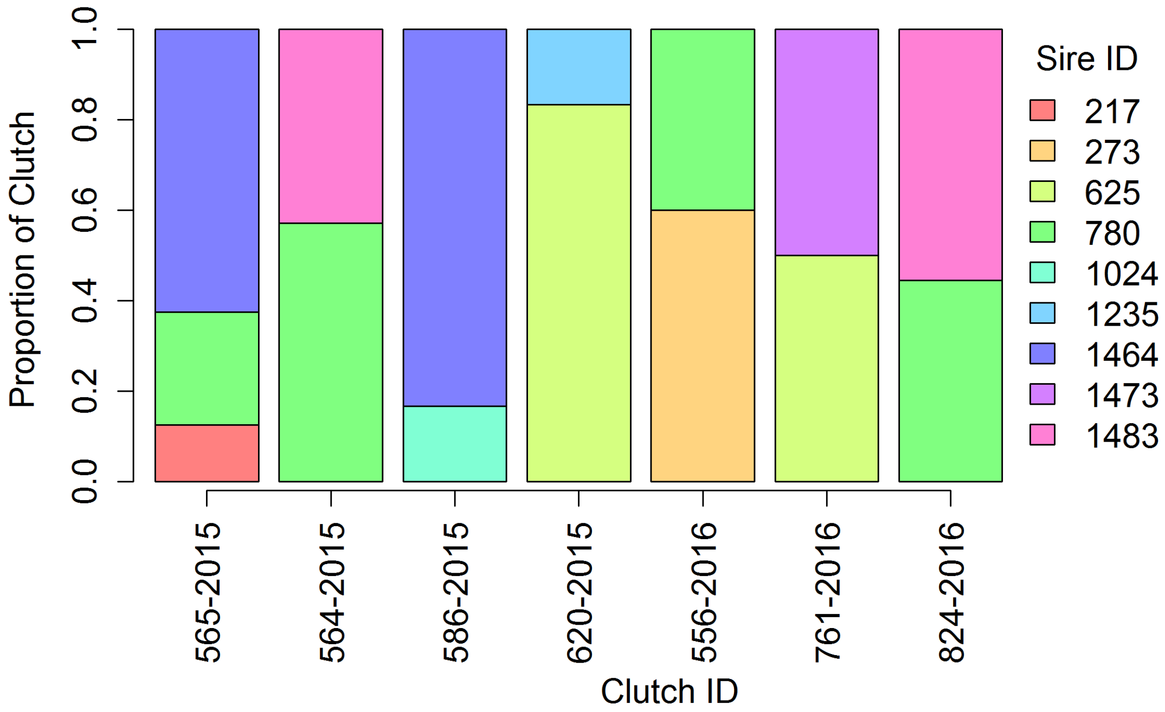

Figure 2.8. Sire contributions in multiple paternity gopher tortoise nests collected from Archbold Biological Station, Highlands Co., Florida during 2015-2016.

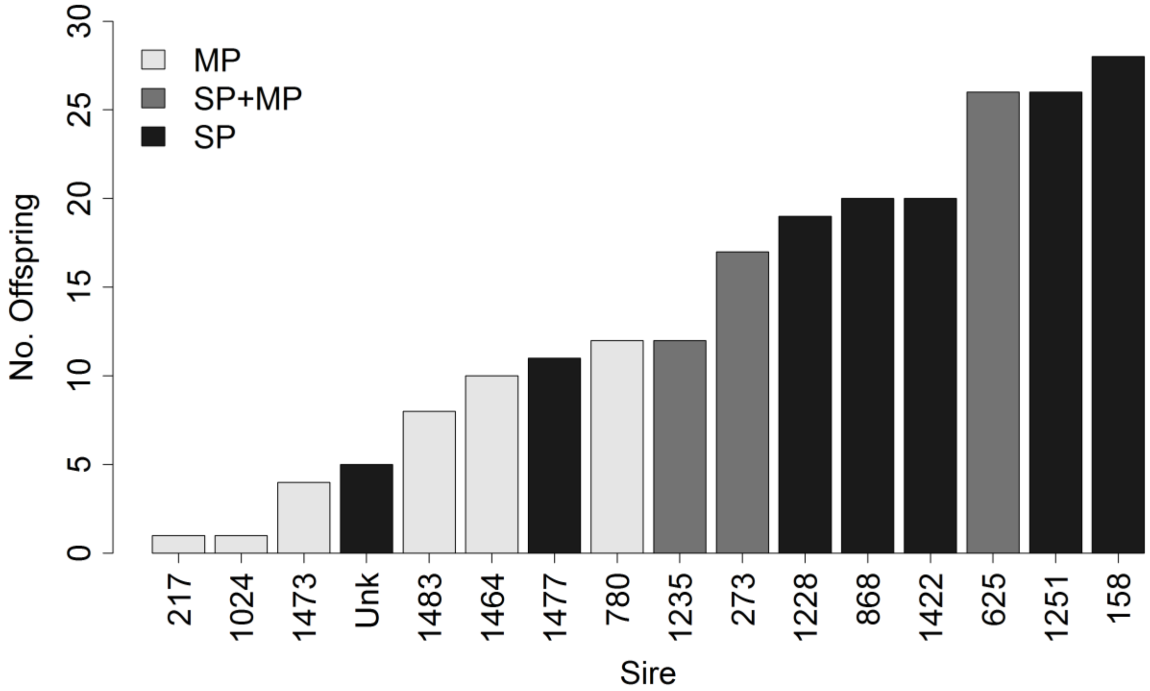

Figure 2.9. Paternity strategies of male gopher tortoises and total offspring produced by males from gopher tortoise nests collected at Archbold Biological Station, Highlands Co., FL during 2015-2016. MP refers to male that produced offspring solely in clutches with multiple sires, SP+MP to sires that produced offspring in both SP and MP clutches, and SP to those males the produced only single sire clutches.

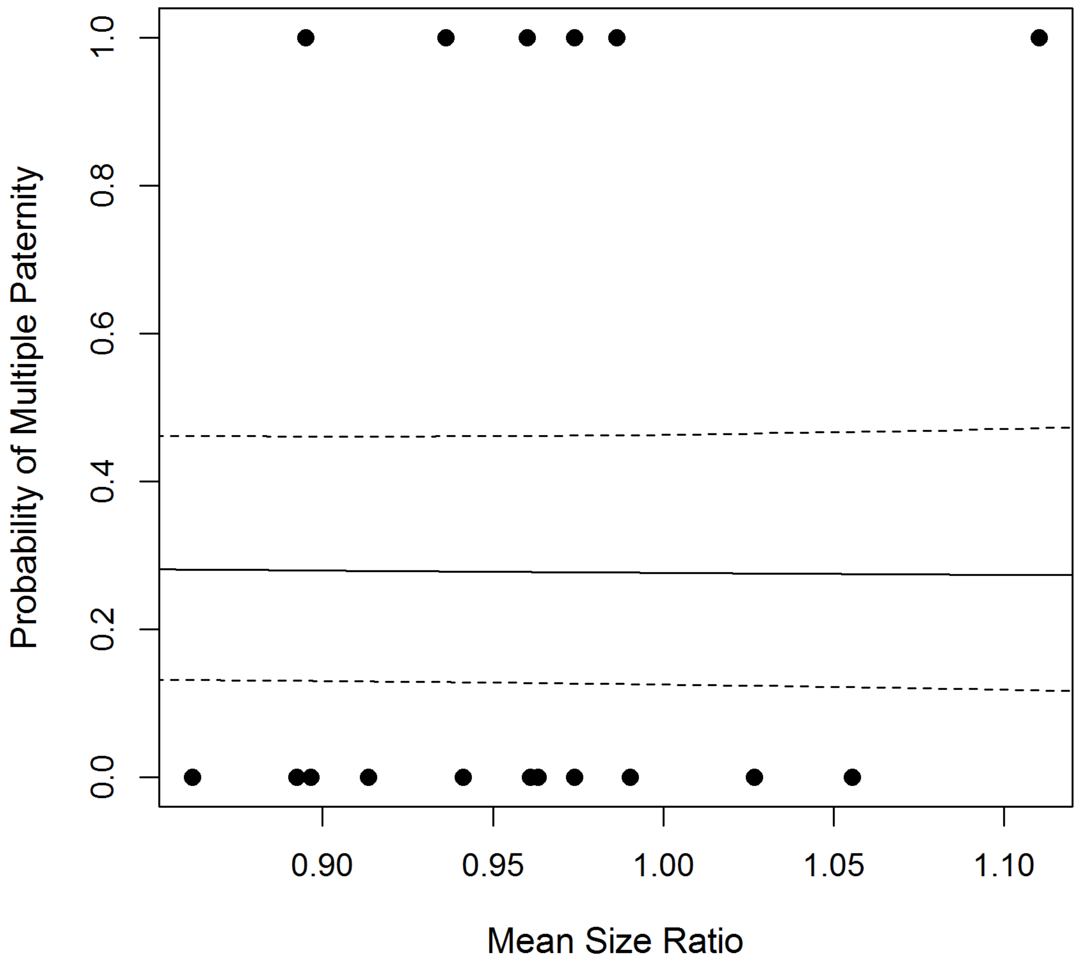

Figure 2.10. The mean effect (solid line) of male:female body size ratios on probability of producing a clutch with multiple sires with 95% confidence intervals (dashed lines) from gopher tortoise nests collected at Archbold Biological Station, Highlands Co., FL during 2015-2016.

CHAPTER 3

CONCLUSIONS

In this study, we provide strong molecular evidence of male size dependence of siring success and evidence for weak size dependence of reproductive success in the gopher tortoise. We found no support for the theory that large male dominance of smaller females drives multiple paternity in the gopher tortoise. Based on molecular evidence of polyandry in our study, we suggest that neither scramble competition polygyny nor harem defense polygyny adequately describe the mating system of this species.

Body size effects on reproductive success have been reported for both males and females of other reptiles (Schuett 1997; Pearse *et al.* 2002; Friesen *et al.* 2014). Selection by males of larger females provides opportunities to fertilize more eggs because larger females can produce larger clutches (Congdon & Gibbons 1985). Body size of males may also provide a cue to females regarding male fitness. Conversely, male body size can provide an advantage in competitive interactions and provide greater access to mates or resources (Olsson & Shine 1996; Schuett 1997; Shine *et al.* 2000). While effects of male size on dominance have not been tested in the gopher tortoise, in its western congener, the *G. agasizzii*, larger males are more likely to be dominant and receive access to mates than are smaller males (Niblick *et al.* 1994). Additional support for our conclusion of size effects on siring success have been found by Tuberville *et al.* (2011) that larger males are more likely to sire offspring than smaller males, though not necessarily more likely to produce more offspring. Interestingly, while size effects on siring success have been detected based on parentage analysis, behavioral observations reveal that larger males are not necessarily more likely to have more mounting opportunities (Boglioli *et al.* 2003) or be more central to social groups (Guyer *et al.* 2014) than smaller males. Berry & Shine (1980) proposed that male size in terrestrial turtles provides an advantage for competitive interactions between males. While male combat has been reported as common in the gopher tortoise (Douglass 1986; Guyer *et al.* 2014), other studies suggest that male combat is less common than previously thought (Boglioli *et al.* 2003). Of the tortoises in the United States, the gopher tortoise is considered to exhibit the least aggression between conspecifics (Guyer *et al.* 2014). Further observations of competitive interactions between males will likely provide information on how these interactions impact male opportunities for reproduction.

In our study, male contributions to individual MP clutches were more evenly distributed between males than previously reported in the gopher tortoise (Tuberville *et al.* 2011). Females may be able to selectively allocate sperm towards egg fertilization (Friesen & Mason 2013). An alternate explanation may be last male (most recent mate) precedence, which has been experimentally documented in snakes (Friesen & Mason 2013) but not definitively in turtles. If last male precedence was driving distribution of male contribution within clutches, we would expect to see more skew between the primary sire and secondary sire. In this study, the mean proportion sired by the primary male in our study was closer to 60%. This pattern may be the result of stored sperm depletion (Pearse *et al.* 2001). However, so little is known about the mechanism and function of sperm storage in *Gopherus* species that the mechanism remains unknown.

Increases in population density may be the consequence of decreases in available habitat due to destruction, fragmentation or degradation of preferred habitat. Density-dependent effects on mating systems have been documented in various taxa (reviewed by Kokko & Rankin 2006). Population declines in the gopher tortoise can result in areas of poor habitat low density populations or small patches of remnant suitable habitat with high population densities as individuals leave degraded areas. While low tortoise densities result in decreased mating interaction rates (Guyer *et al.* 2012), higher population density may alter distribution of females and result in greater access to potential mates as distance to nearest female decreases. However, little is known about how variation in population density affects mating systems or reproductive success in this species.

Douglass' (1986) description of the *G. polyphemus* mating system as a harem defense system was based on this particular high-density population at ABS, where harem defense might be possible, albeit loosely enforceable given the slow-moving nature of tortoises. Similar high-density situations can occur either because tortoise densities increase in small, remnant patches of suitable habitat as surrounding areas continue to degrade, or they can occur as a result of translocations of animals from one locale to another. Tortoises are one of the most heavily translocated and relocated species in the United States (Birkhead *et al.* 2005). To ensure that moved populations stay in the relocation site, they must be penned for a period of time while they acclimate to their surroundings and settle in (Tuberville *et al.* 2005). Individuals may be penned at densities of up to 17 tortoises/hectare or greater (B. Cozad, *pers. comm*). At such high densities, males may more effectively defend a group of females from competing males and the skew in male siring success may be pushed towards the largest males. In contrast, Johnson et al. (2009) described the behavioral mating system of gopher tortoise based on populations at lower densities than the focal population of this study as well in a different habitat type. In such low-density populations, given that size and similar external characteristics in theory are supposed to be less important in scramble competition polygynous systems, size shouldn't necessarily be the main factor that drives who sires. In addition to a higher population density, the sex ratio of the current ABS population is heavily skewed toward females (1:2). Most reported sex ratios in tortoise populations, while slightly female-biased, range from 1:1.0-1:2.7 (Amatuli 2012; Rostal *et al.* 2014). Changes in the operational sex ratio (OSR) can result in increased competition between males for access to females as male bias increases; though ultimately competition may decrease when OSR bias reaches (1.99:1) and the frequency of competitive interactions becomes energetically unsustainable (Weir *et al.* 2011). However, in this study population, the OSR bias is female-biased. Further investigation into the altered OSRs and population densities in the gopher tortoises is merited to better understand their effects on the mating system.

While our study provides valuable insight into the mating dynamics of the gopher tortoise, further observations of gopher tortoise behavior are necessary to truly understand which traits are under sexual selection in this species. Behavioral studies will provide information on mate choice and selection that cannot be seen solely through use of molecular data. Combining molecular and behavior studies will likely provide resolution on the mating system of this species.

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