Male Body Size Predicts Reproductive Success But Not Within-Clutch Paternity Patterns in Gopher Tortoises (Gopherus polyphemus)

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Abstract

In many vertebrates, body size is an important driver of variation in male reproductive success. Larger, more fit individuals are more likely to dominate mating opportunities, skewing siring success and resulting in lower effective population sizes and genetic diversity. The mating system of the gopher tortoise (Gopherus polyphemus) has been characterized as both female-defense and scramble-competition polygyny. Mating systems are typically not fixed and can be influenced by factors such as population density, demographic structure, and environmental conditions; however, most populations will have a predominant strategy that results from local conditions. We assessed how male body size influences patterns of paternity and reproductive success in a natural population of gopher tortoises in Florida, United States. Using microsatellites, we assigned parentage of 220 hatchlings from 31 nests collected during 2 reproductive seasons. Larger males were significantly more likely to sire offspring and sired more offspring than smaller males; however, the likelihood of a clutch being multiply sired was unrelated to male body size. We also found evidence of mate fidelity across years. Although paternity patterns in this high-density population are more consistent with defense polygyny, female monopoly by males was incomplete, with both large and small males contributing to multiply sired clutches. Additional behavioral data are needed to clarify the role of female mate selection in paternity outcomes. The context-dependence of mating systems underscores the need to compare parentage patterns across populations and to recognize the potential for more than 1 strategy to be employed within a single population.
most successful males. The degree of sexual selection on traits and which traits are important will vary by species depending on the specific context for mate competition for males and female choice (Bateman 1948; Arnold and Duvall 1994; Jennions and Petrie 1997; Andersson and Simmons 2006). One trait that is often positively correlated with enhanced fitness in vertebrates is body size (Clutton-Brock 1988). In females, larger body size is typically correlated with increases in fecundity or quality of offspring (Hailey and Davies 1987; Rotzemel and Castellon 2014; Nafus et al. 2015)—both of which enhance female fitness. Large body size can also impart direct or indirect fitness advantages to males. Depending on the mating system, larger males may have higher fitness because of female choice, because they are more successful at defending females or resources important for breeding, or some combination of the 2 (Blouin-Demers et al. 2005).

Although body size is an important phenotypic trait affecting mating and reproductive success in many taxa (Hughes 1985; McElligott et al. 2001; Serbezov et al. 2010), its effect on fitness is especially evident in species with indeterminate growth and variable growth rates, because in those species, mature individuals exhibit a wider range of body sizes (Congdon and van Loben Sels 1993; Madsen and Shine 2006; Congdon et al. 2013; Arsovski et al. 2018). Reptile species typically have indeterminate growth (Congdon et al. 2013) and most have polygynous mating systems (Bull 2000; Uller and Olsson 2008). In many reptile species, male body size drives reproductive success through female selection for larger males (Hunt et al. 2009; Cutuli et al. 2014) or through competitive interactions between males that afford the winner increased access to females (Schuett 1997). In other species, mating systems more closely approximate scramble-competition polygyny (Graves and Duvall 1990; Weatherhead et al. 2002; Friesen and Mason 2013), in which fitness is dictated by strategies that increase access to females during the breeding period and not necessarily by specific male phenotypes (Alcock 1980; Schwagmeyer and Woontner 1986). Therefore, these 2 polygynous mating systems can have different outcomes in terms of selection for body size. We expect that in a population dominated by a scramble-competition mating system, body size of competing males should be less important than other factors, such as male courtship effort expended per female, or male movement rate. Conversely, male body size is more likely to determine reproductive success in populations dominated by a female- or resource-defense mating system, where high densities of males can lead to frequent male-male competition and/or rejection of comparatively smaller individuals by females (Emlen and Oving 1977).

Quantifying size-dependent reproductive success in species with different mating systems has been challenging in part because many species show evidence of multiple paternity (i.e., the inclusion of more than 1 sire in a single clutch of eggs; henceforth, MP). MP is impossible to infer from behavioral observations of individual interactions; yet, it has been recorded in many species of reptiles using molecular parentage assignment methods (Pearse and Avise 2001; Roques et al. 2006; Uller and Olsson 2008; Lance et al. 2009; Duran et al. 2015; Farke et al. 2015), indicating that it is potentially a widespread phenomenon. The degree of MP varies greatly both among taxa and within species (Pearse and Avise 2001). Evaluating the fitness benefits of morphological traits such as body size requires sufficient within-population genetic sampling to accurately assess the contributions of individual males to shared clutches.

In this study, we assessed the influence of body size on male reproductive success in the gopher tortoise (Gopherus polyphemus), a long-lived, terrestrial turtle native to the southeastern United States. Two mating system types have been reported for the gopher tortoise: female-defense polygyny (Douglas 1986) and scramble-competition polygyny (Johnson et al. 2009). Douglas (1986) described the gopher tortoise mating system as a loose or incipient female-defense polygyny. Female-defense polygyny can arise when females are spatially clumped such that males can feasibly defend multiple females (Emlen and Oving 1977). However, Johnson et al. (2009) characterized this species’ mating system as scramble-competition polygyny, in which males primarily attempt to outcompete other males for access to females via mate-searching rather than male combat and female defense (Schwagmeyer 1988). At this second study site, female dispersion likely precluded defense of multiple females from other males.

Existing studies provide varying levels of support for each hypothesized mating system, likely because mating systems are not fixed, and can be influenced by factors such as population density, demographic structure, and environmental conditions. The relative importance of male body size in influencing siring or mating success might be expected to vary, depending on context. A larger-male advantage in siring success was observed in 2 previous studies examining parentage patterns of gopher tortoises based on genetic assignment (Moon et al. 2006; Tuberville et al. 2011). Additionally, behavioral observations revealed that larger, socially dominant males defended burrows, but that smaller males utilized neighboring burrows or cohabited with females in the absence of the dominant males (Douglas 1986). Similarly, both telemetry data and long-term mark-recapture studies suggest that smaller or younger male gopher tortoises may be precluded from maintaining burrows or home ranges through competition with larger males (McRae 1981b; Tuberville et al. 2014). Collectively, these findings demonstrate that male body size can be an important determinant in the outcome of social interactions, thereby influencing male string or reproductive success.

However, other observations of gopher tortoise populations indicate that in some contexts male size may be less important for mate acquisition. Male home range sizes and movement distances are not correlated with body size (Eubanks et al. 2003; Castellón et al. 2018), and yet, male home ranges are consistently larger than female home ranges (McRae 1981b; Diemer 1992), potentially providing a wider range of males from which females might choose rather than those that reside just in a female’s immediate vicinity. Moreover, males of varying sizes are equally likely to initiate courtship visits with spatially isolated females (Boglioli et al. 2003). In cases where scramble-competition polygyny is the dominant mating strategy, males of more variable sizes (as just large males) will experience reproductive success.

Here, we test whether genetic patterns in siring and reproductive success of males and rates of MP across 2 nesting seasons suggest predominance of scramble-competition polygyny or female-defense polygyny in a natural population of gopher tortoises in south-central Florida. Specifically, we hypothesized that a mating system dominated by female-defense polygyny would result in 1) higher siring success (defined as whether a male produces offspring) for larger males, 2) higher reproductive success (defined as the number of offspring sired per male) for larger males, and 3) low incidence of MP, with cases of MP more likely in smaller males. If female defense is effective, we would expect larger males to monopolize matings and be responsible for most singly sired clutches while smaller males would contribute to multiply sired clutches. Conversely, a mating system dominated by scramble-competition polygyny would result...
in similar siring success, reproductive success, and rates of MP across male size classes.

Materials and Methods

Focal Species and Study Site

Gopher tortoises are long-lived, social reptiles that typically aggregate in clusters of burrows referred to as colonies (McCoy and Mushinsky 2007). Males interact agonistically with other males, engaging in combat (Douglass 1986), and typically have larger home ranges than females, conducting regular forays from their burrows in search of mating opportunities (McRae et al. 1981b; Diemer 1992). Females are typically courted by multiple males within a mating season (up to 26 visits by 6–12 males) and much of the courtship takes place at the female’s burrow (Boglioli et al. 2003; Johnson et al. 2007). Females can store sperm from multiple males for several years (Palmer and Guillette 1988).

Archbold Biological Station (ABS) is a 2102-ha site located at the southern end of the Lake Wales Ridge in Highlands County, Florida. The Station encompasses Florida scrub, southern ridge sandhill, and flatwoods habitats (Abrahamson et al. 1984). Our study population is centered on a site within ABS known as Hill Garden (hereafter, HG), a 7-ha area of human-modified, old-field habitat surrounded by approximately 50 ha of southern ridge sandhill (known as Red Hill) in varying stages of restoration following decades of fire suppression (Figure 1). 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 previous observational studies of mating behaviors (Douglass 1986) and clutch size variation (Ashton et al. 2007).

Figure 1. The Hill Garden sampling area at Archbold Biological Station, Highlands Co., FL (gray in inset map) with locations of 2015 and 2016 nests.

Data Collection

In each of 2 breeding seasons, we located and collected eggs from nests in HG and adjacent units. Female tortoises often place their nests in the burrow apron (i.e., the mound of sand just outside the burrow), although they also oviposit in open sandy sites away from the burrow (Landers et al. 1980; Butler and Hull 1996). We searched the aprons of active, inactive, and collapsed burrows in and around HG during May–July 2015 and 2016. We expended more search effort at burrows used by 37 radio-telemetered females (i.e., all females residing in the study area as of spring 2015), conducting nest searches at each of these burrows up to 5 times. In addition, we searched all remaining burrow aprons in the study area at least once in July after oviposition had likely occurred. To prevent depredation, we covered nests with plastic hardware cloth (2- × 2-cm mesh size) staked to the soil before reburying the nest to its original depth. We left all nests in situ until the second week of July in 2015 and until the first week of July 2016, when we found the first clutches of fully hatched eggs. We then excavated all nests and placed eggs in Sterilite containers filled with ddH2O and perlite substrate (1:1 by mass) maintained in incubators at 29 ± 1 °C and ~80% humidity until hatching.

At hatching, we collected <0.2-ml blood samples for genotyping and parentage analyses from the subcarapacal vein of each live hatchling (Hernandez-Divers et al. 2002), corresponding to less than 5% mass of each individual (Mader 2006). We also dissected inviable eggs to salvage tissue from embryos when possible. As part of an ongoing mark-recapture study at ABS, we attempted to capture (by hand or using wire cage traps) and collect blood (1–2 mL) from all potential breeding adults in our study area for this and a concurrent study (Yuan et al. 2015). Over the course of 3 annual censuses conducted in 2012–2016, we collected blood from all 112 subadult or adult tortoises encountered in HG and surrounding units, that is, the entire section of the Station shown in Figure 1. We also 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). We stored all blood samples in lysis buffer (100 mM Tris Base, 100 mM EDTA, 150 mM NaCl, 1% SDS) and embryonic tissue in 70–90% EtOH at −20 °C until DNA extraction. We extracted whole genomic DNA using QIAGEN DNeasy Blood and Tissue kits (QIAGEN N.V., Hilden, Germany).

Genotyping and Parentage Analyses

We initially screened 15 polymorphic microsatellite loci previously optimized for the tortoise population at ABS (Yuan et al. 2015) to genotype all hatchlings and adults (Schwartz et al. 2003; Tuberville et al. 2011; Kreiser et al. 2013). We used GeneMarker (v. 2.6.7) to genotype 220 offspring and 98 adults and reviewed and visually confirmed all calls made by GeneMarker. We calculated allele frequencies using CERVUS (Kalinowski et al. 2007) and included all potential dams and sires to capture all alleles present in the population. We excluded loci with low polymorphic information content (PIC < 0.382), high frequency of null alleles (F(null) > 0.05), or those that failed to adhere to Hardy–Weinberg equilibrium. The final 11 selected loci (see Supplementary Table S1 online) had high exclusion probabilities (the probability of excluding an unrelated individual as a parent) for both individual parents and combined parent pairs to increase likelihood of accurate parentage assignment (see Supplementary Table S2 online). We tested all combinations of loci for linkage disequilibrium (LD) using GenAlEx (Peakall and Smouse 2006; Peakall and Smouse 2012) and used Bonferroni-corrected P-values to adjust for multiple comparisons.
After assigning genotypes, we used 2 likelihood-based programs, CERVUS (Kalinowski et al. 2007) and COLONY (Jones and Wang 2010), to assign parentage of hatchlings. CERVUS uses a pair-wise likelihood approach, assigning the parent pair with the highest likelihood score. COLONY operates on a pedigree-reconstruction approach with known sibship included. COLONY allows for more efficient incorporation of error rates, reducing the potential for incorrect assignment because of null alleles or genotyping errors. When combined, the 2 programs provide a powerful approach to assigning parentage to offspring (Karaket and Poompuang 2012).

In CERVUS, we simulated analyses of 10,000 hatchlings with 47 candidate dams and 51 candidate sires. Given the intensity of mark-recapture sampling at ABS, we estimated that 98% of potential dams and 95% of potential sires had been accounted for in our sample. Individuals were only included in the analysis if at least 6 of 11 loci were successfully genotyped. 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. We used allele frequencies and null allele frequency rates calculated in CERVUS as input into COLONY. In COLONY, we included no prior for sibship size. We used the recommended parameters for run length (medium) and number of threads (1; Jones and Wang 2010). Finally, for eggs collected from the same nest, we assumed that hatchlings were siblings or half siblings. For those individuals, we applied a 2-locus mismatch threshold. We included a flat error rate of 0.01 to account for other potential sources of error (including mutation; Hoffman and Amos 2004; Christie et al. 2014) in our data. We compared assignments from both programs to assess convergence of results. Exclusion-based approaches to parentage assignments (Richard et al. 2005; Serbezov et al. 2010; Davy et al. 2011) can be sensitive to mismatches at even just 1 locus (Karaket and Poompuang 2012). With the addition of more loci, accuracy of CERVUS increases while accuracy of COLONY assignments may decrease (Karaket and Poompuang 2012). Given that we 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 2 programs. To estimate the power of our methods, we conducted 2 comparisons: 1) within COLONY, we compared the assignment results of dams with and without known sibship; and 2) we compared assignment results of dams and sires between programs. Each potential assignment was treated as a binomial test in which mismatches in assignments were counted as “0” values. For each comparison, we calculated the probability of convergence and confidence intervals.

We reviewed genotypes of any hatchling that presented mismatches in parentage assignment between the 2 programs. For clutches for which multiple females were assigned to a single clutch in CERVUS, we compared genotypes of each hatchling to the assigned sire and majority dam. If there were <2 allele mismatches with the candidate dam for all hatchlings not assigned the majority dam, we assigned the majority dam to the clutch, contingent on assignment of that dam to the clutch in COLONY. Any parent pairs assigned to offspring that did not meet the 95% critical delta value in CERVUS but were still assigned as the most likely parent pairs were reviewed for allele mismatches. If less than 2 mismatches occurred across all loci and the results were convergent with those from COLONY, the parent pair was assigned. Of the 31 clutches, mismatches in assignment of females occurred in 2 offspring across 2 clutches. For male assignments, of the 31 clutches we reviewed, nonconcordance in assignment between the 2 programs occurred in 7 offspring across 5 clutches. Mismatches in assignment occurred in samples from both offspring (n = 5) and inviable eggs (n = 4). Assignment of additional sire(s) to the clutch by both programs was checked for number of mismatches between primary sire and offspring. If offspring had mismatches at >2 loci, then we assigned an additional sire to the clutch (Lance et al. 2009).

Statistical Analyses

We used a hierarchical zero-inflated Poisson (ZIP) mixture model to evaluate male size (SCL) effects on siring success (whether a male was a sire or nonsire) and reproductive success (count of offspring sired). We selected the ZIP model 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. 2003; Kéry 2010). The logistic component of the model evaluated the effect of sire size on whether a male was successful in siring any sampled offspring, resulting in a binary outcome of sire or nonsire. 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 per sire, evaluating each 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 phenomenon that only some males are successful in siring any offspring; hence, our data have many zeroes (i.e., males not assigned as sires to any offspring in our sample). Whether an individual sired offspring (sired) is drawn from a Bernoulli distribution with the probability, \( p_{\text{sire}} \), which is based on a linear model with mean-centered sire size (centered around the sample mean; \( n = 51 \)) as the predictor variable (i.e., larger males are predicted to be more likely to successfully sire offspring) and is estimated by the logit,

\[
\text{sired} \sim \text{Bernoulli}(p_{\text{sire}}),
\]

\[
\text{logit}(p_{\text{sire}}) = \beta_0 + \beta_1 \text{SCL},
\]

The effect of male body size on reproductive success (number of offspring sired by those males who were confirmed sires; number of offspring pooled across years) is illustrated by the following equations where total offspring sired (\( t \)) is drawn from a Poisson distribution with probability, \( p_{\text{sire}} \), which is based on a linear model with mean-centered sire size (centered around the sample mean; \( n = 51 \)) as the predictor variable (i.e., larger males are predicted to be more likely to successfully sire offspring) and is estimated by the logit,

\[
t \sim \text{Poisson}(\lambda),
\]

\[
\text{zip}_i = \lambda_i \ast \text{sired}_i,
\]

\[
\text{logit}(\lambda_i) = \beta_0 + \beta_1 \text{SCL}_i.
\]

In addition, we evaluated the effect of male body size on probability of contributing to a clutch with multiple sires (MP) or being the sole sire in a clutch (single paternity; SP) using a Bernoulli logistic regression. We excluded males that contributed to both MP and SP clutches. Paternity outcome (MP vs. SP) of a clutch (\( MP \)) is drawn
from a Bernoulli distribution with probability $p_{M\text{P}}$, which is a function of male body size ($SCL_i$),

$$MP_i \sim \text{Bernoulli}(p_{M\text{P}}),$$

$$\text{logit}(p_{M\text{P}}) = b_0 + b_1SCL_i.$$

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

**Results**

**Nest searching**

We searched the aprons of 149 tortoise burrows in 2015 and 101 tortoise burrows in 2016. We collected 126 eggs from 17 clutches in 2015 and 129 eggs from 18 clutches in 2016. Clutch sizes were similar in 2015 (mean 8.73 ± 2.05, range 6–12 eggs) and 2016 (mean 8.11 ± 2.54, range 5–15 eggs; Table 1). Hatching success varied among clutches but overall hatching success (total eggs hatched/total eggs incubated = 78%) was similar between years.

**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 un-hatched eggs (15 clutches). In 2016, we collected 115 samples from 93 hatchlings and 22 un-hatched eggs or deceased hatchlings (16 clutches). We attempted to genotype all samples at the selected loci but excluded 6 samples from 2015 and 5 samples from 2016 due to poor amplification, resulting in 110 samples from each year for parentage analysis. Frequency of null alleles at our 11 microsatellite loci ranged from −0.0518 to 0.0404 (see Supplementary Table S2 online). PIC (range 0.385–0.883; see Supplementary Table S2 online) was greater in loci with more alleles ($k$; range 3–15; see Supplementary Table S2 online). We found no evidence of LD.

**Parentage Assignment**

We identified 22 unique dams and 17 unique sires that contributed to the 2015–2016 clutches. When comparing results with and without

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<th>Offspring genotyped</th>
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<td>1477</td>
<td>287</td>
</tr>
<tr>
<td>603–2016</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>1531</td>
<td>270</td>
<td>1242</td>
<td>263</td>
</tr>
<tr>
<td>761–2016</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>1922</td>
<td>314</td>
<td>625 (4), 1473 (4)</td>
<td>285, 300</td>
</tr>
<tr>
<td>798–2016</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>Unk. Dam</td>
<td>—</td>
<td>1235 (4), 1487 (1)</td>
<td>286, 260</td>
</tr>
</tbody>
</table>

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 un-hatched eggs when possible), assigned dam and sire and their respective SCL. For clutches with multiple sires (MP), the number of offspring attributed to each sire is denoted in parentheses after Sire ID. Clutches are sorted by Dam ID to facilitate comparison of parentage between years.
known sibship included in analyses, probability of convergent results was high (0.9818 ± 0.0177). When comparing assignment of dams and sires between COLONY and CERVUS, probability of convergent assignments was 0.9590 ± 0.0185 (±1 SE). For a single clutch from 2016, we were unable to assign a known (i.e., sampled) female within the population with confidence (Table 1). Most genotyped males (n = 34; Figure 2) did not sire any of the genotyped offspring in our sample. Of those males with assigned offspring, individual males sired a total of 2–28 offspring (Figure 2) and contributed to 1 to 5 of the genotyped clutches (Table 1). The average number of offspring attributed to successful males was similar between 2015 and 2016, although the mean number and variance of assigned offspring among successful sires were slightly greater in 2015 than 2016 (8.46 ± 5.3 and 7.85 ± 3.4 offspring, respectively). All females for which clutches were assigned in both 2015 and 2016 (n = 9) produced offspring with the same males in both years (Table 1). Seven of these 9 females produced offspring with just a single male. One female produced MP clutches with the same 2 males (n = 7). However, 1 clutch (565–2015) required assignment of at least 3 males to explain offspring genotypes (Figure 4).

Of the 17 males known to sire offspring, 7 individuals (mean SCL 294 ± 20 mm) contributed exclusively to multiply sired clutches, whereas another 7 males (295 ± 23 mm) were assigned exclusively to singly sired clutches. Three males (292 ± 9 mm) contributed to both multiply sired and singly sired clutches (Figure 5). Proportional contributions of males to MP clutches were variable. The average contribution of the primary male was 66.5 ± 12.7% (Figure 4). Three clutches exhibited skewed distributions with 1 male siring at least 80% of the offspring in the clutch (Figure 4). In 75% of MP clutches that had a predominant male (sired > 50% of clutch), the primary male was smaller than the other contributing male(s). We found no significant effect of male size on likelihood of contributing to MP or SP clutches (see Supplementary Figure S1 online). The model reached convergence on all parameters across 3 chains of 100,000 iterations with a 1,000-iteration burn-in period. Likewise, mean SCL of females that produced SP clutches (314 ± 20 mm) was similar to mean SCL of females that produced MP clutches (318 ± 13 mm).

**Multiple Paternity**

For estimating frequency of MP, we excluded 2 clutches from female 1245 (one from 2015 and one from 2016) that were too small for analyses (<3 offspring genotyped). Thus, we calculated rates of MP in 2015 and 2016 based on 14 and 15 clutches, respectively. The frequency of MP was similar in 2015 and 2016 (26.7% and 28.6%, respectively). The overall rate of MP across years was 27.6% (8/29 clutches). Most MP clutches were explained by assignment of 2 males (n = 7). However, 1 clutch (565–2015) required assignment of at least 3 males to explain offspring genotypes (Figure 4).

**Male Body Size and Siring Success**

Size of sexually mature males ranged from 209 to 330 mm SCL with an average of 275.6 ± 26.8 mm (mean ± 1 SD). Sires were on average 28 mm larger than nonsires (294 ± 21 and 266 ± 25, respectively). The Bernoulli regression on siring success (probability of being a sire or nonsire) indicated that siring success increased with sire size (see Supplementary Table S3 online; Figure 3A) and that males larger than 294 mm (15 males in this sample) had at least a 50% probability of siring offspring. Of 15 males in our sample with SCL > 294 mm, more than half (n = 10) produced offspring. Among successful sires, the effect of size on expected number of offspring was slightly positive, indicating that larger sires were slightly more likely than smaller sires to sire more offspring (see Supplementary Table S3 online; Figure 3B). The combined model reveals that at larger sizes, male gopher tortoises are more likely to experience both increased siring and reproductive success (Figure 3C). We achieved model convergence on all parameters with 50,000 iterations of 3 chains with 1,000-iteration burn-in.

**Discussion**

By characterizing the genetic mating system of the gopher tortoise, we sought to clarify whether reproductive strategies in our study population were more characteristic of female-defense or scramble-competition polygyny. Our data indicate that gopher tortoises in this population are primarily adopting a defense polygyny system. Based on the offspring we sampled, siring success was heavily skewed towards a subset of candidate males. We found that male body size (SCL) was a significant predictor of whether males...
sired any offspring, as well as the number of offspring successful males sired. As expected, we detected both singly and multiply sired clutches, though we found no significant relationship between male body size and likelihood of contributing to singly or multiply sired clutches. Lastly, we documented potential mate fidelity across years in all females for which we collected nests in both years. Although our data are consistent with female defense being the dominant strategy employed by males in this population, we cannot yet distinguish if males are effectively defending individual females or the territories within which females reside. Thus, future studies of individual behavior will be fruitful for determining the actual mechanisms by which larger males achieve higher reproductive success.

Our results corroborate previous findings that larger males achieve greater siring success (Moon et al. 2006; Tuberville et al. 2011). Although we found overlap in the body sizes of sires (260–330 mm) and nonsires (209–305 mm) in our population, sires were on average 28 mm longer than nonsires, an even greater size discrepancy between sires and nonsires than reported by Tuberville et al. (2011), ~10 mm. Because we did not find the nests of all 37 telemetered individuals, it is possible that candidate males with zero offspring in our analysis did in fact sire offspring in other unsampled clutches. However, we assume that our sample is representative of the population as a whole, given the range in body sizes of both males and females identified as parents and because we have no reason to expect that dams of undetected nests would exhibit divergent mate-selection strategies or rates of MP. The Hill Garden and surrounding areas are small enough that males should be able to access most females over the breeding season (McRae et al. 1981b; Diemer 1992); yet, large males had an advantage in siring and reproductive success. Thus, larger males may be more likely to succeed in competitive interactions with other males, as observed in the closely related desert tortoise (*Gopherus agassizii*; Niblick et al. 1994). The enhanced mating opportunities afforded larger males are indicative of a female-defense mating system and would presumably result in greater reproductive success.

The frequency of MP we observed in our study (27.6 %) is similar to the frequency reported by Moon et al. 2006 (28%) for...
a population in Florida but is only half the frequency reported by Tuberville et al. (2011) (57%) for a gopher tortoise population in Georgia. These differences may be a result of differences among populations in the spatial distribution of females, which affects the strength of selection on male size (Emlen and Oring 1977). Although larger males in our population had greater rates of siring success, they did not completely monopolize matings with their female partners. Contrary to our expectations, larger males were no more likely than smaller males to contribute to MP versus SP MP clutches. Therefore, although male size confers advantages to males, defense of either the female or territory is not complete, or females are exerting a role by also mating with smaller males when the opportunity arises. The function of MP in reptiles is highly debated. MP likely confers genetic benefits to females (Uller and Olsson 2008) via increased hatching success (Chrysemys picta; McTaggart 2000) and increased genetic diversity of offspring (Jennions and Petrie 2000). The effects of shared paternity on male fitness in our study population are unclear, as males with the greatest reproductive success contributed to both MP and SP clutches with no clear likelihood of one over the other. However, the fact that large males in our population have a fitness advantage in terms of siring and reproductive success, yet small males still contribute to paternity through multiply sired clutches underscores the many influences on male and female mating decisions that ultimately lead to different mating systems predominating in different populations.

One of the most intriguing and unexpected patterns that emerged from our study was the stability in parent pairs between years, with all 9 females whose clutches we sampled in consecutive years producing offspring sired by the same males each year. To the best of our knowledge, our study is the first to report paternity patterns in consecutive clutches from multiple female Gopherus tortoises. The ability of female gopher tortoises to store sperm (Palmer and Guillette 1988) may explain the stability of pairs across years. However, stability in paternity patterns may also occur if males are effectively able to defend females from other males in a female-defense mating system. In reptile species that lack sperm storage capacity, mate fidelity (i.e., remating) appears to be the mechanism for repeated clutches sired by a single male (Rawlinson 1974; Stow and Sunnucks 2004). For example, multiyear mate fidelity has been observed in reptiles without documented long-term sperm storage, such as the American alligator (Alligator mississippiensis; Lance et al. 2009) and the wood turtle (Glyptemys insculpta; Bouchard et al. 2016). Given the frequency of social interactions and the extended mating season of gopher tortoises (Douglass 1986; Moore et al. 2009), male gopher tortoises in our study population have ample opportunity to remate with females. Recent examination of gopher tortoise social behaviors has revealed complex structure, including formation of restricted social groups or cliques (Guyer et al. 2014), which may further contribute to mate fidelity. Douglass (1986) observed males at ABS visiting the same females in subsequent years, reinforcing the idea that mate preference or mate fidelity may play a role in this species. The formation of longer-term pairs may confer a reproductive advantage to males by securing access to particular females. Concurrent evaluation of mating behavior and parentage patterns will be needed to determine which mechanism(s)—repeated use of stored sperm, mate fidelity, or both—are driving repeated siring of clutches in the gopher tortoise.

Unexplained variation remained in predicting the degree of polyandry, siring probability, and reproductive success, especially of males that had the highest degree of success (those with the most offspring). Considering females may be visited by as many as 14 males in 1 mating season (Boglioli et al. 2003), female choice may be a contributing factor in determining the outcome of mating in this species, particularly if females are unreceptive to attempted matings by certain males. Furthermore, gopher tortoises exhibit female-biased size dimorphism (McRae et al. 1981a), which likely precludes coercive matings in this species. In a study based on behavioral observations of gopher tortoises, larger males did not necessarily experience increased mounting success compared with smaller males (Boglioli et al. 2003). Although size is clearly an important factor to consider, variation in male courtship effort allocated among females, similar to what we might expect in a scramble-competition polygynous system, may still be important (Testudo hermanni; Cutuli et al. 2014). Our results confirm that mating systems are dynamic and context-dependent and thus may differ across populations within a species, depending on the sizes of mating individuals, sex ratio, and population density (Carrete et al. 2006, Kokko and Rankin 2006). The gopher tortoise population in our study area exhibits...
a female-biased sex ratio (1.5:1, Betsie Rothermel, unpublished data). Although theoretically this female bias should rely sexual selection and decrease reproductive skew (Kokko and Rankin 2006), we observed high reproductive skew in the number of successful sires, which would suggest that males are effectively defending females, or females are exceptionally choosy despite a smaller pool of males. The density of adult tortoises in HG is approximately 6 tortoises/ha (Betsie Rothermel, unpublished data), 4–20 times greater than the densities of gopher tortoises at sites where the mating system has been described as scramble-competition polygyny (Johnson et al. 2009; Geyer et al. 2012). Increases in population density may sufficiently concentrate females to make female defense a feasible reproductive tactic in terms of male fitness (Emlen and Oring 1977). Nevertheless, our results are consistent with Douglass’s (1986) observation that HG females may still be dispersed enough that individual males cannot completely defend a harem given the need to allocate courtship effort among multiple females. Although ultimately our study shows support for characterizing the gopher tortoise mating system as female-defense polygyny, the variation among observed mating systems in the gopher tortoise is suggestive of the potential for plasticity of mating systems within a species. Indeed, different individuals within a population may employ alternative mating tactics (Noble et al. 2013). Within-species mating system plasticity as a function of density has been observed in birds (Carrete et al. 2006), fishes (Mobley and Jones 2007; Casalini et al. 2010), and mammals (Yoccoz et al. 2002; Iossa et al. 2008; Streetfeld et al. 2011). Our data confirm patterns consistent with behavioral observations in the gopher tortoise (Douglass 1986), while conflicting with others (Johnson et al. 2009), further underscoring the need to combine molecular and behavioral techniques to assess mating system dynamics.

Our study represents the largest study of parentage for an in situ population of gopher tortoises and the most complete sampling of a single population to date. Overall our results provide evidence of female-defense polygyny in the gopher tortoise, but also reveal non-size-dependent polyandry in a fraction of the population. We recommend incorporating additional aspects of gopher tortoise biology and methods of study to provide a more comprehensive view of how range-wide variation in population characteristics may affect mating dynamics in this and other long-lived, group-living reptiles.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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Data Availability

We have archived microsatellite genotypes, assignment data, and size data in Dryad (doi:10.5061/dryad.31bc37q). Metadata for this and other projects related to the long-term tortoise study at Archbold Biological Station can be found at http://www.archbold-station.org/html/datapub/data/data.html.

References


