

Multiyear multiple paternity and mate fidelity in the American alligator, *Alligator mississippiensis*

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Abstract

We examined multiple paternity during eight breeding events within a 10-year period (1995–2005) for a total of 114 wild American alligator nests in Rockefeller Wildlife Refuge in south-west Louisiana. Our goals included examining (i) within population variation in multiple paternity among years, (ii) variation in multiple paternity in individual females and (iii) the potential for mate fidelity. To accomplish this, in the current study, eggs were sampled from 92 nests over 6 years and analysed along with 22 nests from a previous 2-year study. Genotypes at five microsatellite loci were generated for 1802 alligator hatchlings. Multiple paternity was found in 51% of clutches and paternal contributions to these clutches were highly skewed. Rates of multiple paternity varied widely among years and were consistently higher in the current study than previously reported for the same population. Larger females have larger clutches, but are not more likely to have multiply sired nests. However, small females are unlikely to have clutches with more than two sires. For 10 females, nests from multiple years were examined. Seven (70%) of these females exhibited long-term mate fidelity, with one female mating with the same male in 1997, 2002 and 2005. Five females exhibiting partial mate fidelity (71%) had at least one multiple paternity nest and thus mated with the same male, but not exclusively. These patterns of mate fidelity suggest a potential role for mate choice in alligators.

Keywords: alligator, mate fidelity, mating systems, microsatellites, multiple paternity

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Introduction

Multiple mating by females has been reported from a wide range of taxa, including classes of all vertebrates and many invertebrates (Birkhead & Møller 1998), but has been most extensively studied in birds (see reviews by Westneat *et al.* 1990; Birkhead & Møller 1992, 1995; Westneat & Sherman 1997; Møller & Cuervo 2000; Griffith *et al.* 2002). In birds, the proportion of extra-pair paternity (EPP) varies as a function of variation in male quality and of the ability of females to choose freely the highest quality male (Petrie & Kempenaers

1998). However, multiple ecological and environmental factors can influence occurrence of EPP, with different factors operating at the levels of individual, population and species (Griffith *et al.* 2002). Some analyses attribute up to 55% of the variation in EPP in birds to phylogeny (Arnold & Owens 2002; Westneat & Stewart 2003). Therefore, the causes and consequences of EPP vary both intra- and interspecifically in birds.

Differences in EPP patterns among species can be partially explained by social mating system, with rates of EPP highest in socially monogamous species, intermediate in socially polygynous species and the lowest in lek-mating systems (Petrie & Kempenaers 1998). This pattern is associated with the decrease in direct benefits (e.g. resources, male parental care) received by females from their mates under the different mating systems.

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Other ecological factors, such as degree of breeding synchrony, could also influence interspecific variation in EPP (Petrie & Kempenaers 1998).

Marked variation in EPP has been reported among populations of the same bird species. For example, in their 4-year study of blue tits, *Parus caeruleus*, Kempenaers *et al.* (1997) found evidence that females gain indirect genetic benefits by mating multiply. However, a 5-year study (Krokene *et al.* 1998) on a different population of blue tits found no evidence for indirect genetic benefits and instead suggested females mate multiply for fertility insurance. Likewise, within a single population, EPP patterns can also vary among individuals and across time. For example, in tree swallows, *Tachycineta bicolor*, individual females were highly consistent in both the number of males they mated with and the proportion of resulting extra-pair young (Whittingham *et al.* 2006). On the other hand, Westneat & Mays (2005) found that variation in EPP in red-winged blackbirds, *Agelaius phoeniceus*, was partially explained by which male, rather than female, attended a nest. The high level of variation in EPP variation observed in birds both within and across populations highlights the complexity of multiple mating by females and the need for testing specific hypotheses concerning how ecology and social systems influence female mating behaviour (Westneat & Mays 2005).

In contrast to birds, few studies have investigated patterns of multiple mating in reptiles (reviewed in Uller & Olsson 2008). Parental care is typically lacking in reptiles – males provide no direct resources to females or offspring and there is little evidence that females gain indirect genetic benefits (Uller & Olsson 2008). Perhaps as a result, polygyny, rather than social monogamy, is the most common mating system in reptiles (Bull 2000; Uller & Olsson 2008). Overall, multiple paternity (MP) levels are high and differ among taxa with an average of 42% in turtles and 55% in squamates (Uller & Olsson 2008). Thus it may be more appropriate to describe most reptilian mating systems as polygynandrous, and not just polygynous.

In reptiles, studies of intra-specific variation in MP are uncommon, but demonstrate the complexity of mating patterns both among and within populations. High levels of among-population variation in MP have been reported in some species, including garter snakes, *Thamnophis sirtalis* (McCracken *et al.* 1999; Garner *et al.* 2002) and olive ridley sea turtles, *Lepidochelys olivacea* (Jensen *et al.* 2006). In their review of MP in reptiles, Uller & Olsson (2008) suggest that MP rates are determined by the interaction of mate-encounter rates, female costs of multiple mating and selection on males to mate multiply. Overall, MP was positively correlated with mate-encounter rates. In several species of reptiles,

females investing more in reproduction are more likely to have multiply sired broods (e.g. *Chrysemys picta*: Pearse *et al.* 2002; *Nerodia sipedon*: Prosser *et al.* 2002; *T. sirtalis*: Garner *et al.* 2002; *Thamnophis elegans*: Garner & Larsen 2005; *Lacerta vivipara*: Fitze *et al.* 2005; Fitze & Le Galliard 2008; Eizaguirre *et al.* 2007), leading to the common suggestion that intersexual conflict and male preference for these females drives multiple mating in reptiles (reviewed in Uller & Olsson 2008).

Unlike most reptiles, female crocodylians (but not males) exhibit extensive parental care including nest attendance and defence of hatchlings (Kushlan & Simon 1981; Hunt & Watanabe 1982; Hunt 1987). Crocodylians, then, may be more similar in behaviour to birds without male parental care, in which copulation frequency (Birkhead *et al.* 1987) and EPP are low, whereas female choice is high (Petrie & Kempenaers 1998). In particular, female American alligators, *Alligator mississippiensis*, have ample opportunity to assess males and choose their preferred mate: male alligators form dominance hierarchies and during courtship, both males and females perform stereotypical mating behaviours (Garrick & Lang 1977; Joanen & McNease 1989), and females can terminate courtship (Garrick & Lang 1977). In southwest Louisiana, female American alligators typically spend most of their time in small isolated ponds within the marsh interior. During April and May, they move into deeper water where courtship and mating take place (Joaen & McNease 1970, 1972). Females exhibit varying degrees of nest site fidelity with some nesting in the same general area as they did in previous years and some nesting in the same location over multiple years (Elsey *et al.* 2008). Most nesting behaviour occurs 1 month after the peak mating period. In some cases, females produce clutches in successive years, but more typically they experience some nonbreeding years (Elsey *et al.* 2008). The suite of reproductive behaviours exhibited by alligators illustrates how different their mating system is from most other reptiles. As a result of their position as primitive archosaurs and their evolutionary relationship to birds, crocodylians may provide important insight into the ancestral behavioural patterns for birds (e.g. Tullberg *et al.* 2002).

Since its discovery in American alligators (Davis *et al.* 2001), MP has subsequently been found in Morelet's crocodile, *Crocodylus moreletti* (McVay *et al.* 2008) the broad-snouted caiman, *Caiman latirostris* (Amavet *et al.* 2008) and the saltwater crocodile, *Crocodylus porosus* (Lewis *et al.* personal communication). MP was found in 50% of clutches for Morelet's crocodile and broad-snouted caiman and in 69% of saltwater crocodile clutches, but all three studies had small sample sizes. In the only detailed study of MP in crocodylians published to date, Davis *et al.* (2001) found 32% of American

alligator clutches to be multiply sired, a value much lower than levels of MP found in most turtles and squamates. Similarly, low levels of MP are found in some marine turtles, in which breeding population densities are low (Uller & Olsson 2008 and references therein). Low breeding densities and encounter rates cannot explain the low incidence of MP in the study of Davis *et al.* (2001) at Rockefeller Wildlife Refuge (RWR). At RWR and along the Louisiana coast in general, alligators are abundant. Accurate density estimates are not available, but nest surveys indicate a range of ~24 000–43 000 nests along the coast over the course of this study and ~100 a year at RWR with one nest per 10 acres in peak years (unpublished data from RME and the Louisiana Department of Wildlife and Fisheries). Females move freely through male territories, presumably leading to high mate-encounter rates. In addition, Davis *et al.* (2001) did not find a relationship between MP and either female size or clutch size suggesting that intersexual conflict also cannot fully explain observed patterns of MP in alligators.

Intrapopulation variation in MP and mating behaviour is common (Petrie & Kempenaers 1998), thus a larger study is required to determine what drives MP in alligators. If mate-encounter rates and intersexual conflict do actually drive MP, then we predict higher levels of MP than found by Davis *et al.* (2001). On the other hand, if the low values of MP are consistently observed, then alligator mating behaviour may be similar to that of bird species that, like alligators have maternal, but no paternal care. In several lek-mating species of birds, females show consistency in their choice of males (Trail & Adams 1989; Pruett-Jones & Pruett-Jones 1990; Rintamäki *et al.* 1995; Sæther *et al.* 2005) and the same may be expected of alligators. The samples for the study by Davis *et al.* (2001) came primarily from 1 year. Thus, to better understand the role of MP and female mating behaviour in alligators, we expanded upon that study by investigating the same population additionally for 6 years. Specifically, our goals include examining (i) within population variation in MP among years, (ii) variation in MP in individual females and (iii) the potential for mate fidelity.

Materials and methods

Sample collection

Samples for this study were collected between 2000 and 2005 by staff at RWR, a 32 000 ha coastal marsh located in southwestern Louisiana. The refuge boundaries and predominant vegetation have been described previously (Joanen & McNease 1969). The RWR alligator population is free ranging, dense and subjected to a variety of

human impacts. Human impacts include development along canals constructed for oil exploration activities, impoundments created to manipulate water depth for wildlife management and annual harvests of nuisance alligators.

Alligator nests were located by helicopter, marked with PVC pipes and plotted on aerial maps to facilitate egg collections by ground crews. Female alligators attending the nests were captured, measured and sampled as described in Davis *et al.* (2001). Eggs were collected from each nest and incubated at RWR's field laboratory as described in Joanen & McNease (1987). All clutches were incubated in separate containers and identified by location and attending female. Within a few days, after hatching a 0.5–1.0 mL blood sample was obtained from each hatchling. Samples were placed in DNA lysis/storage buffer (1:1 by volume for blood; buffer = 100 mM Tris, pH 8, 100 mM EDTA, 1% SDS) and frozen at –20°C until DNA extraction. In an attempt to examine multiple nests from individual females, we collected eggs from specific nest sites over several years because females show some nest site fidelity (Elsey *et al.* 2008). In most cases, identity of the attendant female could be confirmed in the field based on previously placed web tags, but blood samples were also collected for confirmation via genotyping.

DNA extraction

DNA was extracted from red blood cells using one of the two methods. In the first method, a mud extraction (adapted from Boom *et al.* 1990; Höss & Paabo 1993; and Höss 1994) consisted of a pre-extraction digestion in 400 µL of Tris-high EDTA (THE; 100 mM Tris, pH 8, 100 mM EDTA) with 1% SDS and 6 µL of proteinase K (10 mg/mL) added to approximately 0.1 g of red blood cells in lysis buffer. The samples were placed in a 52°C incubator on a rotator for 2 h to overnight. Two hundred microlitres of the digested DNA was added to 400 µL of GuSCN extraction buffer (4 M GuSCN, 40 mM Tris, pH 6.4, 20 mM EDTA, 1% Triton X-100) and 75 µL of MUD (diatomaceous earth suspended in water), then placed in a 52°C incubator on a rotator for 1 h to overnight. The supernatant was discarded after the samples were vortexed and then centrifuged for 10 min. The resulting pellets were washed twice with a 1-mL aliquot of 70% ethanol, then vortexed and centrifuged for 2 min removing the supernatant after each wash. The pellets were then dried at ambient temperature, re-suspended in 125 µL Tris-low EDTA (TLE) (10 mM Tris, pH 8, 0.2 mM EDTA), vortexed and centrifuged for 5 min. The liquid was removed and used for DNA amplification.

In the second method, a salt-modified mud extraction was adapted for use with 96-well plates. Blood samples

were digested prior to extraction as described above. In each well of a filter plate (Millipore, MAHVN45), 35 µL of a digested sample was added to 150 µL of saturated NaCl and 15 µL of MUD. The plate was incubated at 55°C for at least 5 min, then centrifuged for 1 min or until all liquid had migrated into the discard plate. The resulting pellets were washed twice by adding 175 µL of washing ethanol (70% ethanol supplemented with 100 mM NaCl, 10 mM Tris, 1 mM EDTA) to each well, centrifuging for 1 min and removing the liquid in the discard plate between each wash. The plates were then incubated at 55°C until pellets and filters were completely dry (5–20 min). To elute the DNA, 50 µL TLE was added to each well, and the plate was incubated at 55°C for at least 5 min. The filter plate was then centrifuged for at least 1 min, with a 96-well polymerase chain reaction (PCR) plate secured below it (in place of the discard plate) as a recovery plate.

Multiplex microsatellite amplification and detection

Alligators were genotyped using primer pairs for loci Amiu-202, -203, -229, -231 and -244 (Table 1). From each nest, we genotyped a minimum of 10 and an average of 20 hatchlings. For DNA amplification by the PCR, only the forward primer oligos of each microsatellite primer pair were labelled with one of the three colours: yellow (NED), blue (FAM) and green (VIC). The reverse primers were pigtailed with GTTT to ensure that the extra A was always added to the PCR product by nontemplate activity of Taq DNA polymerase (primers with this sequence include pgtl in the primer name).

Amplification of the microsatellite locus was usually carried out in two independent single-locus reactions

for loci Amiu-229 and -244, and a three-locus multiplex for loci Amiu-202, -203 and -231. Each of the three 25 µL reactions contained: 2.5 µL of 10× buffer without MgCl₂, 2.5 µL of BSA, 2.0 µL MgCl₂, 1.5 µL dNTPs (25 mM each), 0.2 µL of Taq DNA polymerase (Sigma Jumpstart, 2.5 units/µL) and 2 µL DNA. In addition, the three-locus multiplex reaction contained 1 µL Amiu-202-hex (10 µM), 1 µL Amiu-202-pgtl (10 µM), 0.5 µL Amiu-203-ned (10 µM), 0.5 µL Amiu-203pgtl, 1.5 µL Amiu-231-fam (10 µM), 1.5 µL Amiu-231pgtl (10 µM) and 8.3 µL of distilled water. The single-locus 229 reaction contained: 1.5 µL Amiu-229-fam (10 µL), 1.5 µL Amiu-229pgtl (10 µM) and 11.3 µL of distilled water. The single-locus 244 reaction contained: 1.0 µL Amiu-244-ned (10 µM), 1.0 µL Amiu-244pgtl (10 µM) and 12.3 µL of distilled water.

For all loci scored, PCR conditions were optimized using a touchdown protocol on a GeneAmp PCR system 9700 thermocycler (Applied Biosystems) or Eppendorf Mastercycler Gradient thermal cycler at 55°C. The optimized amplification profile was: 5 min at 95°C, 30 s at 95°C, 30 s at 55.0°C, 30 s at 72°C for five cycles, 30 s at 95°C, 30 s 55.0°C (–0.5°C every cycle), 30 s at 72°C for 21 cycles, 30 s at 95°C, 30 s at 45°C, 30 s 72°C for 15 cycles and 30 min at 72°C extension time.

Prior to 2005, PCR products were inspected via ethidium bromide staining in a 1.2–1.5% agarose gel alongside a 100 base pair ladder (HiLo, Minnesota Molecular) to estimate PCR product yield. For loading onto the ABI Prism 377, a cocktail of 3.0 µL Dextran/formamide, 0.6 µL Promega CXR fluorescent ladder and approximately 10 ng of each PCR product was prepared. This cocktail was denatured by incubation at 95°C for 5 min and placed on ice. Of this, 0.8–1.5 µL

Table 1 Characteristics of microsatellite loci used to assess mating patterns in American alligators within the current study and a previous study by Davis *et al.* (2001), both conducted at Rockefeller Wildlife Refuge

Locus	N _A	EP ₁	EP ₀	f(null)	GE	Source
Current study						
Amiu-202	19	0.79	0.65	–0.035	0.016	2
Amiu-203	8	0.55	0.37	0.072	0	2
Amiu-229	9	0.40	0.22	0.078	0.002	3
Amiu-231	9	0.48	0.31	–0.032	0.002	3
Amiu-244	9	0.66	0.49	–0.104	0.009	3
Davis <i>et al.</i> (2001)						
Amiu-6	7	0.52	0.34	—	—	1
Amiu-8	9	0.43	0.26	—	—	1
Amiu-15	5	0.28	0.12	—	—	1
Amiu-17	16	0.72	0.57	—	—	1
Amiu-18	7	0.59	0.41	—	—	1

N_A = number of alleles; EP₁, EP₀ = exclusion probabilities when 1 or 0 parents are known, respectively; f(null) = estimated null allele frequency calculated in Cervus 3.0; GE = estimated genotyping error rate based on comparing genotypes of mothers and their offspring. 1 = Glenn *et al.* (1998); 2 = Davis *et al.* (2001); 3 = Subaluski *et al.* (unpublished data, available from the authors).

was loaded into the wells of a 0.2-mm thick 4.5% polyacrylamide gel (12 cm well-to-read length) and the amplicons separated over a 1.5-h period. GeneScan and GeneMapper programs (Applied Biosystems, Inc.) were then used to identify and genotype microsatellite fragments. All peaks were inspected visually for confidence in genotyping.

Starting in 2005, 1 μ L of PCR product was mixed with 6.65 μ L of distilled water, 6.65 μ L HiDi loading buffer and 1.0 μ L of Naurox-pgtl (modification of DeWoody *et al.* 2004) size standard. Samples were then denatured for 5 min at 95°C. The samples were run on a 3130xl Genetic Analyzer (Applied Biosystems, Inc.) with a 50-cm array using Pop-7 and freely standard fragment analysis. Fragment sizes (alleles) were determined by comparison with the internal Naurox size standard for each sample using GeneMapper 3.7 or 4.0 software (Applied Biosystems). All genotypes were visually inspected to verify that the correct alleles were called.

Statistical analyses

Allele frequencies for the RWR population were estimated using the genotypes of all adult females captured in the study. Alleles that were represented in clutch, but not sampled among females (i.e. uncommon alleles from the fathers), were accounted for by adding the new alleles directly to the allele distribution for that locus only. Alternative methods of accounting for such alleles were investigated (e.g. adding a hatchling genotype, which included the uncommon allele), but were found to have little effect on the results or interpretation of results. Frequencies of null alleles were estimated using Cervus 3.0.3 (Kalinowski *et al.* 2007).

Single-locus exclusion probabilities for individual hatchlings, when one parent is known, or both parents are unknown, were calculated using the program GERUD 2.0 (Jones 2005) for the five loci used in this study and for comparison for the five loci used in Davis *et al.* (2001) (Table 1). Simulations were run in GERUDSIM 2.0 to determine our power to detect MP with different combinations of loci and different numbers of hatchlings. Simulations were run with 10, 20, or 30 offspring and a reproductive skew such that the primary male fathered 8, 17 and 26 offspring respectively. Simulations were run on our combinations of loci and those used by Davis *et al.* (2001).

When GERUD could not assign all hatchlings in a clutch to the female guarding the nest, each hatchling genotype was compared with the genotype of the attending female to determine whether incompatible offspring were because of either mutation or genotyping error. Inconsistent genotypes were attributed to

genotyping error or mutation if they were inconsistent with all other genotypes within a clutch at one or more loci in only one individual in a clutch. Paternity analyses were performed in GERUD 2.0 and then confirmed by visual inspection of hatchling genotypes. A clutch was considered to exhibit MP if the following conditions were met: (i) all hatchling genotypes within a locus contained at least one maternal allele that was consistent within the clutch; (ii) the remaining paternal alleles could not be accounted for by one father because of inconsistencies in Mendelian inheritance; and (iii) hatchlings containing an inconsistent allele in their genotype at one locus had inconsistent alleles in other loci, or shared that inconsistent allele with at least one other nest-mate at that locus. In nests with MP, the most common paternal allele(s) was (were) considered to be from the primary father. Paternal alleles that were different from those of the primary father were considered to be alleles of the secondary or tertiary fathers. When a single allele from one hatchling required the addition of a secondary or tertiary father to explain the hatchling's genotype, we considered that allele to be a mutation or a genotyping error and did not add a father to the clutch. For all the clutches, we determined the number of hatchlings attributable to each father. For cases in which it was not possible to assign each hatchling unambiguously to a specific father, we calculated the maximum number of hatchlings that any one father could have sired. We then calculated the percent of each clutch that could have been sired by primary, secondary and tertiary males, which, in some cases, sums to more than 100%. For clutches sired by two or three males, we used Wilcoxon rank-sum tests to compare the fertilization success of primary and secondary males. In addition, we compiled all of the paternal genotypes generated by GERUD 2.0 and examined them for redundancy to estimate the yearly and cumulative number of males that successfully mated with the females in our study.

We calculated the percentage of MP vs. single paternity (SP) clutches observed in the RWR population each year to determine consistency among years. To identify potential biological correlates of MP, we used Wilcoxon rank sum tests to compare the size of females that had MP and SP clutches and to compare the size of MP and SP clutches. To compare the sizes of females that had nests sired by one, two, or three males, we used a Kruskal-Wallis test. The females used in this study represent a subset of those used for a study that examined the relationship between female length and clutch size (Elsley *et al.* 2008). We repeated the regression analysis on our subset and report those data for accuracy. For all statistical comparisons, alpha was set at 0.05.

Variation in female behaviour and mate fidelity

Over the course of this study, we attempted to examine paternity across multiple years for individual females. Two clutches from 1997 come from a separate study (Davis *et al.* 2001) and were in the same nest locations as nests for this study and so we re-analysed them with the loci used in this study. For clutches of females sampled more than once, the consistency of breeding strategy (single vs. MP) and the inferred paternal genotype(s) were compared. Sires with identical inferred genotypes were considered to be the same male. We define mate fidelity as occurring when the same paternal genotype is inferred for clutches from the same female during different years. We also compared paternal genotypes from all clutches to determine if males were siring nests from more than one female during any given year. We calculated the probability of identity in GenAlEx6 (Peakall & Smouse 2006) by including genotypes of all adult females.

Results*Population variation in MP*

We genotyped a total of 1802 alligator hatchlings from 92 alligator clutches. Details concerning the loci used in

our study are provided in Tables 1–3. Overall, two loci had positive null allele frequencies (Table 1), but all loci conformed to Hardy–Weinberg Equilibrium. The combination of loci yielded a strong exclusion probability (0.99, Table 3) and a multilocus probability of identity of 2.4×10^{-6} . Simulations (GERUDSIM 2.0) correctly identified two fathers in more than 93% of all clutches, regardless of whether 10, 20 or 30 offspring were sampled. In no cases did GERUDSIM overestimate the number of fathers; thus, our estimates of MP are conservative.

Overall we found MP in 51% of all clutches, ranging from a low of 40% in 2001 and a high of 67% in 2002 (Table 4). Davis *et al.* (2001) found lower levels of MP. Combining our data with those in the study of Davis *et al.* (2001) yields an overall estimate of 47% MP. Only two males were required to explain the hatchling genotypes in 70% of the MP clutches, whereas three males were required for the remaining clutches. However, based on the results of GERUD simulations and our conservative approach to identify multiply sired clutches, it is possible that we underestimated the number of fathers in some clutches.

When the GERUD results yielded more than one possible combination of paternal genotypes, we only considered the most likely combination of genotypes. In 87% of MP clutches, there was an obvious primary male responsible for >50% of the offspring. Interestingly,

Table 2 Allele frequency data for the five microsatellite loci used in this study

Allele	Allele frequencies				
	Locus 202	Locus 203	Locus 231	Locus 229	Locus 244
1	0.0010	0.3176	0.0539	0.0042	0.0208
2	0.0441	0.0521	0.4613	0.0210	0.0208
3	0.0050	0.3176	0.1077	0.0294	0.1458
4	0.0251	0.0352	0.0815	0.6008	0.2500
5	0.1214	0.1588	0.2445	0.0420	0.2083
6	0.0883	0.0267	0.0180	0.0420	0.1042
7	0.1986	0.0788	0.0276	0.0588	0.1667
8	0.1324	0.0133	0.0055	0.1597	0.0208
9	0.0221			0.0420	0.0625
10	0.1103				
11	0.0301				
12	0.0662				
13	0.0662				
14	0.0221				
15	0.0130				
16	0.0090				
17	0.0221				
18	0.0010				
19	0.0221				

Frequencies were generated from adult female alligators. Alleles that were represented in clutches, but not sampled among attendant females (i.e. uncommon alleles from the fathers), were accounted for by adding the new alleles directly to the allele distribution for that locus only.

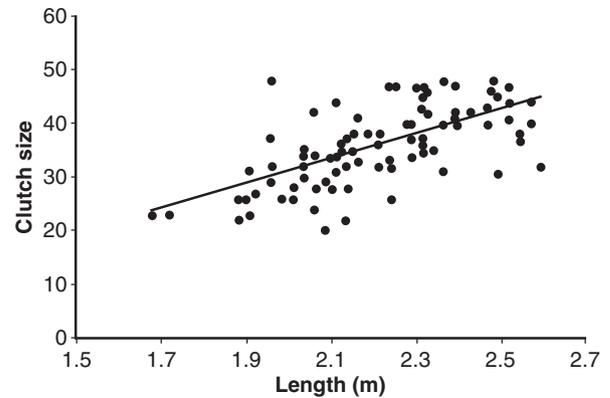
Table 3 Combinations of microsatellite loci employed in this study and a previous study by Davis *et al.* (2001) and their power to detect multiple paternity in American alligators

Loci examined	N_C	N_O	EP_1	S_{10}	S_{20}	S_{30}
Current study						
202/203/229/231/244	92	1802	0.99	0.991	0.996	0.996
Davis <i>et al.</i> (2001)						
6/8/15/17/18	22	643	0.98	0.967	0.979	0.985

N_C = number of clutches examined; N_O = number of offspring genotyped; EP_1 = multilocus exclusion probabilities when one parent is known; S_{10} , S_{20} , S_{30} = the proportion of simulated (GERUDSIM) clutches, in which the correct number of fathers was inferred when 10, 20 or 30 offspring are sampled respectively from a total of 1000 simulations.

although primary males lost significant paternity in clutches with three sires relative to those with only two sires (Wilcoxon rank-sum test, $Z = 213.5$, $P = 0.0005$), the secondary males did not lose paternity with the addition of a third sire (Wilcoxon rank-sum test, $Z = 341$, $P = 0.91$). From the GERUD output, we could estimate the number of males successfully breeding with our sampled females each year and determine which males were represented in multiple years (Table 4). On average, 1.6 unique male genotypes contributed to each nest. In any given year, only 7.7–25% of the male genotypes contributing to sampled clutches were also represented in previous years.

We examined several factors as potential ecological correlates of MP. Number of eggs per clutch ranged from 17 to 48 with larger females producing larger clutches ($r^2 = 0.42$, $F_{1,87} = 63.43$, $P < 0.0001$; Fig. 1). However, MP clutches (35.7 ± 1.1 eggs) were not significantly larger than SP clutches (35.1 ± 1.2 eggs; Wilcoxon rank-sum test, $Z = 1867.5$, $P = 0.70$). Additionally, females with MP clutches were not significantly larger

**Fig. 1** Relationship between total lengths of adult female alligators captured at Rockefeller Wildlife Refuge, Louisiana, during 2000–2005 (current study) and the number of eggs in their clutches. Females that were caught multiple times are only represented once. Line shown is from a simple regression ($y = 23.134x - 15.233$, $R^2 = 0.42$, $P < 0.0001$).

in total body length (2.21 ± 0.03 m) than females with SP clutches (2.19 ± 0.03 m; Wilcoxon rank-sum test; $Z = 1699.5$, $P = 0.84$). When we separate MP clutches into those sired by two or three males, there is no significant difference in the size distribution of females with nests sired by three males (2.27 ± 0.05 m), two males (2.19 ± 0.04 m) or one male (size listed above; Kruskal–Wallis test; $\chi^2 = 1.97$, $P = 0.37$). However, there does appear to be a difference in the minimum size of females mating with one (1.68 m), two (1.88 m) or three (2.08 m) males.

Individual variation in MP and mate fidelity

We were able to examine clutches from multiple years for 10 females. In all cases, the female was captured and positively identified each year she was sampled. In addition, all females were genotyped. Based on our

Table 4 Incidence of multiple paternity in 22 American alligator clutches examined by Davis *et al.* (2001, 92) clutches examined in this study and the combined analysis of 114 clutches

Paternity	Davis <i>et al.</i> (2001)			Current study							Combined data sets
	1995	1997	Total	2000	2001	2002	2003	2004	2005	Total	Total (<i>n</i>)
Multiple	33	32	32	40	35	62	55	55	61	51	47 (54)
Single	67	68	68	60	65	38	45	45	39	49	53 (60)
No. nests	3	19	22	10	20	13	11	20	18	92	114
No. males	n/a	n/a	n/a	16	28	25	16	29	33	133	n/a

The % of multiple paternity (MP) and single paternity (SP) clutches each year is reported. The total columns include the mean % of MP and SP clutches and the total number of clutches examined for the combined data sets. The total number of nests sampled each year is reported with the estimated number of males that contributed to those nests. The number of males contributing each year does not sum to the total number of males because some males were represented in multiple years and redundant genotypes were removed.

multilocus probability of identity (2.4×10^{-6}), any two individuals with the same genotype were considered to be the same alligator. We had clutches from three seasons for two females and from two seasons for eight females for a total of 22 clutches. Two females consistently had SP clutches and two females consistently had MP clutches. The remaining six had both SP and MP clutches (Table 5) – with an SP clutch in the first year we sampled them and MP clutches in subsequent years. Additionally, for 14 clutches, the paternal genotype of the most likely father was the same genotype as the most likely father for at least one other clutch.

Of the 10 females examined, seven exhibited mate fidelity (Table 5). In six of the seven females exhibiting

Table 5 Pattern of mate fidelity in female American alligators at RWR

Female year-nest ID	Mate fidelity	Paternity	Primary male	Secondary male	Tertiary male
Female 1					
2000-S	Y	SP	Male 1*	—	—
2002-C		SP	Male 1*	—	—
2004-F		SP	Male 1*	—	—
Female 2					
1997-192B	Y	SP	Male 2	—	—
2002-M		SP	Male 2	—	—
2005-AA		MP	Male 2	Male 3	Male 4
Female 3					
2004-A	Y	SP	Male 5	—	—
2005-S		MP	Male 5	Male 6	Male 7
Female 4					
2003-Q	Y	SP	Male 8*	—	—
2004-R		SP	Male 8*	—	—
Female 5					
2002-A	N	SP	Male 9	—	—
2004-N		MP	Male 10	Male 11	—
Female 6					
2001-M	Y	MP	Male 12	Male 13	—
2003-F		MP	Male 13	Male 12	—
Female 7					
2001-BB	Y	SP	Male 8*	—	—
2004-Z		MP	Male 8*	Male 14	—
Female 8					
2001-R	N	SP	Male 15	—	—
2005-B		MP	Male 16	Male 17	—
Female 9					
1997-C	Y	MP	Male 1*	Male 18	—
2002-D		MP	Male 1*	Male 19	—
Female 10					
2001-W	N	SP	Male 20	—	—
2005-R		MP	Male 21	Male 22	—

Represented are females from which clutches were collected in more than 1 year.

MP, multiple paternity; SP, single paternity.

*Males that had paternity in more than one female's nest within a breeding season.

mate fidelity, the male she mates with more than one time is the primary male. Females 1 and 2 had mate fidelity across three different seasons. Female 1 had SP clutches in all 3 years and thus showed genetic monogamy with male 1. Female 2 had the same primary male in three different years, but one of her clutches was MP. In contrast, female six showed fidelity to two different males, but those males switched their primary and secondary male roles. From the male perspective, male 1 is the primary male for all three of female 1's clutches and both of female 9's clutches and was therefore the primary male of at least two clutches in 2002. Male 8 is the primary male for both female 4 and female 7's clutches. Two of these clutches were from 2004. Thus, males are successfully mating with multiple females within a single breeding season. We also had two clutches from the same nest area in two different years (2003, 2005) that did not have the same mother, but did have the same father, potentially indicating territory fidelity of males even if females change over time.

Discussion

We have demonstrated high levels of variation in MP among years at the level of both individual females and the overall population. Additionally, we have found the first evidence for partial mate fidelity in any crocodylian species. Together, these findings illustrate the complex nature of alligator mating behaviour.

Female alligators in the RWR population routinely lay multiply sired clutches of eggs. The percent of MP clutches in the population varies widely among years (40–67%). Overall, we found an average of 51% MP at RWR, which is substantially higher than the findings of 32% MP by Davis *et al.* (2001). Given the similar power to detect MP in both studies, the observed differences cannot be explained by choice of markers. Rather, it is clear that alligator mating behaviour can be highly variable among years and studying MP for only 1 or 2 years can yield an incomplete understanding of mating strategies. At this point, we do not have data to address why MP levels are so variable. However, in the coastal area of Louisiana, the number of alligator nests varies greatly from year to year (~20 000 in 2006 and ~43 000 in 2007; data courtesy of N.Kinler, Louisiana Department of Wildlife and Fisheries, Grand Chenier, LA, unpublished data). Water levels and conditions fluctuate and if fewer females are able to secure suitable nesting sites, the competition among males seeking a mating opportunity may increase and lead to higher MP. Based only on the findings of Davis *et al.* (2001), alligators have lower average incidence of MP than turtles (42%) or squamates (55%; Uller & Olsson 2008). However, by continuing the study for multiple years

and combining our data, we see that the average incidence of MP in alligators is similar to other reptiles, but is highly variable across seasons.

In their review, Uller & Olsson (2008) suggest that the amount of MP in reptiles should correlate with mate-encounter rates. For example, in the extremely dense mating populations of olive ridley sea turtles, levels of MP reach 90% as compared with 30% in low-density sites (Jensen *et al.* 2006). Alligators occur in high densities in much of coastal Louisiana, where there were an estimated 41 392 nests in 2005 (Elsey *et al.* 2008). As the RWR population of alligators is dense and females can move freely among male territories, mate-encounter rates are presumably high, providing both males and females with ample opportunity to mate multiply and to exercise mate choice. If intersexual conflict and high mate-encounter rates drive MP in alligators, then we would predict levels of MP to be reduced in populations with a lower population density than RWR. Supporting this idea, MP was found in only four of 16 nests (25%) in a low-density population (~4 nests per year along 53 km of shoreline) of alligators in South Carolina (Davis *et al.*, personal communication). If density is a driving factor in crocodylian mating systems, studies in additional populations of varying density would be informative.

Individual female alligators produce clutches with inconsistent patterns of paternity. Forty percent of the females had only SP or only MP clutches. Interestingly, all of the females that had both SP and MP clutches had their MP clutches in later years of the study. However, it is difficult to determine if the incidence of MP is correlated with female age (or experience) or with environmental conditions, as all of these changes are correlated over the course of the study. Although we did not find a significant difference in the size of females with MP and SP clutches, larger females are equally likely to have clutches with one, two or three sires, whereas smaller females are unlikely to have clutches with three sires. Given the strong positive relationship between female size and clutch size (Fig. 1), it would not be surprising for males to prefer large females. We do not know the specific ages of each female, but as individuals aged, they were more likely to have MP clutches. Although size and age are often presumed to be correlated in reptiles (Halliday & Verrell 1988), after an initial rapid growth period, there is no linear relationship between total length and age in alligators. Thus, MP in alligators could be correlated with age and not size. It is also possible that alligators have size-assortative mating, but we have no data on the males and therefore cannot address that possibility. Further studies will be needed to determine whether MP becomes less frequent as females age and to determine whether larger males mate with larger females.

Sexual conflict has been previously suggested to drive multiple mating in snakes (Prosser *et al.* 2002; Hosken & Stockley 2003; Shine *et al.* 2004). For example, in the western terrestrial garter snake, *Thamnophis elegans*, larger females produced larger litters and were more likely to mate multiply (Garner & Larsen 2005). Presumably, males prefer to mate with females that invest more in reproduction and intersexual conflict leads to MP. Similar results have been found in painted turtles, in which larger females produce larger clutches that are more likely to be multiply sired (Pearse *et al.* 2002). However, there is no relationship between either female size or clutch size and tendency to have MP clutches in the RWR alligator population.

We do not know whether female alligators with SP clutches only mated with one male, but on the basis of MP clutches we know that at least half of the females are mating multiply. Gist *et al.* (2008) recently demonstrated that wild female alligators could store sperm within a breeding season, but found no evidence that sperm can be stored from one reproductive season to the next. There is a single report of sperm storage across reproductive seasons in a captive Cuvier's Dwarf Caiman (Davenport 1995), but with the large number of crocodylians in captivity, it is remarkable that there have not been additional observations if such long-term sperm storage were common in crocodylians. Therefore, females with MP clutches are apparently mating with multiple males during a single season, resulting in a high potential for sperm competition. From this study and the study by Davis *et al.* (2001), we also know that males can achieve paternity in more than one female's nest. Thus, although dominant males would benefit by restricting other males' access to females, they can potentially benefit even more by courting additional females.

Our multiyear study allowed us to examine mate fidelity, which has not previously been studied in crocodylians. Over 70% of females sampled during our study displayed partial mate fidelity over multiple seasons. For example, female 1 was sampled in 2000, 2002 and 2004, and male 1 sired all of her offspring in the three clutches. However, females mating repeatedly with one particular male did not always mate only with that male, thus it is not a case of exclusive mate fidelity. In >90% of the MP clutches in our study, there is an obvious primary father and in females for which we have clutches for multiple years, the primary father is often consistent among years. For example, male 1 was the primary male for female 9's two clutches, but was not the sole sire for either clutch. We do not know if the primary male is determined by copulation frequency, sperm precedence and/or timing of copulation with ovulation, or sperm competition and/or selection. Dominance hierarchies exist among male alligators, and

future studies should examine whether the primary males are socially dominant. In captivity, females prefer dominant males, but will mate with subdominant males when the dominant male is with another female. Based on the assumption that females are not able to store sperm between mating seasons, RWR female alligators are mating with the same male across multiple breeding seasons.

Although some females produced offspring exclusively with specific males, those males sired offspring with additional females; therefore, alligators do not exclusively display genetic monogamy. Rather our data demonstrate that both males and females mate multiply, but apparently not randomly. There are several potential explanations for our finding of mate fidelity. Although highly unlikely, given the population density, it could be random chance that the same males and females mated over several seasons. However, a combination of several observations suggests that random mating is an unlikely explanation for our data. During the mating season, males maintain much larger home ranges (452–12 560 acres) and travel more on a daily basis than females (Joanen & McNease 1970, 1972). Our study site covers 2000 acres, thus most of the males in RWR can easily move through the entire area and could potentially encounter all breeding females. On average 100 females nest in the study area each year. We sampled a small portion of the nesting females each year and by examining the genotypes of associated putative sires, we estimated the number of males that successfully bred with our sampled females each year. On the basis of these data, we genotyped offspring from 133 different males over the course of the study, and an annual average of 1.6 males per clutch. If ~100 females and ~160 males are successfully mating in our study site each year and we only sample 10–20% of nests each year, the odds of sampling clutches resulting from the same mated pairs of alligators is incredibly small if mating is random. Although we have no direct observation or evidence of mate choice, it seems unlikely that the same pairs of alligators are mating together over long periods of time because of either random chance or lack of alternative mates.

Females can move through the territories of rival males and at RWR, there are high densities of reproductively mature males, providing individual females the opportunity to encounter multiple males during the breeding season. Also, we do not think that males are routinely forcing copulations because alligator courtship is highly stereotypical and females have been observed terminating courtship (Garrick & Lang 1977; Vliet 2001). On the basis of these combined observations, we conclude that the mating system in alligators at RWR is not characterized by a few dominant males monopolizing

matings. In addition, an important criterion for captive female alligators to reproduce successfully is acceptance of their mate (Joanen & McNease 1971). For example, a large (2.13 m) female killed two smaller (1.98 m, 1.83 m) males and then mated with a large wild male (3.05 m) that was caught and introduced to her pen. Similarly, captive females and males in pens separated by over 183 m have called to one another rather than court their pen-mates. When a female does not nest for several years, replacing the male in her pen can rapidly result in successful reproduction (Joanen & McNease 1971). One reason that cryptic female choice has been championed in reptiles is a general lack of precopulatory female choice (Olsson & Madsen 1995; Tokarz 1995). However, our data and observations from the literature suggest that there is potential for active choice by alligators and indicate that future studies designed to examine choice should be conducted. Future studies should include behavioural observations of both sexes and DNA analysis of males to determine whether females are consistently choosing dominant males. In addition, they should directly assess whether our finding of partial mate fidelity is because of consistent choice of mates or if both sexes display consistent choices of breeding areas resulting in repeated pairings.

Long-term mate fidelity has been well documented in birds (Black 1996), but is uncommon in reptiles (Bull 2000). Among the reptilian examples, long-term mate fidelity is associated with monogamy. Long-term mate fidelity has been documented in several species of the monophyletic *Egernia* group of lizards, of which most species display social monogamy (Bull 2000; Gardner *et al.* 2002; O'Connor & Shine 2003; Stow & Sunnucks 2004; Chapple & Keogh 2005). In one species of this group, the sleepy lizard (*Tiliqua rugosa*), males and females form monogamous pairs that can last up to 10 years (reviewed in Bull 2000). Painted turtles are the only other reptilian species that we are aware of, in which females lay clutches sired by the same male across multiple years. However, in this system, it has been assumed that females are using stored sperm and not mating with the same male in multiple years (Pearse *et al.* 2001). The alligator mating system is quite different from the above examples in that both males and females are mating multiply within a season, but with partial mate fidelity among seasons. This is not a case of social monogamy or sperm storage across breeding seasons, but of consistent mate pairings. Ideally, future studies could examine clutch size and hatchling success rates and attempt to determine whether a female is more likely to mate with the same male repeatedly, if her first nest with him is successful.

Crocodylians are the sole surviving reptilian archosaurs, a group of diapsids that includes dinosaurs and

other ancient reptiles that gave rise to birds (Brochu 2001). Thus, crocodylians are in a uniquely informative phylogenetic position to provide information about the ancestral mating systems of birds and many dinosaurs. In recent studies, MP was discovered in Morelet's crocodile (McVay *et al.* 2008), broad-snouted caiman (Amavet *et al.* 2008) and saltwater crocodiles (Lewis *et al.* personal communication). Studies of mating systems of other crocodiles are underway (e.g. the freshwater crocodile, *Crocodylus johnstoni*; N. FitzSimmons and T. Tucker, personal communication). Additional studies of crocodylians will provide a deeper understanding of how mating systems may contribute to the evolution of successful lineages and extinction.

Conclusions

We have conducted a multiyear mating system study on a dense population of alligators. Previous work (Davis *et al.* 2001) first documented MP in this species, yet found relatively low levels of MP in alligators at RWR, leading us to speculate that mate-encounter rates cannot explain MP in alligators and to suspect that precopulatory female choice is pervasive. The data reported here extend the study for additional years and document that MP is very common, but highly variable from year to year. Multiple paternity in alligators may be driven by selection on males to mate multiply coupled with a low cost to females. More studies in areas with lower density and lower mate-encounter rates will help elucidate the factors contributing to MP in alligators. By examining offspring from multiple years, we also found evidence for partial mate fidelity. Females were found to mate with the same male over several breeding seasons. Overall, both male and female alligators appear to mate multiply, but nonrandomly. There is much to be learned about alligator and crocodylian mating behaviour. Studies incorporating genotyping of males together with descriptions of male dominance hierarchies will assist in determining the role of female choice and male social status.

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References

- Amavet P, Rosso E, Markariani R, Pina CI, Saidman BO (2008) Microsatellite DNA markers applied to detection of multiple paternity in *Caiman latirostris* in Santa Fe, Argentina. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, **309 A**, 637–642.
- Arnold KE, Owens, IPF (2002) Extra-pair paternity and eggs dumping in birds: life history, parental care, and the risk of retaliation. *Proceedings of the Biological Society*, **269**, 1263–1269.
- Birkhead TR, Møller AP (1992) *Sperm Competition in Birds*. Academic Press, London.
- Birkhead TR, Møller AP (1995) Extra-pair copulation and extra-pair paternity in birds. *Animal Behaviour*, **49**, 843–848.
- Birkhead TR, Møller AP (1998) *Sperm Competition and Sexual Selection*. Academic Press, New York.
- Birkhead TR, Atkin L, Møller AP (1987) Copulation behavior of birds. *Behaviour*, **101**, 101–138.
- Black JM (1996) Introduction: pair bonds and partnerships. In: *Partnerships in Birds: The Study of Monogamy* (ed. Black JM), pp. 3–20. Oxford University Press, Oxford.
- Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, van der Noordaa J (1990) Rapid and simple methods for purification of nucleic acids. *Journal of Clinical Microbiology*, **28**, 495–503.
- Brochu CA (2001) Progress and future directions in archosaur phylogenetics. *Journal of Paleontology*, **75**, 1185–1201.
- Bull CM (2000) Monogamy in lizards. *Behavioral Processes*, **51**, 7–20.
- Chapple DG, Keogh JS (2005) Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. *Molecular Ecology*, **14**, 1215–1227.
- Davenport M (1995) Evidence of possible sperm storage in the caiman, *Paleosuchus palpebrosus*. *Herpetological Review*, **26**, 14–15.

- Davis L, Glenn T, Elsey RM, Dessauer HC, Sawyer RH (2001) Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. *Molecular Ecology*, **10**, 1011–1014.
- DeWoody JA, Schupp J, Kenefic L, Busch J, Murfitt L, Keim P (2004) Universal method for producing ROX-labeled size standards suitable for automated genotyping. *BioTechniques*, **37**, 348–352.
- Eizaguirre C, Laloi D, Massot M, Richard M, Federici P, Clobert J (2007) Condition dependence of reproductive strategy and the benefits of polyandry in a viviparous lizard. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **274**, 425–430.
- Elsey RM, Trosclair PL III, Glenn TC (2008) Nest-site fidelity in American alligators in a Louisiana coastal marsh. *Southeastern Naturalist*, **7**, 737–743.
- Fitze PS, Le Galliard JF (2008) Operational sex ratio, sexual conflict and the intensity of sexual selection. *Ecology Letters*, **11**, 432–439.
- 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**, 2451–2459.
- Gardner MG, Bull CM, Cooper SJB, Duffield GA (2002) High levels of genetic monogamy in the social Australian lizard *Egernia stokesii*. *Molecular Ecology*, **11**, 1787–1794.
- Garner TWJ, Larsen KW (2005) Multiple paternity in the western terrestrial garter snake, *Thamnophis elegans*. *Canadian Journal of Zoology*, **83**, 656–663.
- Garner TWJ, Gregory PT, McCracken GF *et al.* (2002) Geographic variation of multiple paternity in the common garter snake (*Thamnophis sirtalis*). *Copeia*, **2002**(1), 15–23.
- Garrick L, Lang JW (1977) Social signals and behavior of adult alligators and crocodiles. *American Zoologist*, **17**, 225–239.
- Gist DH, Bagwill A, Lance V, Sever DM, Elsey RM (2008) Sperm storage in the oviduct of the American alligator. *Journal of Experimental Zoology*, **309A**, 581–587.
- Glenn TC, Dessauer HC, Braun MJ (1998) Characterization of microsatellite DNA loci in American alligators. *Copeia*, **3**, 591–601.
- Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11**, 2195–2212.
- Halliday TR, Verrell PA (1988) Body size and age in amphibians and reptiles. *Journal of Herpetology*, **22**, 253–265.
- Hosken DJ, Stockley P (2003) Benefits of polyandry: a life history perspective. *Evolutionary Biology*, **33**, 173–194.
- Höss M (1994) More about the silica method. *Ancient DNA Newsletter*, **2**, 10–12.
- Höss M, Paabo S (1993) DNA extraction from Pleistocene bones by silica-based purification method. *Nucleic Acids Research*, **21**, 3913–3914.
- Hunt RH (1987) Nest excavation and neonate transport in wild *Alligator mississippiensis*. *Journal of Herpetology*, **21**, 348–350.
- Hunt RH, Watanabe ME (1982) Observations on maternal behavior of the American alligator, *Alligator mississippiensis*. *Journal of Herpetology*, **16**, 235–239.
- Jensen MP, Abreu-Grobois FA, Frydenberg J, Loeschcke V (2006) Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. *Molecular Ecology*, **15**, 2567–2575.
- Joanen T, McNease L (1969) Nesting ecology of alligators in Louisiana. *Proceedings of the 23rd Annual Conference of the Southeastern Association of Game and Fish Commissioners*, **23**, 141–151.
- Joanen T, McNease L (1970) A telemetric study of nesting female alligators on Rockefeller Wildlife Refuge, Louisiana. *Proceedings of the 24th Annual Conference of the Southeastern Association of Game and Fish Commissioners*, **24**, 175–193.
- Joanen T, McNease L (1971) Propagation of the American alligator in captivity. *Proceedings of the 25th Annual Conference of the Southeastern Association of Game and Fish Commissioners*, **25**, 106–116.
- Joanen T, McNease L (1972) A telemetric study of adult male alligators on Rockefeller Refuge, Louisiana. *Proceedings of the 26th Annual Conference of the Southeastern Association of Game and Fish Commissioners*, **26**, 252–275.
- Joanen T, McNease L (1987) The management of alligators in Louisiana. In: *Wildlife Management: Crocodiles and Alligator* (eds Webb GJW, Manolis SC, Whitehead PJ), pp. 33–42. Surrey Beatty and Sons, Chipping Norton, NSW, Australia.
- Joanen T, McNease L (1989) Ecology and physiology of nesting and early development of the American alligator. *American Zoologist*, **29**, 987–998.
- Jones AG (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Molecular Ecology Resources*, **5**, 708–711.
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.
- Kempnaers B, Verheyen GR, Dhondt AA (1997) Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. *Behavioral Ecology*, **8**, 481–492.
- Krokene C, Rigstad K, Dale M, Lifjeld JT (1998) The function of extrapair paternity in blue tits and great tits: good genes or fertility insurance? *Behavioural Ecology*, **9**, 649–656.
- Kushlan JA, Simon JC (1981) Egg manipulation by the American alligator. *Journal of Herpetology*, **15**, 451–454.
- McCracken GF, Burghardt GM, Houts SE (1999) Microsatellite markers and multiple paternity in the garter snake *Thamnophis sirtalis*. *Molecular Ecology*, **8**, 1475–1479.
- McVay J, Rodriguez D, Rainwater T *et al.* (2008) Evidence of multiple paternity in Morelet's Crocodile (*Crocodylus moreletii*) in Belize, CA, inferred from microsatellite markers. *Journal of Experimental Zoology, Part A: Ecological Genetics and Physiology*, **309A**, 643–648.
- Møller AP, Cuervo JJ (2000) The evolution of paternity and paternal care. *Behavioral Ecology*, **11**, 472–485.
- O'Connor D, Shine R (2003) Lizards in 'nuclear families': a novel reptilian social system in *Egernia saxatilis* (Scincidae). *Molecular Ecology*, **12**, 743–752.
- Olsson M, Madsen T (1995) Female choice on male quantitative traits in lizards – why is it so rare? *Behavioral Ecology and Sociobiology*, **36**, 179–184.
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

- Pearse DE, Janzen FJ, Avise JC (2001) Genetic markers substantiate long-term storage and utilization of sperm by female painted turtles. *Heredity*, **86**, 378–384.
- Pearse DE, Janzen FJ, Avise JC (2002) Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. *Behavioral Ecology and Sociobiology*, **51**, 164–171.
- Petrie M, Kempenaers B (1998) Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology and Evolution*, **13**, 52–58.
- Prosser MR, Weatherhead PJ, Gibbs HL, Brown GP (2002) Genetic analysis of the mating system and opportunity for sexual selection in northern water snakes (*Nerodia sipedon*). *Behavioral Ecology*, **13**, 800–807.
- Pruett-Jones SG, Pruett-Jones MA (1990) Sexual selection through female choice in Lawes' Parotia, a lek-mating bird of paradise. *Evolution*, **44**, 486–501.
- Rintamäki PT, Alatalo RV, Höglund J, Lundberg A (1995) Mate sampling behaviour of black grouse females (*Tetrao tetrix*). *Behavioral Ecology and Sociobiology*, **37**, 209–215.
- Sæther SA, Baglo R, Fiske P, Ekblom R, Höglund J, Kålås JA (2005) Direct and indirect mate choice on leks. *American Naturalist*, **166**, 145–157.
- Shine R, Phillips B, Langkilde T, Lutterschmidt DI, Wayne H, Mason RT (2004) Mechanisms and consequences of sexual conflict in garter snakes (*Thamnophis sirtalis*, Colubridae). *Behavioral Ecology*, **15**, 654–660.
- Stow AJ, Sunnucks P (2004) High mate and site fidelity in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology*, **13**, 419–430.
- Tokarz R (1995) Mate choice in lizards: a review. *Herpetological Monographs*, **9**, 17–40.
- Trail PW, Adams ES (1989) Active mate choice at cock-of-the-rock leks: tactics of sampling and comparison. *Behavioral Ecology and Sociobiology*, **25**, 283–292.
- Tullberg BS, Ah-King M, Temrin H (2002) Phylogenetic reconstruction of parental-care systems in the ancestors of birds. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, **357**, 251–257.
- Uller T, Olsson M (2008) Multiple paternity in reptiles: patterns and processes. *Molecular Ecology*, **17**, 2566–2580.
- Vliet KA (2001) Courtship behavior of American alligators *Alligator mississippiensis*. In: *Crocodylian Biology and Evolution* (eds Grigg GC, Seebacher F, Franklin CE), pp. 383–408. Surrey Beatty and Sons, Chipping Norton, New South Wales, Australia.
- Westneat DF, Mays HL Jr (2005) Tests of spatial and temporal factors influencing extra-pair paternity in red-winged blackbirds. *Molecular Ecology*, **14**, 2155–2167.
- Westneat DF, Sherman PW (1997) Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioral Ecology and Sociobiology*, **41**, 205–215.
- Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology and Systematics*, **34**, 365–396.
- Westneat DF, Sherman PW, Morton ML (1990) The ecology and evolution of extra-pair copulations in birds. In: *Current Ornithology* (ed. Power DM), pp. 331–369. Plenum Press, New York.
- Whittingham LA, Dunn PO, Stapleton MK (2006) Repeatability of extra-pair mating in tree swallows. *Molecular Ecology*, **15**, 841–849.

S.L.L. studies local scale processes such as breeding strategies, survivorship, and dispersal and their combined effects on the maintenance and distribution of genetic diversity within and among populations. In addition, she examines the effect of environmental contaminant exposure on the interplay of behavior, demography, and population genetics. T.D.T. studies the conservation and management of reptile and amphibian populations with an emphasis on translocations and reintroductions. She also is interested in using genetics and long-term mark-recapture data as tools to better understand the ecology and life history of reptiles and amphibians. R.M.E. and P.L.T. study alligator ecology, physiology, nesting behavior, harvest management, egg ranching, and captive husbandry. In addition they both are very interested in waterfowl management. T.C.G. develops and uses DNA technologies to make discoveries and solve problems in ecology, evolution, and environmental health. Massively parallel sequencing technologies and non-traditional biomedical model organisms are current areas of emphasis. L.D. is interested in the application of genetic analyses to conservation practice. In particular she examines the conservation genetics of captive and wild red pandas and the molecular phylogeny of the orchid genus *Spiranthes* with implications for endangered taxa.

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