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Seasonal monogamy and multiple paternity in a wild population of a territorial reptile (tuatara)

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Investigating the mating system of a population provides insight into the evolution of reproductive patterns, and can inform conservation management of threatened or endangered species. Combining behavioural and genetic data is necessary to fully understand the mating system and factors affecting male reproductive success, yet behavioural data are often difficult to collect for threatened species. In the present study, we use behavioural data and paternity analyses to characterize the mating system of a high density population of a long-lived, ancient reptile (tuatara, *Sphenodon punctatus*). We further investigate the phenotypic traits (including body size, body condition, tail length, and ectoparasite load) that affect male reproductive success. Our behavioural data reflect a seasonally monogamous system with low levels of polyandry and polygyny that are consistent with male mate guarding. Male reproduction is highly skewed (only 25–30% of males are successful), and body size is the primary predictor of male reproductive success. Based on the genetic data, multiple paternity was found in only 8% of clutches, and the results of the paternity analyses showed monandrous clutches from socially polyandrous females. Our behavioural and genetic results revealed complexities in female mating patterns that support the potential for cryptic female choice or sperm competition. This warrants further experimental investigation into the mechanisms underlying reptile fertilization and the disparities between social and genetic polyandry in wild populations. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **98**, 161–170.

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INTRODUCTION

Investigating the mating system of a population or species provides insight into the evolution of reproductive patterns across taxa. Knowledge of the mating system can also inform conservation management because natural variation in mating success, the number and sex ratios of mating populations, and reproductive skew affect population genetic diversity (by influencing the effective population size; Anthony & Blumstein, 2000) and can be critical for successful captive breeding programs (Moore *et al.*, 2008b).

Combining paternity data with behavioural data is necessary to achieve a complete understanding of the mating systems and the factors affecting male reproductive success and offspring fitness in natural populations. Yet collecting mating data for many threatened or endangered reptiles is notoriously difficult because the animals are often cryptic and occur at low population densities (Morrison, Keogh & Scott, 2002). Therefore, many recent studies have relied solely on genetic analyses to explain mating patterns, and these have revealed high incidences of multiple paternity in reptiles (Uller & Olsson, 2008). However, inferring female mating patterns from paternity

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alone can be misleading. Paternity and multiple mating are not directly linked because single paternity clutches can result from multiply mated females if sperm competition or cryptic female choice is occurring (Eberhard & Cordero, 2003; Uller & Olsson, 2008).

Non-avian reptiles provide a good contrast to well-studied avian systems because they mostly lack parental care, which is one of the primary influences on the mating patterns of birds (Griffith, Owens & Thuman, 2002). Few reptiles are monogamous (i.e. the close association of one male and one female, often involving cooperation in breeding; Wickler & Seibt, 1983), and even fewer show long-term pair bonding (but see lizards in the social genera *Tiliqua* and *Egernia*; Bull, 2000; Bull, Cooper & Baghurst, 1998; Chapple, 2003). Furthermore, multiple paternity occurs at extremely high frequencies in some reptile populations (e.g. 95–100% for some turtles; Jensen *et al.*, 2006; Zbinden *et al.*, 2007), and is generally dependent upon: (1) mate encounter rates; (2) the degree of pair bonding; and (3) clutch size. Predictions from well-studied lizards are that larger species may be more likely to be monogamous because the spatial organization of large male lizards only allows for defence of one female (or one female's home range; Stamps, 1983).

In the present study, we combine behavioural and genetic data to examine the mating system and variation in male reproductive success for a wild population of tuatara (*Sphenodon punctatus*). Tuatara are the sole living representatives of the ancient reptilian order Rhynchocephalia, which diverged from their sister taxa (squamates) approximately 250 Myr ago (Rest *et al.*, 2003). Endemic to mainland New Zealand and the outlying islands, the range of tuatara is now restricted to approximately 30 small offshore islands mainly as a result of predation by introduced mammals. Tuatara are sexually dimorphic and highly territorial and, similar to many lizard systems, their mating system is characterized by intense male–male competition (Gillingham & Miller, 1991), no male parental care, and nesting that occurs outside of home ranges (Shine, 1988; Cree *et al.*, 1991). Tuatara differ from most lizards in that they are long-lived (80–100 years), and breeding intervals are prolonged for females (i.e. asynchronous reproduction occurs every 2–5 years, with 8–10 months from insemination to oviposition; Cree, Cockrem & Guillette Jr, 1992).

Because tuatara have medium to large body sizes relative to ecologically similar, territorial species, and territories of male tuatara only overlap an average of four females (not all of which are receptive every year; Cree *et al.* 1992; Moore, Daugherty & Nelson, 2009), we expect that wild tuatara may exhibit a high degree of seasonal monogamy with long-term polygyny (Stamps, 1983). Furthermore, male reproductive success is likely to be based on large body size (Cree *et al.*, 1992; Finch & Lambert, 1996) because large males monopolize local areas where females are most dense (Moore *et al.*, 2009), meaning that reproductive skew may be high (i.e. because sexually mature male body size is highly variable). We also examine the role of parasite loads (which can be affected by testosterone; Folstad & Karter, 1992), body condition and tail autotomy (which can have energetic and social costs; Martin & Salvador, 1993; Chapple & Swain, 2002) on male mating success. In a study of a captive population of tuatara, male reproductive success was highly skewed toward the largest male (of four), and the frequency of multiple paternity was low (18%, or three of 16 clutches; Moore *et al.*, 2008b). However, multiple paternity has yet to be found in wild tuatara (Hay & Lambert, 2008). By examining the social and genetic mating system of this archaic reptile, we aim to shed new light on patterns of mating system evolution in vertebrates, particularly reptiles, and to inform future conservation efforts for tuatara.

MATERIAL AND METHODS

STUDY SITE AND ANIMALS

The study took place on Stephens Island (Marlborough Sounds, 40°40'S, 174°00'E), where the largest population of tuatara occurs (i.e. an estimated 30–50 000 individuals, over 90% of extant tuatara; Gaze, 2001). Stephens Island is a 150-ha island composed of two distinct habitat types with markedly different densities of tuatara: remnant native coastal forest (approximately 2700 tuatara ha^{-1}) and grassland pasture (approximately 200 tuatara ha^{-1}; Moore *et al.*, 2009) that was cleared in the early 1900s for grazing livestock.

On Stephens Island, tuatara occupy home ranges year-round, except when females migrate to nesting rookeries. Mating, which peaks in March (austral autumn), occurs in home ranges, and is a long process (taking anywhere from 60–90 min; J.A. Moore, unpubl. data) that involves a conspicuous courtship followed by copulation (via cloacal apposition, as tuatara lack an intromittent organ), with males remaining mounted on top of females, shuddering periodically, for up to 1 h until the female moves away (Gillingham, Carmichael & Miller, 1995; Fig. 1). The spatial structure of Stephens Island tuatara provides no evidence for sneaker males (i.e. small males that do not defend territories, and rove throughout multiple home ranges) (Moore *et al.*, 2009).

In November 2004, three circular study plots (314–615 m^2) were located from an accessible track

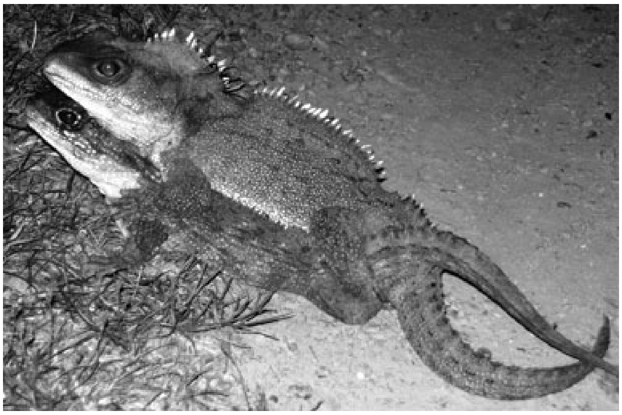

Figure 1. Tuatara (*Sphenodon punctatus*) mating on Stephens Island, New Zealand. Mating is a lengthy process involving courtship and copulation, with the male remaining mounted on top of the female, as shown here, for up to 1 h. Photo courtesy of Jeanine Refsnider.

running through a section of remnant forest on Stephens Island (Moore *et al.*, 2007, 2009). All tuatara in the study plots were captured by hand for marking and measuring, and capture location, snout–vent length (SVL), tail length (VT), regenerated tail length (R), mass, and sex were recorded. Blood samples (0.1–1.0 mL) were taken from the caudal vein/artery of all captured tuatara and stored at −80 °C. Tuatara have two types of ectoparasites (mites, *Neotrombicula* spp., Acari: Trombiculidae: Goff, Loomis & Ainsworth, 1987; ticks, *Amblyomma sphenodonti*: Barker & Murrell, 2004). Mites have an obvious peak in abundance during the tuatara mating season (Godfrey, Bull & Nelson, 2008). We counted mites and ticks for each individual because parasites may affect health or condition (S. Godfrey, pers. comm.) or reflect immunocompetence from high testosterone levels (Folstad & Karter, 1992; Salvador *et al.*, 1996). When more than 100 mites were present, we counted the number of mites within a defined area, and estimated mite loads based on the total infected area of the animal. Ectoparasite counts were conducted after each mating season (Godfrey *et al.*, 2008). A passive integrated transponder (PIT) tag (AVID Identification Systems, Inc.) was inserted subcutaneously anterior to the left rear leg for future identification of individuals. A subset ($N = 100$) of these animals, as well as mated pairs from 2005 and 2006, were marked using a unique coloured bead tag inserted through the nuchal crests (Fisher & Muth, 1989) to allow for identification of individuals from a distance. Individuals without bead tags were identified by PIT tags and, from a distance, by individual idiosyncrasies in tail, head, or crest morphology.

FIELD DATA COLLECTION

Mating activity was monitored during the peak of three consecutive mating seasons (5–30 March 2005, 28 February to 28 March 2006, and 27 February to 27 March 2007). Monitoring was limited to tracks because of the fragile nature of the study site (i.e. it is heavily burrowed by seabirds and tuatara). Accessible tracks in the forest were monitored daily by one or two observers from 13.00 h until dark (approximately 21.00 h), by slowly walking along tracks and visually surveying for courtship or mating activity. All courtships were observed until there was an outcome (i.e. mating occurred or the pair was interrupted by the female or a rival male). Most unsuccessful courtships ended quickly. However, successful matings took up to 1 h; thus, there is a small chance that we might have missed some mating activity if multiple pairs were mating simultaneously in different areas. Unmarked pairs of tuatara that were opportunistically observed mating were captured by hand, PIT-tagged, measured, weighed, and blood samples and ectoparasite counts were taken after mating concluded. Pairs were returned immediately after processing to their capture locations. Handling and processing did not appear to affect behavioural interactions because some pairs were observed remating less than 30 min after release. In 2007, monitoring was expanded to include low density pasture sites. A 500-m long dirt road running through the pastures was surveyed each night after dark until approximately 00.00 h. Activity of pasture and forest tuatara differs. Pasture tuatara rarely emerge from their burrows during daylight hours because of a lack of cover from avian predators, whereas forest tuatara are active during the day and night, but activity in the forest shifts to foraging after dark (J. Moore, unpubl. data; Gillingham *et al.*, 1995). Thus, forest and pasture surveys were conducted during peak mating activity for each habitat type.

STATISTICAL ANALYSIS

To examine the effects of individual phenotypic differences on male mating success, we performed a binary logistic regression analysis in R (R Core Development Team, 2006) with mating success (successful = 1, unsuccessful = 0) as the dependent variable. We chose to implement a binary logistic regression analysis because so few males had multiple partners. Males were categorized as successful if they were ever observed mating. Males were categorized as unsuccessful if they were monitored for ≥ 2 mating seasons, in our focal study plots, and were never observed mating. We excluded some marked males on the periphery of the study plots because behavioural observations were limited. Predictor variables included SVL, body condition (defined as the residuals from a regression of log-transformed mass/log-transformed SVL), tail length ratio (defined as VT/VT ± SVL), mite load, and tick load. We defined mite load as the residuals from a linear regression of mite count/(SVL ± VT) to control for body size. A comparable statistic was calculated for tick load. After correction for body size, mite and tick loads were not correlated with SVL or mass. We did not include mass in the logistic regression analysis because it is highly correlated with SVL ($r = 0.94$, $P < 0.001$). Models were tested in a backward stepwise manner, starting with the full model, with variables removed in an iterative process based on their relative Akaike's information criterion (AIC) values. AIC values were rescaled to ΔAIC for interpretation. The best model was selected based on a ΔAIC of zero, and terms were analysed for significance within the models using analysis of variance (ANOVA).

To examine whether successful males differed with respect to habitat type (or population density), we compared mean log-transformed SVL and mass, body condition, tail length ratio, and mite and tick loads of successful males in the pastures to successful males in the forest using an ANOVA. Although the mean size of forest males is slightly smaller than pasture males (N. Nelson, unpubl. data), this is driven primarily by the larger range of sizes at the lower end of forest males (because of their higher density). Body sizes of mated individuals did not differ between years (male: $F_{2,51} = 1.4$; female: $F_{2,50} = 2.32$, $P > 0.1$), so data were pooled across years, within habitat types. We used only the first record from individuals that were measured multiple times. Data are reported as the mean ± SE and all data were checked for normality and homogeneity of variances, or were appropriately transformed. $P < 0.05$ was considered statistically significant.

PATERNITY ANALYSIS

In November 2005 (nesting season), ten gravid females that had been observed mating in the previous mating season were fitted with backpack radio transmitters and tracked to their nests. Females were allowed to lay eggs naturally and nests were excavated to collect eggs. Many nest sites proved inaccessible and complex; thus, we were only able to collect partial clutches from three females in 2005. One of these clutches failed, probably because they had been disturbed by another female prior to collection. In October 2006 and 2007, we located ten more gravid females that had been observed mating during the previous mating seasons, and induced oviposition by injecting oxytocin (concentration $10\ \mathrm{IU\ mL^{-1}}$, $10\ \mathrm{IU\ kg^{-1}}$ body mass) intraperitoneally. We held females overnight in cardboard boxes and collected eggs as they were laid throughout the night. Females were then returned to their capture locations. All eggs were incubated at Victoria University of Wellington, and toe-clips were collected from all hatchlings the following year and stored at −80 °C until processing.

Genomic DNA was extracted from toe-clips of hatchlings (one per individual) and blood from mothers and candidate fathers (5–10 μL) using a proteinase K phenol–chloroform protocol (Sambrook, Fritsch & Maniatis, 1989). DNA was quantified using a Nanodrop ND-1000 spectrophotometer. Seven highly polymorphic microsatellite loci were amplified using polymerase chain reaction (PCR) (*C2F*, *C11P*, *E11N*, *H5H*, *A12N*, *C12F*, *H4H*; Aitken *et al.*, 2001; Hay & Lambert, 2008) in 15-μL reactions. Reactions and PCR conditions were conducted *sensu* Moore *et al.* (2008b) and Hay & Lambert (2008). Amplified products were combined for genotyping and were run on an ABI 3730 Genetic Analyzer (Applied Biosystems, Inc.). Alleles were visualized using Genemapper software (Applied Biosystems, Inc.) and sizes were manually scored by the same observer (J.A.M.).

We checked offspring genotypes manually to confirm maternity. Paternity was assigned for all offspring based on seven locus genotypes using the computer program CERVUS, version 3.0 (Marshall *et al.*, 1998; Kalinowski, Taper & Marshall, 2007), which calculates LOD scores (i.e. the log of the overall likelihood that the candidate parent is the true parent). Positive LOD scores indicate that the putative father is more likely to be the true father than not be the true father, and vice versa for negative LOD scores. Candidate fathers were those that were observed mating with each female and, for females in our focal study plots, we also included males that lived within close spatial proximity to each female. Clutches were determined to have multiple paternity if more than one offspring per clutch was assigned to a different father with > 95% confidence, and that this assignment was based on two or more loci (i.e. to account for potential offspring-parent mismatches due to microsatellite mutation).

RESULTS

From three consecutive mating seasons, we recorded a total of 97 mating events from 75 pairs (62 males, 69 females) in the forest ($N = 54$ pairs) and pasture ($N = 21$ pairs) on Stephens Island. Using behavioural data from the forest only, where animals were intensively monitored, the average frequency of seasonal polyandry and polygyny was low (7% of mated females were polyandrous and 9% of mated males were polygynous; Table 1). No individuals of either sex had more than two mates per season. On average,

Table 1. Behavioural mating patterns of tuatara (*Sphenodon punctatus*) in remnant forest (Keepers Bush), on Stephens Island during three mating seasons, including the percentage of females with multiple partners per season (percent polyandry) and the percent of males with multiple partners per season (percent polygyny), frequency of remating with the same partner, and the percent of males that are successful (male reproductive skew)

Season	Percent polyandry*	Percent polygyny*	Remating†		Male reproductive skew‡
2005	11.1 (18)	4.8 (21)	14.0 (22)		10.9 (6)
2006	0.0 (10)	14.3 (7)	40.0 (10)		11.9 (5)
2007	9.1 (22)	9.1 (22)	22.7 (22)		9.1 (5)
Average	6.7 ± 3.4	9.4 ± 2.8	25.6 ± 7.6	Overall§	29.1 (14)

*Total number of mated females (polyandry) and males (polygyny) are shown in parentheses.

†Percentage of pairs that mated > 1 time per season, total number of mated pairs are shown in parentheses.

‡Percentage of males that were successful, total number of successful males are shown in parentheses.

§Number of males that mated in any season over the total number of focal males (not the sum of skew per season, as some males mated in multiple seasons).

Table 2. Characteristics of successful male tuatara (*Sphenodon punctatus*) in the low density pasture habitat and the high density forest habitat on Stephens Island

	Pasture ($N = 18$)		Forest ($N = 44$)	
	Mean ± SE	Range	Mean ± SE	Range
Mass (g)*	552.8 ± 49.2	300–707	635.4 ± 16.6	470–830
Snout–vent length (mm)	251.5 ± 7.3	213–273	259.5 ± 2.9	229–292
Body condition	–0.5 ± 0.2	–1.4–0.4	0.05 ± 0.2	–2.1–1.5
Tick load	0.06 ± 0.01	0.002–0.1	0.04 ± 0.01	0.004–0.3
Mite load	0.6 ± 0.3	0–2.6	1.3 ± 0.2	0–4.5
Tail length ratio*	0.4 ± 0.03	0.3–0.5	0.5 ± 0.01	0.2–0.5

*Significant differences between forest and pasture males ($P < 0.05$).

we observed 24% of pairs remating at least once and up to five times throughout the season. Only one female was observed mating in multiple seasons (in 2005 and again in 2007) and she was polyandrous. We marked a total of 201 individuals (107 males, 87 females, and seven juveniles) in our focal study plots. Only 29% of males within the focal study plots were ever successful, leaving the majority of males unsuccessful in all three seasons (Table 1). Of the successful males, only 14% of males were observed mating in multiple seasons, and all of these were polygynous (i.e. had different mates each season). No male was observed mating in all three seasons.

The best logistic regression model of successful ($N = 62$) versus unsuccessful ($N = 52$) males included SVL and tail length ratio (ΔAIC = 0.0). The next best model, which was competitive with the best model, included SVL, tail length ratio, and body condition (ΔAIC = 0.93). The only predictor that was significant in the best models was SVL ($\beta = 0.10$, $z = 4.4$, $P < 0.0001$). Mean SVL for successful males was 258.0 ± 2.7 mm (range = 213–292 mm) and 221.0 ± 3.5 mm (range = 165–263 mm) for unsuccessful males. Successful males had significantly shorter tails relative to their body length than unsuccessful males (mean tail length ratio, successful males = 0.45 ± 0.008, unsuccessful males = 0.48 ± 0.006; $F_{1,112} = 4.7$, $P = 0.03$). Only 14% of males (ten unsuccessful and six successful) had complete tails (i.e. had never autotomized a portion of their tail).

There were no significant differences in SVL ($F_{1,60} = 3.6$, $P = 0.06$), body condition ($F_{1,60} = 0.6$, $P = 0.4$), mite load ($F_{1,41} = 1.9$, $P = 0.2$), or tick load ($F_{1,41} = 0.9$, $P = 0.3$) between successful males in the forest and the pasture. On average, successful males in the forest were significantly heavier (mean mass forest males = 628.3 ± 14.2, pasture males = 559.6 ± 25.2, $F_{1,60} = 6.8$, $P = 0.01$) and had longer tails (mean tail length ratio forest males = 0.47 ± 0.009, pasture males = 0.42 ± 0.02, $F_{1,60} = 5.9$, $P < 0.02$) than successful males in the pasture (Table 2).

Paternity was assigned to a total of 76 offspring (from 12 clutches; genotypes presented in the Supporting information, Table S1). Based on the

Table 3. Summary of data from clutches sampled from wild female tuatara (*Sphenodon punctatus*) on Stephens Island. Every clutch is from a different female

Clutch	Year	Hatchlings (*N*)	Sired by observed (candidate) father (*N*)	Sired by unobserved father (*N*)	Multiple paternity
GG	2005	5	5	0	No
BW	2005	2	2	0	No
PGW	2006	10	10	0	No
YPY	2006	4	0	4	No
PWB	2006	8	8	0	No
WY	2006	10	10	0	No
C	2007	4	4	0	No
B	2007	10	10	0	No
E	2007	6	0	6	No
F	2007	4	2	2	Yes
A	2007	7	7	0	No
D	2007	6	6	0	No

behavioural data, only one of these females was polyandrous; mothers of the other 11 clutches were only observed mating with one male. Paternity was assigned to candidate fathers with $> 95\%$ confidence for 64 offspring (from nine clutches). The LOD scores (i.e. the sum of the log-likelihood ratios of each locus) from these 64 offspring were all positive and in the range 0.9–7.4. The father's genotype matched predictions based on behavioural observations for 84.2% of the offspring ($N = 64$, 95% confidence interval = 76.0–92.4%; Table 3). Two clutches ($N = 10$ offspring) were sired by unsampled males that were never observed mating with the females (i.e. offspring had negative LOD scores and mis-matched candidate father alleles at 2–4 loci per individual), and the mate based on behaviour was not represented in the clutch. Multiple paternity was found in one clutch ($N = 4$ offspring), with paternity split equally between the male that we observed mating with the female, and an unknown (unsampled) male. No clutches had more than two sires. All offspring in the clutch from the polyandrous female (based on behaviour) were sired by one of the males with which she was observed mating. Based on the genetic data, 8% of females were polyandrous (Table 3).

DISCUSSION

The results obtained in the present study support three main findings. First, the predominant mating system for territorial tuatara on Stephens Island is seasonal monogamy with polygyny across seasons. Second, all mating activity is dominated by a small proportion of males (25–30%, that may change over time), and male mating success depends largely upon body size. Lastly, multiple paternity occurs at a very low frequency (8.3% of clutches), and comparing the social and genetic mating systems has revealed complexities in mating patterns of individuals.

VARIANCE IN MALE REPRODUCTIVE SUCCESS

It is not surprising that large body size is the strongest phenotypic determinant of mating success for male tuatara because this is the case in many reptilian taxa that are characterized by male-male competition (Cooper & Vitt, 1993; Shine *et al.*, 2000; Lebas, 2001; Stapley & Keogh, 2005; Salvador *et al.*, 2008). A more intriguing finding is that successful males had shorter tails, relative to their body size, than unsuccessful males, which is contrary to previous findings of tail autotomy having negative consequences for reptile energy stores (Dial & Fitzpatrick, 1981; Doughty & Shine, 1998), survival (Fox & McCoy, 2000), locomotor performance (Chapple & Swain, 2002), social status (Martin & Salvador, 1993), and reproductive success (Martin & Salvador, 1993; Hofmann & Henle, 2006; Salvador *et al.*, 2008). If the costs of tail autotomy are outweighed by the benefits of large body size, losing a tail probably has a negligible effect on social status and reproductive success. Tail loss may also be a byproduct of age (i.e. the longer a male competes in this system, the greater the likelihood of losing a significant proportion of his tail). Successful males may also be more aggressive and more prone to fighting (e.g. for fallow deer, *Dama dama*; McElligott & Hayden, 2000), which may increase the frequency of tail loss in reptiles.

SOCIAL MATING SYSTEM

Very few reptiles show within-season monogamy, and even fewer have long-term pair fidelity (Bull, 2000).

Monogamy in vertebrates is usually assumed to be maintained through parental care (Emlen & Oring, 1977), mate guarding (Stamps, 1983), or because unattended females are somehow disadvantaged. In reptiles, long-term monogamy appears to have evolved only in highly social lizards (of the Australian *Egernia* and *Tiliqua* genera) that exhibit indirect parental care and benefit from the increased vigilance of aggregative family living (Bull & Pamula, 1998; Bull, 2000; Chapple, 2003; Chapple & Keogh, 2006). Tuatara parental care is negligible, aside from the occasional short period of nest guarding by females (Refsnider *et al.*, 2009), and unattended females do not appear to be disadvantaged in any way (J.A. Moore, pers. observ.). Seasonal monogamy in tuatara may be facultative and the result of strong mate guarding (Moore *et al.*, 2009). Male tuatara can defend multiple females (Moore *et al.*, 2009) but, because the female reproductive interval is so long (approximately 4 years; Cree *et al.*, 1992), only one of these females is likely to be receptive in any given season. This means that successful males vary in the number of partners that they have per year, depending on the receptivity of the females to which they have regular access. For female tuatara, male territoriality and mate guarding limits access to other potential mates (similar to territorial lizards like *Anolis sagrei*; Tokarz, 1998), resulting in mostly seasonally monogamous pairings with long-term polygyny dominated by large males. However, females do not always return to the same home burrows (i.e. territories) after nesting, which should increase their access to new mates and result in long-term polyandry.

Genetic mating system

The present study is the first to document multiple paternity in a wild population of tuatara, although the phenomenon is widespread throughout other reptiles (Uller & Olsson, 2008). Hay & Lambert (2008) genotyped eight tuatara clutches from two populations (Stephens and North Brother Island) and found only single paternity clutches. Their results, when combined with the results obtained in the present study, suggest that the frequency of multiple paternity is very low in wild tuatara populations. A higher frequency of multiple paternity (18.8% of clutches) was found in a small captive population of tuatara (Moore *et al.*, 2008b) but comparisons are difficult because the population dynamics of wild and captive populations are quite different, and the number of individuals in captive populations is markedly smaller than most wild populations. The lowest rates of multiple paternity in wild reptile populations are found in: (1) the social *Egernia* and *Tiliqua* skinks (with multiple paternity of clutches in the range 2.6–25%; Bull *et al.*, 1998; Gardner, Bull & Cooper, 2002; Stow & Sunnucks, 2004; Chapple & Keogh, 2005); (2) species with low mate encounter rates (e.g. 0–10% of clutches in some populations of Leatherback turtles, *Dermochelys coriacea*; Rieder *et al.*, 1998; Dutton, Bixby & Davis, 2000; Crim *et al.*, 2002); and (3) species with small clutch sizes (Uller & Olsson, 2008). Tuatara do not have a strong social structure (Moore *et al.*, 2008a), and some populations occur in high densities. Thus, the low rates of multiple paternity in tuatara are most likely the result of small clutch sizes (averaging nine eggs, Cree *et al.*, 1992) combined with low mate encounter rates due to territoriality and mate guarding rather than low population density (e.g. for territorial *Ctenophorus* lizards; Lebas, 2001). The results obtained in the present study only hint at the potential for density effects in the mating system of tuatara because smaller males appear to have a better chance of mating success at lower densities (in the pasture habitat), and the one multiple paternity clutch was from a female living in this habitat. Future research should aim to better understand the links between population density, the mating system, and rates of multiple paternity in wild reptile populations (Kokko & Rankin, 2006).

The present study underlines the importance of combining behavioural data with genetic data to obtain a complete picture of the mating system of a population or species. The integration of molecular studies has overturned long standing paradigms in avian behavioural ecology (e.g. that birds are monogamous; Petrie & Kempenaers, 1998; Griffith *et al.*, 2002) and a better understanding of the mating systems of non-avian reptiles may support equally contentious insights (e.g. that females play an active role in mating or that they do benefit from polyandry; Madsen, 2008; Uller & Olsson, 2008). When taken alone, our behavioural and genetic estimates provided similar levels of polyandry at the population level (7–8% of females). Results were less consistent at the individual level. For example, some socially polyandrous females would have gone undetected if we had only examined paternity, which revealed monandrous clutches. Similarly, had we relied on behaviour alone, we would have missed some instances of multiple paternity or polyandry that were revealed by the paternity results. When combined, the social and genetic data reveal a much higher level of multiple mating by female tuatara (approximately 25% of females).

The occurrence of single paternity clutches from females with two social mates suggests that either sperm competition or cryptic female choice is present in tuatara. Unfortunately, we only had one monandrous clutch from a polyandrous female where we sampled both mates. The primary male, who sired all of her offspring, was monogamous and remated with the female multiple times throughout the season. The secondary male was polygynous that season, and only mated with the sampled female once, and we were unable to sample the offspring from the second female. Although speculative, these results suggest that two mating strategies may have evolved for male tuatara: (1) to focus on one female and remate with her throughout the season to ensure fertilization or (2) to mate once or twice with multiple females in a territory, while risking losing paternity to other males. Spatial and behavioural data do not support a sneaky mating strategy, as males do not rove freely throughout territories and mating is always preceded by the stereotypical, conspicuous courtship display (Gillingham *et al.*, 1995; Moore *et al.*, 2009). Further experimental data are needed to fully elucidate post-copulatory phenomena in female tuatara, and in reptiles generally.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Genotypes of individual tuatara seperated by clutch, including maternal (F-) and putative paternal (M-) genotypes (in bold). Allele mismatches between offspring and putative sires are underlined. Note the putative null allele in locus *C12F* for individual WY05.