

# Multiple Paternity and Breeding System in the Gopher Tortoise, *Gopherus polyphemus*

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## Abstract

Little is known about the reproductive behaviors and the actual outcomes of mating attempts in the gopher tortoise (*Gopherus polyphemus*). We examined the mating system and reproductive behaviors of a population of gopher tortoises in central Florida. Using microsatellite markers, we assigned fathers to the offspring of seven clutches and determined that multiple fathers were present in two of the seven clutches examined. We found that gopher tortoises exhibited a promiscuous mating system with larger males fertilizing the majority of clutches. The advantage of larger males over smaller males in fertilizing females may be a result of larger males winning access to females in aggressive bouts with other males or larger males may be more attractive to females. Clutches produced by larger females tended to be sired by a single male, whereas clutches of smaller females tended to be sired by multiple males.

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The gopher tortoise (*Gopherus polyphemus*) is one of four native North American tortoise species generally occupying dry, sandy habitats of the southern United States and northern Mexico. *G. polyphemus* ranges in the southeast United States south from the extreme corner of southwestern South Carolina to the tip of the Florida peninsula, west to Louisiana, and north to about the middle of Mississippi, Alabama, and Georgia (Auffenberg and Franz 1982). Wild tortoises generally live for 40–60 years and become reproductively active at 10–20 years of age (Epperson and Heise 2003; Mushinsky et al. 1994). Although believed to be previously abundant, the gopher tortoise is declining in numbers, mostly due historically to harvesting and recently to extensive habitat loss (Auffenberg and Franz 1982; Diemer 1986). Consequently, the species is of conservation concern and is protected in a variety of ways throughout its range. *G. polyphemus* internationally is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Inskipp and Gillett 2003); federally is listed by the U.S. Fish and Wildlife Service as threatened in Louisiana, Mississippi, and west of the Tombigbee and Mobile Rivers in Alabama (U.S. Fish and Wildlife Service 1986); and in the state of Florida is considered a Species of Special Concern by the Florida Fish and Wildlife Conservation Commission (2005).

Habitat loss in Florida is occurring at an alarming rate. Between 1936 and 1987, urban land area increased 500% in Florida (Kautz 1993), and since 1970, the human population of Florida has increased by more than 3 million persons every decade (Bureau of Economic and Business Research 2000). Also between 1936 and 1987, longleaf pine forest, the prime tortoise habitat, declined in area by 88% (Kautz 1993) with the remaining forests area now small and fragmented (Noss 1989). Because of these trends, aggressive conservation efforts are needed to ensure the survival of the gopher tortoise.

In general, sound conservation efforts should include as much information as possible on the biology and ecology of a species of interest. In particular, a detailed understanding of the elements of the mating system can be critical to the persistence of a species. How mates are selected determines in large part the fate of genetic variation, and genetic variation, in turn, factors into the presumed long-term evolutionary health of species (Dudash and Fenster 2000; Gilpin and Soulé 1986; Lande and Barrowclough 1987). For turtles in general, monogamous pair-bond formation is uncommon (Pearse and Avise 2001). Parental investment is minimal, and the male's contribution to the offspring is principally the donation of sperm. To the contrary, more promiscuous types of

mating systems, such as polygyny and polyandry, are common in turtles (Crim et al. 2002; Jessop et al. 1999; Valenzuela 2000). In a polygynous system, a single male copulates with multiple females, whereas in a polyandrous system, a single female copulates with multiple males. There exist, therefore, differing expectations for patterns of paternity in clutches produced in polygynous versus polyandrous systems; multiple paternity should be the norm in polyandrous systems but not necessarily so in polygynous systems. Although female turtles apparently do not gain direct mating benefits, such as nuptial gifts or paternal care of offspring, possible indirect benefits to mating with more than one male would include gaining “good genes” (Kempnaers et al. 1992; Otter and Radcliffe 1996; Watson 1998), avoidance of genetic incompatibility (Zeh JA and Zeh DW 1996), fertility insurance (Orsetti and Rutowski 2003), and increased genetic diversity of offspring (Byrne and Roberts 2000; Madsen et al. 1992).

Many turtle species are known to store sperm for long periods of time. For example, a captive female box turtle continued to produce viable offspring 4 years after being isolated from males and thus a source of sperm (Ewing 1943). The desert tortoise (*Gopherus agassizii*) has been shown to store sperm for at least 2 years (Palmer et al. 1998; Pearse and Avise 2001). Because of sperm storage, females could engage in a form of temporal polyandry. Multiple paternity in gopher tortoises, therefore, could result from inter- as well as intra-annual copulations. Gopher tortoises exhibit mating behaviors from March to September; egg laying, however, is only known to occur in May and June (Butler and Hull 1996; Douglass 1976; Iverson 1980). It seems likely then that sperm from late summer or fall mating is stored and used to fertilize eggs the next spring. Therefore, even females mating with only a single male within a season, but not between seasons, would be effectively polyandrous. Clearly, sperm storage could lead to a higher probability or degree of multiple paternity even in a strictly monogamous species. Thus, multiple paternity in gopher tortoises may be an example of temporal as well as single-season polyandry, the combined effect of which can be estimated indirectly by assessing the degree of multiple paternity of natural clutches.

Very little is known about the mating system of gopher tortoises. Male dominance hierarchies as related to mating have been documented; larger males most often are the victors in aggressive male-male interactions (Douglass 1976; McRae et al. 1981). Douglass (1976) hypothesized that dominant males might also maintain a loose harem (but see Boglioli et al. 2003). Given the general low density of females and unfavorable energetics, McRae et al. (1981) suggested that it would be unlikely for a male to defend several courted females, and the results of a study by Boglioli et al. (2003) supported scramble competition polygyny. Instances of male courtship behaviors (e.g., head bobbing) have been observed and are commonly reported in wild tortoise populations, although subsequent copulation rarely has been observed except in captive settings (Auffenburg 1966; Douglass 1976; Wright 1982). Male-male confrontations, in which the participants attempt to overturn one another, also are observed during courting season (Hailman 1991; personal observa-

tion). The importance of these behaviors, however, is largely unknown. Field observation can identify dominant males on an incident-by-incident basis, but rarely can they reveal whether dominance leads to greater reproductive success. For this, an assessment of the actual paternity of the offspring in a clutch is necessary. Molecular genetic techniques able to identify single individuals are providing answers to a variety of questions concerning mating systems, such as paternity (Roques et al. 2004), maternity (Jones et al. 1999), and mating behaviors (Strausberger and Ashley 2003). Microsatellite loci are by far the most frequently used genetic tool in the investigation of specifics of mating systems. These loci mutate quickly and, therefore, are highly polymorphic (Dallas 1992) making them especially useful in identifying individuals (Queller et al. 1993). The probability of identifying or excluding from consideration specific individuals often is extremely large with multilocus microsatellite genotypes. Given this, microsatellite markers are an ideal genetic tool for assaying multiple paternity.

Here, we report the results of a microsatellite locus genotyping study examining the mating system of a population of gopher tortoises in central Florida. In particular, we addressed the following questions: Does multiple paternity occur in gopher tortoise clutches, and, if so, is the fertilization of a single clutch evenly divided among the males? Do some males fertilize more clutches than others, and, if so, is fertilization success related to male size?

## Methods

### Sample Collection

Our field site was the University of South Florida Ecological Research Area (ERA), a 200-ha reserve located in Hillsborough County in west central Florida (28.05°N, 82.20°W). Approximately 20 ha of sandhill habitat within the ERA have been exposed to controlled burning since 1976 (Mushinsky 1992). This area is separated into 11 plots that are burned on 1-, 2-, 5-, or 7-year cycles or left as unburned controls (Mushinsky 1985). Tortoises are distributed through the upland habitat of the ERA but are most commonly found in the plots subjected to control burns.

A population of about 280 tortoises occupies the ERA (Mushinsky et al. 1994). All plots were trapped for tortoises during the course of this study from April to August of 2001 and again in 2002, and all active and inactive burrows (based on Mushinsky and McCoy 1994) in each plot were located and marked. The width of each burrow was measured at a depth of 500 mm and used as an estimate of the carapace length (CL) of the resident tortoise (Wilson et al. 1991). Burrows greater in width than the minimum CL of a sexually mature individual of either sex (i.e., males at 177 mm; Diemer and Moore 1994; Mushinsky et al. 1994) were pit trapped by burying at the burrow entrance a 9.5-L bucket camouflaged with brown fabric and sand with the opening level with the surface of the ground. While set, the traps were checked every 2 h during daytime. Individuals also were captured by hand when encountered outside their burrows.

Sex and stage of maturity in gopher tortoises can be inferred readily by the size of the carapace. Tortoises larger than 240-mm CL display sex-specific degrees of plastral concavity (PC). The PC of females is less, and that of males greater, than 6 mm deep (Mushinsky et al. 1994). Females in the ERA are reproductive at ~240-mm CL (Mushinsky et al. 1994) and males at CL from 177 to 230 mm (Diemer and Moore 1994).

Mass and CL were assessed for all sexually mature and subadult tortoises captured (Mushinsky et al. 1994). Differences between various groupings of individuals were tested using Student's *t* tests. The CL and mass of the mothers and the fathers assigned to the clutch based on the genetic data for single- and multiple-sire clutches, as well as clutch characteristics, also were compared using Student's *t* tests. A blood sample was obtained from the brachial sinus with a heparinized 21-gauge needle and a 10-cc syringe. After collection, blood was stored in a lysis buffer (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 50 mM ethylenediaminetetraacetic acid, 1% w/v polyvinylpyrrolidone, and 0.2% v/v 2-mercaptoethanol) until DNA extraction. Unless already identified, all individuals were numbered and uniquely marked by notching the marginal scutes, and all individuals were released at the site of capture. The overall goal was to genotype all sexually mature males in the population (i.e., the paternal pool) and genotype and capture all sexually mature females.

To determine if they were carrying shelled eggs, sexually mature females were taken to the laboratory and radiographed with one pulse from The Inspector X-ray Source, Model 200 (Golden Engineering, Centerville, Indiana), with a source output of 3 mR at 1 ft per pulse. Females with completely shelled eggs were given an injection of 1.5 units per 100-g body mass of 3% oxytocin to stimulate oviposition (Ewert and Legler 1978; Iverson J, personal communication). After oviposition, blood was drawn and stored for DNA extractions, and mothers were returned to the site of capture.

Eggs were incubated in the laboratory in nests of vermiculite with a 1:1 w/v ratio of vermiculite to water (Burke et al. 1996) and the temperature maintained at ~30°C (Burke et al. 1996; Demuth 2001). Eggs were inspected daily during incubation and hatchlings removed when discovered. Eggs that did not hatch after 120 days were removed and opened to remove the embryo, if present, and frozen for later DNA extraction. Live hatchlings were maintained in aquaria with a 12:12 light:dark cycle and fed a diet of vegetables supplemented with vitamin and mineral powders. Blood samples were obtained from hatchlings by cardiocentesis with a heparinized 26-gauge needle and a 1-cc syringe (Jacobson E, personal communication). Hatchlings were given individual markings in the same manner as adults and released at the burrow where the mother was captured.

### Genotype Analysis

Total cell DNA was extracted following a standard phenol/chloroform protocol and ethanol precipitated (Herrmann and Frischauf 1987). All individuals were genotyped at nine microsatellite loci (GP15, GP19, GP26, GP30, GP55,

GP61, GP81, GP96, and GP102) previously characterized by Schwartz et al. (2003). Microsatellite polymerase chain reaction amplification reactions were run with one of two multiplexing primer mixes with reaction mixes and cycling parameters following Schwartz et al. (2003). After amplification, samples were run on an ABI Prism 377 automated DNA sequencer (Perkin Elmer, Applied Biosystems, Inc, Foster City, CA) at Iowa State University. The program, GENESCAN (Perkin Elmer, Applied Biosystems, Inc.), was used to identify and quantify microsatellite peaks, and electropherograms of all individuals at all loci were scored once each by three persons to ensure accuracy in reading and recording of results.

Maternal contribution of each individual in the clutch was determined by comparing the offspring's and the mother's genotypes. Paternal alleles were inferred by removing known maternal allelic contributions from the offspring genotypes, when possible. If among all hatchlings, more than two paternal alleles were found at any locus, the clutch was classified as having multiple sires. For multiply sired clutches, paternity exclusion was done on an individual hatchling basis. In any case, candidate males whose genotype mismatched the paternal contribution of the hatchlings at one or more loci were excluded as potential sires. Individual and combined exclusion probabilities for all loci were calculated with the program CERVUS, version 2.0 (Marshall et al. 1998). When more than one male could not be excluded as the father of a hatchling, we performed a probability analysis on all nonexcluded males. CERVUS uses a log (base e) likelihood algorithm to calculate the likelihood ratio (LOD) of a candidate male being the true parent compared to an arbitrary male. LOD scores are calculated for all possible sires (i.e., nonexcluded males), and the difference between the two most likely candidates ( $\Delta$ ) is calculated and provides an indication of the reliability of the assignment. By simulation, a critical  $\Delta$  score is calculated at either relaxed (80%) or strict (95%) confidence level and corresponds to an estimated frequency of false positives of 20% and 5%, respectively. In multiple sire clutches, it is possible that all males except one are excluded as potential fathers for some, but not all, of the hatchlings. When the unequivocally assigned father was one of the nonexcluded fathers for a clutch mate with more than one potential sire, he was assigned as the father regardless of the  $\Delta$  value.

Allele frequencies were estimated only from wild-caught individuals (i.e., not including hatchlings). The probability of two unrelated individuals sharing the same genotype at all loci (probability of identity) was calculated as in Hanotte et al. (1991). The probability of exclusion (*D*) is the overall probability of detecting multiple paternity and was calculated as in Westneat et al. (1987). This calculation utilizes the frequency of alleles and all possible mating arrangements to compute the probability of detecting when a female has mated with another male besides the candidate father, given the loci used.

### Results

We captured 15 adult females representing approximately 31% of the 49 adult females observed in the ERA between 1988 and 1993. Seven of the 15 were gravid and produced

**Table 1.** Characteristics of mother, clutch, and father for single-sired and multiple-sired clutches

	Single sired					Multiple sired		
	Clutch 18	Clutch 201	Clutch 253	Clutch 378	Clutch 529	Clutch 446	Clutch 523	
Mother ID	18	201	253	378	529	446	523	
Mother CL (mm)	300	314	306	291	317	276	289	
Mother mass (g)	4400	4850	4100	4300	4450	3100	3100	
Father ID	43	343	441	519	346	43 <sup>a</sup>	533	180 <sup>a</sup> 256
Father CL (mm)	290	278	250	264	270	290	263	260 276
Father mass (g)	3800	4000	2800	3500	3400	3800	2800	2850 3500
Total number of eggs	9	12	9	7	9	6		10
Hatching success (%)	100.0	91.6	33.3	57.2	55.0	33.3		0.0
Number of undeveloped eggs	0	1	0	2	0	1		3
Number of eggs genotyped	8 <sup>b</sup>	11	9	4 <sup>b</sup>	9	5		7
% Fertilized by 1 <sup>o</sup> male	100.0	100.0	100.0	100.0	100.0	80.0		57.1

<sup>a</sup> This male sired the largest number of the clutch mates and is designated as the primary male.

<sup>b</sup> A blood sample could not be obtained from one member of this clutch.

clutches with the number of offspring per clutch ranging from 4 to 11 with a mean of  $7.57 \pm 2.44$  (Table 1). A total of 90 individuals were genotyped, including 5 subadults, 15 adult females, 17 adult males, and 53 offspring. The nine microsatellite loci had two to five alleles each, with a mean of  $3.44 \pm 1.01$  alleles per locus and observed per-locus heterozygosity ranged from 0.053 to 0.658 (Table 2). The average probability of two unrelated tortoises sharing the same genotype at all nine loci (probability of identity) was  $3.84 \times 10^{-4}$  (Table 2). The probability of detecting multiple paternity ( $D$ ) with a single locus ranged from 0.026 (at GP96) to 0.410 (GP81; Table 2) and was 0.876 when all nine loci were used. The cutoff  $\Delta$  value estimates for the relaxed and strict confidence limits were  $\Delta_{80\%} = 1.44$  and  $\Delta_{95\%} = 3.10$ , respectively.

### Paternal Assignment

In most cases, the maternal contribution could be unambiguously determined by inspection. In cases where the mother and offspring were both heterozygous for the same alleles, determining unambiguously which allele was the maternal allele was impossible. Males who possessed at least one copy of

either allele, therefore, could not be excluded from the analysis. When a father was assigned to a clutch or an individual, the probability of another unrelated tortoise having the same genotype as the father was extremely low, ranging from  $5.65 \times 10^{-13}$  to  $1.37 \times 10^{-6}$  (Table 3).

The genetic contribution of the male also was often unambiguously identified. In four of the seven clutches, all candidate males but one were excluded because, at one or more of the loci, they could not have contributed the paternal allele to the offspring (Table 3). For one hatchling in a fifth clutch (378), all but one male (519) were excluded as potential sires. For each of the remaining three clutch mates, however, three candidate males could not be excluded based on genotype alone. Results from an analysis with CERVUS indicate that male 519 was the most likely father to all three with at least 80% confidence ( $2.52 \leq \Delta \leq 3.20$ ), so we assigned this male as the sole father of the clutch. In the remaining two clutches, three or more paternal alleles were found at one or more loci, indicating multiple sires for these clutches. For one hatchling in one of these clutches (446), all males except 533 could be excluded as a sire. For the remaining clutch mates, however, male 43 was either the only nonexcluded male (two individuals) or the most likely sire in the likelihood analysis (two individuals) albeit with small  $\Delta$  values ( $0.056 \leq \Delta \leq 0.064$ ). Male 43 also was the sole putative sire for one of the single-sire clutches (Table 3). In the other multiple-sire clutch (523), males 180 and 265 were each either the only nonexcluded male or one of several nonexcluded fathers (Table 3). For one individual in this clutch, all the males included in this survey were excluded based on genotype alone because this hatchling had one allele at each of two loci not seen in any male surveyed.

### Mating System

Reproductive output and hatching success were variable among females. The number of eggs in the clutch did not vary significantly between single- and multiple-sire clutches ( $t_{df=5} = 0.703$ ,  $P = .513$ ; Table 1). The number of undeveloped eggs also did not vary between single- and multiple-sire

**Table 2.** The number of alleles, observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, probability of identity, and probability of exclusion for nine microsatellites in *Gopherus polyphemus*

Locus	Number of alleles	$H_O$	$H_E$	Probability of identity	Probability of exclusion
GP15	5	0.605	0.611	0.210	0.359
GP19	3	0.184	0.295	0.532	0.139
GP26	4	0.289	0.305	0.506	0.163
GP30	3	0.421	0.500	0.366	0.199
GP55	2	0.132	0.125	0.773	0.058
GP61	3	0.184	0.172	0.701	0.082
GP81	5	0.658	0.662	0.168	0.410
GP96	3	0.053	0.052	0.901	0.026
GP102	3	0.632	0.613	0.227	0.326
Overall	$3.44 \pm 1.01$	0.351	0.371	$3.84 \times 10^{-4}$	0.876

**Table 3.** Results of paternity assignment and probability of identity for the seven clutches and females surveyed in this study

Clutch number	Number of offspring	Number nonexcluded	Assigned male	$\Delta^a$	Probability of identity
Putative single sire					
18	8	1	43	—	$1.14 \times 10^{-10}$
201	11	1	343	—	$4.11 \times 10^{-13}$
253	9	1	441	—	$5.65 \times 10^{-13}$
529	9	1	346	—	$2.82 \times 10^{-8}$
378	1	1	519	—	$1.37 \times 10^{-6}$
	3	3	519	2.52–3.20	—
Putative multiple sire					
446	1	1	533	—	$7.07 \times 10^{-12}$
	2	1	43	—	$1.14 \times 10^{-10}$
	2	4 <sup>b</sup>	43	0.06	—
523	1	1	180	—	$5.44 \times 10^{-8}$
	3	10 <sup>b</sup>	180	0.00–0.74	—
	1	1	265	—	$1.58 \times 10^{-8}$
	1	3	265	1.78	—
	1	0	None	—	—

<sup>a</sup> Calculated using CERVUS when there was more than one nonexcluded male and is the range over all offspring for the assigned male. Critical delta values for the relaxed and strict confidence levels are 1.44 and 3.10, respectively.

<sup>b</sup> This is the total number of unique fathers over all of these offspring.

clutches ( $t_{df=5} = -1.641$ ,  $P = .162$ ; Table 1). A negative relationship exists between hatching success and multiple paternity ( $t_{df=5} = 2.254$ ,  $P = .074$ ).

Some morphological differences were noted but were limited to differences among females of single- and multiple-sired clutches. Data on mass and CL were normally distributed and homoscedastic. Males who were assigned as fathers ( $N = 8$ ) had larger CL than unassigned males ( $N = 9$ ;  $t_{df=15} = 2.400$ ,  $P = .030$ ) but did not differ in mass ( $t_{df=15} = 1.335$ ,  $P = .202$ ). Mass ( $t_{df=6} = -0.967$ ,  $P = .371$ ) and CL ( $t_{df=6} = -0.053$ ,  $P = .960$ ) were not significantly different between fathers of single-sire ( $N = 5$ ) and multiple-sire clutches ( $N = 4$ ). Mass ( $t_{df=5} = 6.409$ ,  $P = .001$ ) and CL ( $t_{df=5} = 2.682$ ,  $P = .044$ ) did differ between females inseminated by a single male ( $N = 5$ ) and females inseminated by more than one male ( $N = 2$ ), however. Females inseminated by multiple males on average weighed 1320 g less and had CLs 23.1 mm shorter than females inseminated by a single male (Table 1).

## Discussion

All mothers were genotyped, and no mismatches between known mother-offspring pairs were observed. The mother's contribution to each clutch was identified and removed, and all remaining alleles were designated as paternal alleles. Multiple paternity was observed in two of the seven clutches. In general, the percentage of clutches in which multiple paternity is found varies with species, and sometimes even among populations within a species. For those turtle species that have been studied, the percentage of multiply fertilized clutches ranges from 4% to 100% (Hoekert et al. 2002; Moore and Ball 2002; Pearse and Avise 2001). In this study,

28.6% showed multiple paternity. This number may be conservative, however, as the ability of the loci used in this study to detect multiple paternity was only 87.6%. Thus, for each clutch in which one father was detected, there was a 12.4% chance that another father went undetected. A full characterization of the frequency of multiple paternity in gopher tortoises will require a much more extensive survey including several populations from throughout the range and more individuals per population.

One offspring, in multiple sire clutch 529, could not be assigned to any of the genotyped males. One possibility is that this individual was indeed the offspring of one of the surveyed males but appears different from either the mother or father as a result of new mutation. Because there are two loci each with a novel allele, mutation would have had to have happened independently at each of them concurrently, which is unlikely. A second, more reasonable, possibility is that three different males, one of which was not among the genotyped males, inseminated the female. Between 1988 and 1993, 42 adult males have been identified in the area, but we genotyped only 17 (41%). A third possibility is that the female may be a recent immigrant into the area. Although all individuals in this area have been routinely marked (Mushinsky et al. 1994), the mother of the unassignable offspring was not. It is possible then that in addition to the sperm from the two local males the female was carrying sperm from at least one male outside the ERA. Given the possibility of long-term sperm storage in gopher tortoises, this latter scenario is possible. Regardless, multiple paternity is clearly observed in gopher tortoises and may constitute a frequent mating outcome. We cannot, however, differentiate between intraseasonal and temporal polyandry.

Reports of aggressive interactions between male gopher tortoises have provoked the question of whether or not these

duels influence a male's access to females. If winning males do receive greater access to females, then the victor's fitness would likely be higher. If the chances of winning are positively correlated with size, then larger males would inseminate more females and fertilize more eggs. In this study, one large male (43) fertilized two of the seven gravid females sampled (nearly 30%) and 12 of the 53 eggs (23%). The remaining eight fathers each fertilized only one female and from 1.2% to 20.7% of the eggs. Males that were assigned as sires of the clutches were significantly larger than the unassigned males, suggesting that larger males have a reproductive advantage. Larger males often have been reported as the winners of aggressive interactions (Douglass 1976). Here, we also observed aggressive male-male interactions during the breeding season near the entrances to female burrows. Thus, it is likely that the greater proportion of fertilization attributable to larger males is at least partially a result of winning aggressive encounters and gaining greater access to females.

Adult size also may be a significant factor regulating multiple paternity. Female, but not male, size was significantly different between single- and multiple-sired clutches. Females with multiple-sired clutches were lighter and shorter than females with single-sired clutches (Table 1). At least two explanations exist for this difference. The first involves male guarding behavior. Male-male duels and male-female courtships in gopher tortoises can take a considerable amount of time (Douglass 1976; McRae et al. 1981). Even males that are generally successful in aggressive encounters, therefore, may be temporally limited in the number of females with which they can mate. If males are limited to only a few females, then males may prefer, and preferentially guard, larger females that can produce more eggs (Pearse et al. 2002). If larger males are more likely to select and successfully guard large females, then large males should on average sire clutches from large females. The correlation ( $R = .852$ ,  $P = .067$ ) of female versus male parent mass for single-sired clutches is consistent with this expectation (data available on request). Larger males preferentially guarding larger females also would result in smaller females either being unguarded or ineffectively guarded by small males. It seems possible then that this could result in smaller females being more frequently inseminated by multiple males. After having copulated with a female, a small male could be displaced by a larger male, which, depending on the female's ability to fend off the male, may result in a multiple-sired clutch. Although our sample size is somewhat small, our results are consistent with this in that the two females producing multiple-sired clutches were lighter and had shorter CL than all females with single-sired clutches (Table 1).

A second explanation for differences in female size for single- and multiple-sired clutches involves female receptiveness. In the field, females have been observed blocking their burrow entrances and rebuffing males performing courting behaviors (Douglass 1976; personal observation). Larger females may be better able to turn away attempts by courting males than are smaller females, especially when the courting males are large. If this were the case, then, again, we would expect the incidences of multiple paternity to be higher in

small females, as is observed. It is important to note that we cannot differentiate between explanations involving size versus age (i.e., experience) because age and size are strongly correlated. Smaller and lighter females are likely also to represent younger and less experienced females. Novice females may be engaging in different behaviors simply because they are inexperienced, and any difference may reflect experience and not size, per se. Younger females may also be behaviorally as well as physically less selective in choosing mates.

Regardless of the proximate cause of multiple-sired clutches, a clear result is that promiscuity, both in the form of polyandry and polygyny, is occurring in gopher tortoises. From a conservation standpoint, this is the most positive situation. Increases in the variance of reproductive success (i.e., polygyny) reduce the genetically effective population size ( $N_e$ ) relative to the census size (Wright 1938). Reductions in  $N_e$  can lead to inbreeding and higher probabilities of expressing deleterious recessive alleles resulting in reduced fitness. Even so, the addition of polyandry and a long generation time (i.e., overlapping generations) likely are ameliorating factors (Nunney 1993) partially, if not completely, compensating for any reductions in  $N_e$ . With these initial data, however, we cannot fully determine the evolutionary or conservation implications or how typical the ERA population is of other tortoise populations. However, it does appear that the reproductive output and probability of mating with more than one male are related to female size or experience (at least to the extent that they are correlated). Further studies using other populations and more individuals per population are necessary to fully characterize the consequences of the promiscuous mating system in gopher tortoises.

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