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RESEARCH ARTICLE

Implications of social dominance and multiple paternity for the genetic diversity of a captive-bred reptile population (tuatara)

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Abstract Captive breeding is an integral part of many species recovery plans. Knowledge of the genetic mating system is essential for effective management of captive stocks and release groups, and can help to predict patterns of genetic diversity in reintroduced populations. Here we investigate the poorly understood mating system of a threatened, ancient reptile (tuatara) on Little Barrier Island, New Zealand and discuss its impact on the genetic diversity. This biologically significant population was thought to be extinct, due to introduced predators, until 8 adults (4 males, 4 females) were rediscovered in 1991/92. We genotyped these adults and their 121 captively-bred offspring, hatched between 1994 to 2005, at five microsatellite loci. Multiple paternity was found in 18.8% of clutches. Male variance in reproductive success was high with one male dominating mating (77.5% of offspring sired) and one male completely restricted from mating. Little Barrier Island tuatara, although clearly having undergone a demographic bottleneck, are retaining relatively high levels of remnant genetic diversity which may be complemented by the presence of multiple paternity. High variance in reproductive success has decreased the effective size of this population to approximately 4 individuals. Manipulation to equalize founder representation was not successful, and the mating system has thus had a large impact on the genetic diversity of this recovering population. Although population growth has been successful, in the absence of migrants this population is likely at risk of future inbreeding and genetic bottleneck.

Keywords *Sphenodon* · Mating system · Effective population size · Polygyny · New Zealand

Introduction

Captive breeding is an essential tool for conservation of many threatened and endangered species, but establishing a successful program is often challenging (Snyder et al. 1996). Apart from simply increasing numbers of individuals, a primary goal in captive breeding for species recovery is to maintain the genetic diversity of a population (Ralls and Ballou 1986) to ultimately create a self-sustaining wild population (Ebenhard 1995). Captive breeding programs that are aimed at restoring severely declining populations are often limited to only a small number of founders (Ralls and Ballou 1986), thus furthering the loss of genetic diversity and imposing imminent and/or long-term consequences for fitness and adaptability of the new population (Allendorf and Luikart 2007). Isolated, insular populations may be particularly at risk of losing genetic diversity. Ideally, genetic change in a captive population should be minimized by equalizing founder representation. However, in instances where founders 1) do not breed readily in captivity, or 2) do not accept manipulation (e.g., artificial insemination, isolating mating pairs), retaining remnant diversity in the captive population is challenging (Snyder et al. 1996).

The mating system plays an important role in determining levels of genetic diversity in captive and wild populations (Anthony and Blumstein 2000). For instance, disassortative mate choice can result in balancing selection

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on functional genes (Penn and Potts 1999, Roberts and Gosling 2003), and multiple paternity increases the effective population size (N_e; Sugg and Chesser 1994). Alternatively, social dominance may limit all reproduction to a few successful males, thereby heavily biasing paternity and greatly decreasing genetic diversity and N_e (Hoelzel et al. 1999).

Among reptiles, mating systems are poorly understood. Most reptiles are believed to be polygynous (e.g., Zamudio and Sinervo 2000), but cases of monogamy in lizards do exist (in the genus *Egernia*, Bull 2000, Chapple and Keogh 2005). Because parental care is limited or non-existent, reptile mating systems are usually characterized by social dominance and/or spatial structure. Recent discoveries of genetic polyandry in many reptiles is broadening the understanding of the female's role in the mating system (e.g., Lee and Hays 2004). Furthermore, because females of many long-lived reptiles are capable of storing sperm, multiple paternity has been found at high frequencies in some species (Pearse and Avise 2001). In order to begin to understand the mating system of an ancient reptile species and its impact on the genetic diversity of a small population, we investigate patterns of paternity in a captively bred, insular population (on Little Barrier Island, New Zealand) where manipulation to equalize founder representation was not successful.

Little Barrier Island (LBI; known also by its Māori name, Hauturu) is a 3083 ha volcanic Nature Reserve located in the Hauraki Gulf off the east coast of North Island, New Zealand (36° 12′S, 175° 07′E). Despite a history of habitat modification, human presence, and introduced predators, the island is currently largely forested (Girardet et al. 2001), providing suitable habitat for a number of native species which have been extirpated from the mainland. One such species is the tuatara, a medium-sized, long-lived reptile that is endemic to New Zealand. Tuatara are the sole extant representatives of the ancient reptilian order Sphenodontia (Benton 2000). Although once widespread throughout the mainland, natural populations are now restricted to small offshore islands, primarily due to predation from introduced mammalian predators.

The tuatara on LBI were initially described as morphologically unique (Reischek 1886), and this island population was subsequently elevated to subspecific status in 1943 making it the rarest of the tuatara populations. Allozyme and mitochondrial DNA analyses have since revealed that LBI tuatara are not genetically distinct from other northern island populations of tuatara (Daugherty et al. 1990, Hay et al. 2003, Whitaker and Daugherty 1991). However, as the largest island (by at least an order of magnitude) supporting one of the 32 natural island populations of this threatened reptile, the importance of conserving the LBI population of tuatara is not diminished. Little Barrier is also the only island where tuatara occur in a habitat that may be similar to what they once inhabited on mainland New Zealand.

By the late 1900's, tuatara were thought to be extinct on LBI (due primarily to predation by introduced cats and rats or kiore, *Rattus exulans*) until systematic surveys rediscovered eight adults (4 males, 4 females) in 1991–1992 (Whitaker 1993, Whitaker and Daugherty 1991). These adults were brought into captivity on the island and housed under semi-natural conditions behind a predator proof fence, with the goal of establishing a captive breeding colony to repopulate LBI. Tuatara and rats rarely coexist because the rats limit recruitment (Cree et al. 1995), so the recovered adults likely represented relicts of a population that was headed for extinction (Whitaker and Daugherty 1991). Although extinction threats are often difficult to determine and quantify (Sarrazin and Barbault 1996), in this case there was strong evidence to suggest that rats posed the primary threat to the persistence of tuatara on LBI. As such, the New Zealand Department of Conservation undertook a massive eradication program in 2004 and in 2006 LBI was declared rat free.

Since their rediscovery in 1991/92, the eight LBI tuatara have bred in captivity and have successfully produced over 120 offspring. Eggs were laid in naturally constructed nests in the tuatara enclosure, recovered by the island caretakers, and artificially incubated at Victoria University of Wellington (VUW), after which the hatchlings were returned to LBI and held in captivity until rats were eradicated and they reached an appropriate size (120 mm snout-vent length, SVL) for release onto the island. Because tuatara exhibit temperature dependent sex determination (TSD; Nelson et al. 2004), clutches were split and incubated at two different temperatures to give the offspring group a slightly female biased sex ratio. In an effort to equalize founder representation, the eight adults were initially housed separately as male-female pairs, but after two years of no reproductive activity, they were released into the enclosure as a group. Within this group enclosure, the suspected mothers of each clutch were identified (although not confirmed genetically). However, the fathers (of which there are four candidate males) of each clutch were not identified because mating, which occurs ~8 months earlier, was not observed. Because captive breeding on LBI could not be manipulated, this new population could hypothetically have been fathered by one individual.

Currently, little is known about the mating system of tuatara. Although presumed to be polygynous due to their highly territorial social system (Gillingham et al. 1995), genetic paternity and the female's contribution to the mating system are unknown. To better understand the genetic mating system of tuatara and its potential impacts on genetic diversity of reintroduced populations, we assign paternity to captively bred offspring and their potential parents, and discuss the implications for the future of this highly valued population of a biologically significant reptile.

Methods

Sample collection and genotyping

Tissue samples were collected from all LBI tuatara from the period 1991–2005. These included ~0.5–1.0 ml whole blood taken from the caudal vein/artery of adults, and toe-clips taken from hatchlings that had been incubated at VUW. All samples were stored at −80°C. DNA was extracted from 10 µl of blood, and/or toe-clips (one per individual) using a proteinase K phenol-chloroform protocol (Sambrook et al. 1989). DNA was quantified using a Nanodrop ND-1000 spectrophotometer, and all samples contained sufficient DNA for PCR amplification.

Seven microsatellite loci were amplified using PCR (Table 1; Aitken et al. 2001; Hay and Lambert 2007) in 15 µl reactions. Reactions included approximately 10–50 ng of template DNA, and followed the general thermal cycle of 94°C for 1 min, (94°C for 30 s, 58–63°C for 20 s, 72°C for 30 s) for 35–40 cycles, 72°C for 30 min (see Table 1 for locus-specific details). These loci were previously recognized as the most variable for tuatara on LBI and Stephens Island (Hay and Lambert 2007). Amplified products were multiplexed 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.

Data analysis

Offspring maternity was assumed based on nesting behavior and gravidity of females at the time of egg collection. We checked offspring genotypes manually to confirm maternity. Paternity was assigned for all offspring based on 5 locus genotypes, with all four males considered as candidate parents using the computer program Cervus 2.0 (Marshall et al. 1998). Prior to paternity analysis, we checked loci for null alleles (in Cervus 2.0), and found that one locus (*C2F*) had a high frequency of null alleles (null allele frequency = 0.73). Null alleles (i.e., true alleles that fail to amplify) can cause false exclusion of potential parents by incorrectly typing true heterozygotes as homozygotes (Dakin and Avise 2004). As this locus was not highly variable and had a high probability of null alleles, it was removed from the analysis. Another locus (*E11N*) proved difficult to score (due to non-specific amplification), and was likewise removed from further analysis. 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.

To determine relative genetic diversity in the LBI adults, we compared their number of alleles and observed heterozygosity to the average of three random samples of eight individuals each, from the largest extant population of tuatara (Stephens Island, 30–50,000 individuals). We collected blood samples from 300 tuatara on Stephens Island (for a concurrent genetic study), and genotyped them at the same five loci as the LBI samples. From the Stephens Island samples, three groups of eight samples were randomly selected for comparison to LBI adults. We then determined the mean coefficient of relatedness (r; Queller and Goodnight 1989) of the LBI adults and the three random samples of Stephens individuals, and compared them for significant differences using the computer program GenAlEx (Peakall and Smouse 2006). We compared the parental and offspring generations for significant differences in allele frequencies using the computer program Genepop (Raymond and Rousset 1995), and calculated observed and expected heterozygosities in GenAlEx (Peakall and Smouse 2006).

We also used the program ENDOG v4.0 (Gutierrez and Goyache 2005) to calculate the average relatedness coefficient (AR) of each of the founders based on pedigree data. Average relatedness values (Gutierrez et al. 2003) can be used to indicate the relative genetic contribution of the founders to the population and have been used directly in genetic management of small populations (e.g., Goyache et al. 2003).

We calculated the effective population size (N_e), as a surrogate for the effective number of breeders (N_{eb}; e.g., Fiumera et al. 2002) based on the variance in male and female reproductive success. First, the effective number of males (N_{em}; Kimura and Crow 1963) was calculated using the formula

$$N_{em} = \frac{N_m \bar{k}_m - 1}{k_m - 1 + \frac{V_{km}}{k_m}}$$

where N_m is the census number of breeding males, $\bar{k}_m$is the mean number of offspring produced per male, and V_{km} is the variance in male reproductive success. A similar formula was used to calculate the effective number of females (N_{ef}). N_e was then determined based on the formula

$$N_e = \frac{4N_{em}N_{ef}}{N_{em} + N_{ef}}$$

Heterozygote excess can also be used to calculate the effective number of breeders, based on the theory that when the number of breeders is small, allele frequencies in

Table 1 Tuatara (*Sphenodon*) microsatellite primer sequences (F = forward, R = fluorescently labeled reverse), optimal annealing temperatures (T_A), number of cycles, and optimal $MgCl_2$ concentrations used for PCR reactions in this study

Locus	Primer sequence (5′–3′)	T_A (°C)	No. cycles	$MgCl_2$ (mM)
C1H[a]	F: GTTTCTTTGTCTCATTGCTTTCCCAG R: CCTCTTCTCCGCCTTACACT	60	35	2.5
C2F[a]	F: TCACTGTCAGCAGGCTCTTC R: GAATGCGGGGAATGTGAGG	60	35	2.5
A12N[a]	F: GTTTGTTGGAGAAGGGAGGAGAATAATC R: ATCACTGCTCATTTCAGCC	60	38	2.5
B8P[b]	F: GTTTCTTAGATGGATGATTGGGGGAGT R: AGAATGGGCCAACAAGACAG	58	38	2.5
C11P[b]	F: GTTTCTTAAGTGAAATGGGAAGCTGGA R: GCAATAAGTTCCACCCGTCA	60	40	2.0
E11N[b]	F: GTTTCTTTTTGTGTGAAGAACGCATCC R: CACTCCCCCATTACTGGACA	63	40	2.5
H5H[b]	F: GTTTCTTACTAAACCCCCACTTTGGAG R: GTGTCACCTGCTTCCCAGTT	60	40	2.5

[a] Sequences from Aitken et al. 2001

[b] Sequences from Hay and Lambert 2007

males and females will be different due to binomial sampling error resulting in heterozygote excess in the offspring (Pudovkin et al. 1996). Likewise, the temporal change in allele frequencies between generations can also be used to calculate N_e (Waples 1989). These methods do have limitations (Luikart and Cornuet 1999), and because (1) heterozygote excess was not consistent across all loci in the offspring (we found it in four of five loci), and (2) we can assign maternity and paternity with great confidence in this small population, we relied on variance in reproductive success for directly determining N_e as it is the most accurate estimator in this case.

Results

Five locus genotypes were successfully assigned to all adults ($n = 8$) and 16 clutches (121 offspring) hatched from 1994 to 2005. All loci were polymorphic, and the number of alleles per locus averaged 7.2 for the parents ($n = 8$) and 7.0 for the offspring ($n = 121$). All four founding mothers successfully reproduced, and clutch sizes varied per year and by female (mean size = 9.3 ± 3.8, range = 2–12 eggs). Hatching success averaged 76.4% and was not significantly different between mothers ($F_{(3,13)} = 1.5$, $P = 0.2$) or fathers ($F_{(3,13)} = 1.2$, $P = 0.4$). Because the number of clutches differed between females, the maternal contribution was unequal among release offspring (total number of eggs, proportion; Mrs O = 23, 14.6%; Kowhai = 39, 24.7%; Greta = 40, 25.3%; Whero = 56, 35.4%). From the pedigree data, the genetic contribution of the founding females to the population was as follows; Mrs O = 6.6%, Kowhai = 11.3%, Greta = 11.72%, Whero = 20.31%. On average, LBI females reproduced every two years, which is more frequent than the four year average seen in the wild (Cree et al. 1991, Cree et al. 1992). However, two females produced clutches in consecutive years, indicating that female tuatara are capable of producing clutches every year when in good condition. One of these clutches failed to hatch, and only 2 of the 3 hatchlings survived from the other.

Paternity was assigned to all offspring with >95% confidence, and alleles were manually checked to confirm assignment. All offspring likelihood (LOD) scores (the sum of the log-likelihood ratios of each locus) were positive and ranged from 1.05 to 8.99. Multiple paternity was found in 18.8% of clutches ($n = 3$; Fig. 1). No more than two sires were represented in any clutch, and in multiply sired clutches, the percentage of paternity by the second sire ranged from 33–50% (Fig. 1).

Paternity was not equal among all males, with one male (Punga) siring 77.5% of offspring ($n = 93$). Spike sired 16.7%, Arnie sired 5.8%, and one male (Rudolph) sired no offspring. Punga sired offspring in all but two of the clutches. From the pedigree data, the genetic contribution of each of the founding males to the population was as follows; Punga = 37.11%, Spike = 8.59%, Arnie = 3.52% and Rudolph = 0.78%. Body mass of the founding males generally increased between 1991 and 2006, with Punga eventually outweighing the other males (Fig. 2). Mean observed heterozygosity decreased by 14% from the parent to the offspring generation, which is consistent with a loss

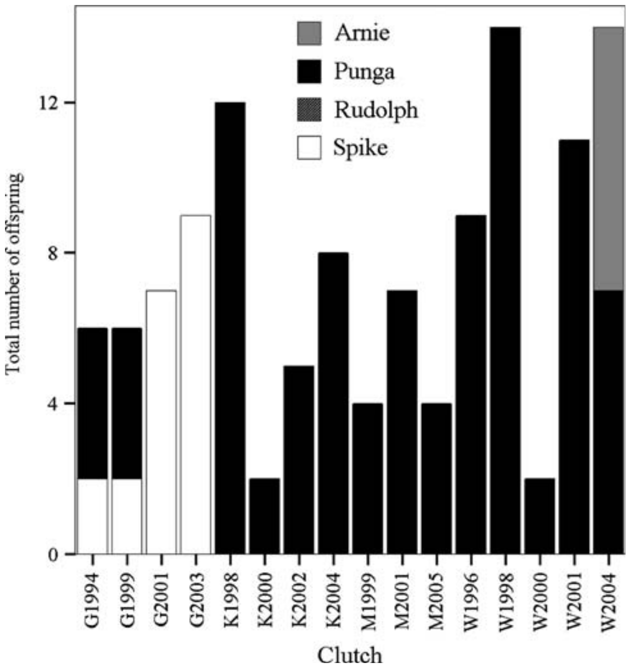

Fig. 1 Relative reproductive success of the four founding male tuatara. Bars represent total number of offspring per clutch (by female W = Whero, M = Mrs O, G = Greta, K = Kowhai, and year) as a proportion of each of the four sires

of genetic diversity due to unequal male contribution (Table 2). One allele was lost from the parent to the offspring generation, yet allele frequencies were not significantly different χ^2 (10, 129) = 16.15, $P = 0.095$.

The average number of alleles per locus and the mean observed heterozygosity for the LBI adults did not differ significantly from Stephens Island tuatara ($F_{(3,16)} = 0.16$, $P > 0.9$), when sample sizes were equal. Compared to a large sample of tuatara from Stephens Island that we genotyped at the same five loci ($n = 50$), private alleles were found in LBI tuatara at 2 of 5 loci (*C11P* and *B8P*). The mean relatedness of LBI adults ($r = 0.065$) did not differ significantly from Stephens Island tuatara (mean $r = 0.033$), indicating that the LBI adults are no more inbred than those of a large, highly dense population (2000 tuatara / ha; Carmichael et al. 1989). Furthermore, the effective number of LBI breeders was lower than the census number of breeders ($N = 8$). Based on the variance in reproductive success, $N_e = 3.77$ ($N_{em} = 1.32$ and $N_{ef} = 3.30$).

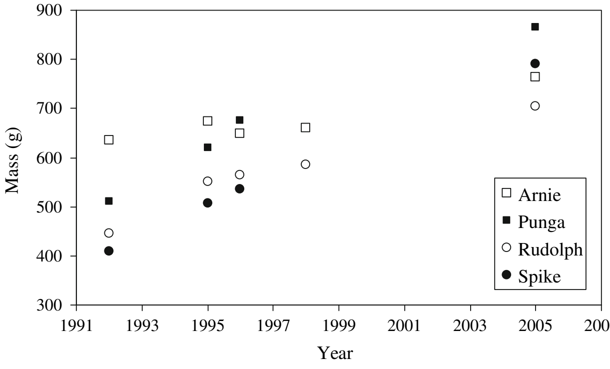

Fig. 2 Body mass of founding male tuatara over time. Punga is the heaviest male and sired 78.8% of the offspring (closed squares = Punga, closed circles = Spike, open squares = Arnie, open circles = Rudolph). Snout-vent length follows a similar pattern of growth

Discussion

This is the first study to document the occurrence of within-season multiple paternity in tuatara. Multiple paternity is common in many taxa including mammals (Eberle and Kappeler 2004), birds, (Gibbs et al. 1990, Jamieson et al. 1994), insects (Bretman and Tregenza 2005), fish (Avise et al. 2002), amphibians (Gopurenko et al. 2006), and in other reptiles (e.g., Davis et al. 2001, Lee and Hays 2004, Morrison et al. 2002, Xu et al. 2005, Zamudio and Sinervo 2000). Our results thus confirm the presence of multiple paternity, and also genetic polygyny in tuatara.

Numerous hypotheses have been presented to explain the benefits conferred to females that exhibit polyandry (see Jennions and Petrie 2000, Reynolds 1996 for review). These include, but are not limited to, (1) improved genetic quality of offspring (via enhanced offspring diversity, genetic compatibility, increased sperm competition, cryptic female choice; Calsbeek and Sinervo 2004, Madsen et al. 1992), (2) transfer of nutrients in seminal fluid, and (3) insurance against male infertility (Levitan and Petersen 1995). In populations or species where the odds of encountering a mate are low, females are expected to exhibit high levels of multiple paternity as a consequence of sperm storage. Although there is no evidence of long-term sperm storage in tuatara (Saint Girons 1983), ovulation occurs ~1–2 months after mating (Cree et al. 1992) thus providing a window for sperm competition or cryptic female choice to occur. The mechanism underlying fertilization success in tuatara remains unknown, but cryptic female choice and/or sperm competition could play a role in paternity determination.

Polyandry, polygyny and social dominance are clearly reflected in paternity of the LBI tuatara population. Social dominance may be based on large body size, as the smallest male was completely restricted from mating. Male-male competition appears to be an important determinant of male reproductive success. Females may also exhibit a preference for larger, more dominant males, a phenomenon which has been shown in other taxa (e.g., *Ambystoma tigrinum tigrinum*, Howard et al. 1997; *Psammodromus algirus*, Salvador and Veiga 2001; *Uta*

Table 2 Number of alleles (Na) and observed heterozygosity (Ho) of offspring and adult Little Barrier Island tuatara by locus

Locus	Na parents	Na offspring	Ho parents	Ho offspring
A12N	7	7	0.63	0.42
C1H	4	4	0.63	0.53
C11P	10	9	0.88	0.89
B8P	10	10	0.88	0.91
H5H	5	5	0.88	0.60
Average	7.2	7.0	0.78	0.67

stansburiana, Calsbeek and Sinervo 2004). Although it is possible that Rudolph, who sired no offspring, was simply infertile, it is unlikely as the rates of infertility in reptile populations are generally very low (Olsson and Shine 1997).

At the population level, social dominance of mating by one or a few males decreases genetic diversity (e.g., Hoelzel et al. 1999). The loss of heterozygosity from the parent to the offspring generation (14%) in the LBI tuatara population is not surprising considering the unequal representation of the founding males. Although there are eight potential breeders in the LBI captive breeding program, based on the variance in male and female reproductive success, the effective size of this population is approximately 4. This small N_e is cause for concern as this growing population is likely to lose diversity at a rapid rate. The presence of multiply sired clutches may help to offset the potential loss of diversity from social dominance by increasing the diversity of offspring within individual clutches and ultimately increasing N_e (Sugg and Chesser 1994).

Because we have included every known individual in the LBI population in our analyses, it is impossible to under- or overestimate current levels of genetic diversity. However, our data span the first 12 years of this breeding program (only a portion of the reproductive lifespan of a tuatara), and it is possible that as male size and condition changes with time, and management options are explored (see below) the dominance structure will be altered significantly. Aside from obvious correlates like body size (i.e., competitive ability), it is currently unknown why some males are consistently more successful than others. Reproductive dominance by these individuals could be beneficial if, for instance, they have greater variation at functional genes, such as the major histocompatibility complex (MHC) (Miller et al. 2007). If this were the case, allowing the natural mating system to play a role, rather than manipulating captive breeding, could actually enhance offspring fitness.

While the small number of remnant LBI tuatara indicates a recent demographic bottleneck, the remaining tuatara still retain relatively high levels of genetic diversity. In a survey of genetic diversity of tuatara populations, MacAvoy et al. (2007) found similar results. Based on a different set of microsatellite markers than we used in our study, MacAvoy et al. (2007) found an intermediate level of genetic diversity in the LBI tuatara ($n = 7$) when compared to 13 other tuatara populations. Tuatara populations exhibit strong genetic structuring across their range, which further emphasizes the need to conserve every remnant population via threat removal and/or successful captive breeding (Aitken et al. 2001, MacAvoy et al. 2007). MacAvoy et al. (2007) concluded that a loss of rare alleles, resulting in a mode shift in allele frequency classes, was indicative of a genetic bottleneck that puts LBI tuatara at risk.

Conservation implications/management recommendations

Conservation breeding programs are common in New Zealand species recovery plans. In some instances, human intervention and manipulation of founder representation are possible (Clout and Craig 1995). However, in cases such as the LBI tuatara, the best managers can do to retain the genetic diversity of a particular population is to repopulate the island with the genetic stock that remains, and any breeding in captivity by these individuals is thus considered a success. Although conservation efforts for tuatara have been extremely successful (Nelson et al. 2002), from a genetic standpoint it would be useful for managers to consider the genetic effects of potentially highly polygynous captive colonies. Many captive-bred populations scheduled for reintroduction are already suffering from genetic bottlenecks, and unequal founder representation would only further this, thus putting these populations at even greater risk. In a captive setting, rotating resident males with a group of females may help to alleviate the effects of dominance and equalize male representation. However, managing on a very fine scale (e.g., isolating specific breeding pairs) has not proven successful in the past, so it is unknown whether breeding would even occur under highly managed circumstances.

Although the remaining LBI tuatara appear to be retaining some remnant diversity, the release group that will repopulate this island is composed of all half and full siblings, which may hinder the future success of this population by reduced reproduction, survival and hatching success resulting from inbreeding depression. Releasing the offspring at geographically distinct sites may help to slow the rate of inbreeding by decreasing the probability of full-sibling mating. Furthermore, removing Punga from the captive colony may help to even out the distribution of founder alleles, although this does not guarantee that all males will be represented in the future. Since rats have been removed from LBI, one new wild tuatara has been sighted on the island. The admixture of new genetic stock, from remnant tuatara still living on LBI outside of captivity, could improve the genetic health of this new population.

In future captive breeding efforts, manipulation of hatchling sex ratios could be beneficial for population growth. Because tuatara exhibit TSD, manipulating sex ratios of release groups (via artificial incubation temperatures) is easily accomplished. Theoretically, an equal sex ratio should maximize N_e (Allendorf and Luikart 2007). However, the adult sex ratio also has an impact on the degree and frequency of polyandry and/or polygyny (e.g., Anthony and Blumstein 2000, Fitze et al. 2005). In small populations where females are the reproductively limiting sex, a female-biased sex ratio can amplify population growth. Lenz et al. (2007) found that female-biased sex ratios (of as much as 0.3 males:1 female) in captive bred Lesser Kestrels, could not only increase N_e, but lead to recovery of the wild population. Because reproductive success is so variable in male tuatara and females have an infrequent, low reproductive output (Cree 1994), a female biased sex ratio (of possibly as much as 0.25 males:1 female) may help to offset the variance in male reproductive success by decreasing intrasexual competition, while maximizing population growth and maintaining genetic diversity.

The concept of translocating individuals or eggs from one population to another (i.e., introducing migrants) has been suggested as a potential means to increase genetic diversity and population demography of some tuatara populations (Allendorf 2001). This could be a way of increasing N_e for the LBI population. Because tuatara exhibit strong phylogenetic structuring across their range, source islands should be the most genetically similar populations that would not be harmed by removal of a small portion of their population. However, it is currently unknown whether this could have negative impacts (e.g., outbreeding depression), so future research and management should aim to explore this direction.

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References

Aitken N, Hay JM, Sarre SD, Lambert DM, Daugherty CH (2001) Microsatellite DNA markers for tuatara (*Sphenodon* spp.). Conserv Genet 2:183–185

Allendorf FW (2001) Genetics and the viability of insular populations of reptiles. NZ J Zool 28:361

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden

Anthony LL, Blumstein DT (2000) Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce Ne. Biol Conserv 95(3):303–315

Avise JC, Jones AG, Walker D, DeWoody JA, Collaborators (2002) Genetic mating systems and reproductive natural histories of fishes: Lessons for ecology and evolution. Ann Rev Genet 36:19–45

Benton MJ (2000) Vertebrate palaeontology. Blackwell Science, London

Bretman A, Tregenza T (2005) Measuring polyandry in wild populations: a case study using promiscuous crickets. Mol Ecol 14(7):2169–2179

Bull CM (2000) Monogamy in lizards. Behav Proc 51(1–3):7–20

Calsbeek R, Sinervo B (2004) Within-clutch variation in offspring sex determined by differences in sire body size: cryptic mate choice in the wild. J Evol Biol 17(2):464–470

Carmichael CK, Gillingham JC, Keall SN (1989) Feeding ecology of the tuatara (*Sphenodon punctatus*) on Stephens Island based on niche diversification. NZ J Zool 16:269 (abstract)

Chapple DG, Keogh JS (2005) Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. Mol Ecol 14(4):1215–1227

Clout MN, Craig JL (1995) The conservation of critically endangered flightless birds in New Zealand. Ibis 1 (supplement 1)

Cree A (1994) Low annual reproductive output in female reptiles from New Zealand. NZ J Zool 21(4):351–372

Cree A, Cockrem JF, Brown MA, Watson PR, Guillette LJ, Newman DG, Chambers GK (1991) Laparoscopy, radiography, and blood analyses as techniques for identifying the reproductive condition of female tuatara. Herpetologica 47(2):238–249

Cree A, Cockrem JF, Guillette Jr. LJ (1992) Reproductive cycles of male and female tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand. J Zool 226:199–217

Cree A, Daugherty CH, Hay JM (1995) Reproduction of a rare New Zealand reptile, the tuatara *Sphenodon punctatus*, on rat-free and rat-inhabited islands. Conserv Biol 9(2):373–383

Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. Heredity 93(5):504–509

Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of tuatara (*Sphenodon*). Nature 347(6289):177–179

Davis LM, Glenn TC, Elsey RM, Dessauer HC, Sawyer RH (2001) Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. Mol Ecol 10(4):1011–1024

Ebenhard T (1995) Conservation breeding as a tool for saving animal species from extinction. Trends Ecol Evol 10(11):438–443

Eberle M, Kappeler PM (2004) Sex in the dark: determinants and consequences of mixed male mating tactics in *Microcebus* *murinus*, a small solitary nocturnal primate. Behav Ecol Sociobiol 57(1):77–90

Fitze PS, Le Galliard JF, Federici P, Richard M, Clobert J (2005) Conflict over multiple-partner mating between males and females of the polygynandrous common lizards. Evolution 59(11):2451–2459

Fiumera AC, Porter BA, Grossman GD, Avise JC (2002) Intensive genetic assessment of the mating system and reproductive success in a semi-closed population of the mottled sculpin, *Cottus bairdi*. Mol Ecol 11(11):2367–2377

Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ (1990) Realized reproductive success of polygynous Red-Winged Blackbirds revealed by DNA markers. Science 250(4986):1394–1397

Gillingham JC, Carmichael C, Miller T (1995) Social behavior of the tuatara, *Sphenodon punctatus*. Herpetol Monogr 9:5–16

Girardet SAB, Veitch CR, Craig JL (2001) Bird and rat numbers on Little Barrier Island, New Zealand, over the period of cat eradication 1976–80. NZ J Zool 28(1):13–29

Gopurenko D, Williams RN, McCormick CR, DeWoody JA (2006) Insights into the mating habits of the tiger salamander (*Ambystoma tigrinum tigrinum*) as revealed by genetic parentage analyses. Mol Ecol 15:1917–1928

Goyache F, Gutierrez JP, Fernandez I, Gomez E, Alvarez I, Diez J, Royo LJ (2003) Using pedigree information to monitor genetic variability of endangered populations: the Xalda sheep breed of Asturias as an example. J Anim Breed Genet 120(2):95–105

Gutierrez JP, Altarriba J, Diaz C, Quintanilla R, Canon J, Piedrafita J (2003) Pedigree analysis of eight Spanish beef cattle breeds. Genet Sel Evol 35(1):43–63

Gutierrez JP, Goyache F (2005) A note on ENDOG: a computer program for analysing pedigree information. J Anim Breed Genet 122(3):172–176

Hay JM, Daugherty CH, Cree A, Maxson LR (2003) Low genetic divergence obscures phylogeny among populations of *Sphenodon*, remnant of an ancient reptile lineage. Mol Phylogenet Evol 29(1):1–19

Hay JM, Lambert DM (2007) Microsatellite DNA loci identify individuals and provide no evidence for multiple paternity in wild tuatara (Sphenodon: Reptilia). Conserv Genet (in press). doi:10.1007/s10592-007-9445-5

Hoelzel AR, Le Boeuf BJ, Reiter J, Campagna C (1999) Alpha-male paternity in elephant seals. Behav Ecol Sociobiol 46(5):298–306

Howard RD, Moorman RS, Whiteman HH (1997) Differential effects of mate competition and mate choice on eastern tiger salamanders. Anim Behav 53:1345–1356

Jamieson IG, Quinn JS, Rose PA, White BN (1994) Shared paternity among non-relatives is a result of an egalitarian mating system in a communally breeding bird, the Pukeko. Proc R Soc B 257(1350):271–277

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21–64

Kimura M, Crow JF (1963) Measurement of effective population number. Evolution 17 (3):279–288

Lee PLM, Hays GC (2004) Polyandry in a marine turtle: females make the best of a bad job. Proc Nat Acad Sci USA 101(17):6530–6535

Lenz TL, Jacob A, Wedekind C (2007) Manipulating sex ratio to increase population growth: the example of the Lesser Kestrel. Anim Conserv 10:236–244

Levitan DR, Petersen C (1995) Sperm limitation in the sea. Trends Ecol Evol 10(6):228–231

Luikart G, Cornuet JM (1999) Estimating the effective number of breeders from heterozygote excess in progeny. Genetics 151(3):1211–1216

MacAvoy ES, McGibbon LM, Sainsbury JP, Lawrence H, Wilson CA, Daugherty CH, Chambers GK (2007) Genetic variation in island populations of tuatara (*Sphenodon* spp) inferred from microsatellite markers. Conserv Genet 8(2):305–318

Madsen T, Shine R, Loman J, Hakansson T (1992) Why do female adders copulate so frequently. Nature 355(6359):440–441

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7(5):639–655

Miller HC, Andrews-Cookson M, Daugherty CH (2007) Two patterns of variation among MHC class I loci in tuatara (*Sphenodon punctatus*). J Hered (in press). doi:10.1093/jhered/esm095

Morrison SF, Keogh JS, Scott IAW (2002) Molecular determination of paternity in a natural population of the multiply mating polygynous lizard *Eulamprus heatwolei*. Mol Ecol 11(3):535–545

Nelson NJ, Cree A, Thompson MB, Keall SN, Daugherty CH (2004) Temperature-dependent sex determination in tuatara. In: Valenzuela N, Lance V (eds) Temperature dependent sex determination in vertebrates, Smithsonian Books, Washington, D.C

Nelson NJ, Keall SN, Brown D, Daugherty CH (2002) Establishing a new wild population of tuatara (*Sphenodon guntheri*). Conserv Biol 16(4):887–894

Olsson M, Shine R (1997) Advantages of multiple matings to females: A test of the infertility hypothesis using lizards. Evolution 51(5):1684–1688

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6(1):288–295

Pearse DE, Avise JC (2001) Turtle mating systems: behavior, sperm storage, and genetic paternity. J Hered 92(2):206–211

Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. Am Nat 153(2):145–164

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote excess in progeny. Genetics 144(1):383–387

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43(2):258–275

Ralls K, Ballou J (1986) Captive breeding programs for populations with a small number of founders. Trends Ecol Evol 1(1):19–22

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Reischek A (1886) Observations on *Sphenodon punctatum*, fringe-back lizard (tuatara). Trans Proc NZ Inst 18:108–110

Reynolds JD (1996) Animal breeding systems. Trends Ecol Evol 11(2):68–72

Roberts SC, Gosling LM (2003) Genetic similarity and quality interact in mate choice decisions by female mice. Nature Genet 35(1):103–106

Saint Girons H (1983) The tuatara: ecological features and some hypotheses concerning its evolution. Bull Soc Zool Fr 108:631–634

Salvador A, Veiga JP (2001) Male traits and pairing success in the lizard *Psammodromus algirus*. Herpetologica 57(1):77–86

Sambrook J, Fritsch E, Maniatis T (1989) Molecular cloning. A laboratory manual. Cold spring harbor laboratory press, Cold Springs Harbor

Sarrazin F, Barbault R (1996) Reintroduction: challenges and lessons for basic ecology. Trends Ecol Evol 11(11):474–478

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD, Miller B (1996) Limitations of captive breeding in endangered species recovery. Conserv Biol 10(2):338–348

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. Genetics 137(4):1147–1155

Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121(2):379–391

Whitaker AH (1993) Research on the tuatara (*Sphenodon punctatus*) of Little Barrier Island, 6–20 October 1992. Page 52. Unpublished report, Auckland Conservancy, New Zealand department of conservation, Auckland

Whitaker AH, Daugherty CH (1991) Research on the tuatara (*Sphenodon punctatus*) of Little Barrier Island, 5–12 February 1991. Page 54. Unpublished report, Auckland Conservancy, New Zealand Department of Conservation, Auckland

Xu QH, Fang SG, Wang ZP, Wang ZW (2005) Microsatellite analysis of genetic diversity in the Chinese alligator (*Alligator sinensis*) Changxing captive population. Conserv Genet 6(6):941–951

Zamudio KR, Sinervo E (2000) Polygyny, mate-guarding, and posthumous fertilization as alternative male mating strategies. Proc Nat Acad Sci USA 97(26):14427–14432