

581

More sires may enhance offspring fitness in Northern Map Turtles (Graptemys geographica)

Nicola Banger, Gabriel Blouin-Demers, Grégory Bulté, and Stephen C. Lougheed

Abstract: Sexual selection theory predicts that males should be promiscuous to maximize their reproductive success, while females should be choosy. Yet females of many taxa often produce progeny sired by multiple males, indicating that promiscuity can be important for the reproductive success of females. Promiscuity may enhance the fitness of females if it increases the genetic quality, or the genetic variety, and thus the viability of their offspring. We quantified the number of sires per clutch in a population of Northern Map Turtles (Graptemys geographica (LeSueur, 1817)) in Lake Opinicon, Ontario, Canada, and tested whether the number of sires affects several metrics of viability in hatchlings. Based on the most conservative estimate, at least 71% of clutches in this population are sired by multiple males, but there was no evidence that larger clutches are sired by more males. Clutches sired by more males had higher hatching success and survival, but the differences were not statistically significant. We did not find any effect of the number of sires on hatchling morphology or locomotor performance. Collectively, our results partially support the hypothesis that promiscuity can increase the reproductive success of female Northern Map Turtles

Key words: multiple paternity, reptiles, genetic benefits, polyandry, promiscuity, Northern Map Turtle, Graptemys geographica.

Résumé : La théorie de la sélection sexuelle prédit que les mâles devraient faire preuve de promiscuité afin de maximiser leur succès de reproduction, alors que les femelles devraient être sélectives. Pourtant, les femelles de nombreux taxons produisent souvent une progéniture issue de mâles multiples, ce qui indique que la promiscuité peut également être importante pour le succès de reproduction des femelles. Elle peut en effet accroître l'aptitude phénotypique des femelles si elle accroît la qualité génétique ou la variété génétique et donc la viabilité de leur progéniture. Nous avons quantifié le nombre de géniteurs par couvée dans une population de tortues géographiques (Graptemys geographica (LeSueur, 1817)) du lac Opinicon (Ontario, Canada) et vérifié si ce nombre avait une incidence sur différentes mesures de la viabilité des bébés tortues. Selon l'estimation la plus prudente, au moins 71 % des nichées dans cette population sont issues de mâles multiples, bien que rien n'indique que les couvées plus imposantes soient issues d'un plus grand nombre de mâles. Les couvées issues d'un plus grand nombre de mâles présentaient un succès d'éclosion et un taux de survie plus élevés, mais ces différences n'étaient pas statistiquement significatives. Nous n'avons noté aucun effet du nombre de géniteurs sur la morphologie ou la performance locomotrice des bébés tortues. Collectivement, nos résultats appuient partiellement l'hypothèse voulant que la promiscuité puisse accroître le succès de reproduction des tortues géographiques femelles. [Traduit par la Rédaction]

Mots-clés : paternité multiple, reptiles, avantages génétiques, polyandrie, promiscuité, tortue géographique, Graptemys geographica.

Introduction

The traditional view of animal mating systems is that males are promiscuous, while females are choosy. In most species, male reproductive success increases in proportion to the number of mating partners obtained, while female reproductive success is limited by how many eggs she can produce (Bateman 1948; but see Snyder and Gowaty 2007). Because a single insemination can typically deliver vastly more sperm than are needed to fertilize all a female's eggs (Trivers 1972), this dichotomy favours males to mate promiscuously and indiscriminately, and females to mate selectively.

Polygynous mating systems, in which males mate with multiple females, are well understood, and their prevalence in almost all taxa accords with evolutionary theory (Andersson 1994). Polyandrous mating systems, in which females mate with multiple males, are harder to understand, and their existence in nature is puzzling. Indeed, mating itself can be costly to females. The

potential cost of mating includes the risk of injury or death (Le Boeuf and Mesnick 1991), exposure to bacteria and other pathogens (Loehle 1997; Westneat and Rambo 2000), and loss of time and energy (Watson et al. 1998). Given these costs, it is often predicted that females should not benefit from multiple mating. and should instead carefully choose their mate. Nonetheless, contrary to expectations, evidence accumulated over the past few decades shows that female promiscuity seems to be the rule, rather than the exception. Mating with multiple males within a single reproductive season has been observed among females of many taxa, including amphibians (Liebgold et al. 2006), reptiles (Madsen et al. 1992; Olsson and Shine 1997; Calsbeek et al. 2007), birds (Foerster et al. 2003), and mammals (Hrdy 1979; Hoogland 1998), and it has been shown that multiple paternity within broods is common (Jennions and Petrie 2000). Although natural selection for multiple mating by males is easily explicable, the selective advantages of multiple mating by females remain poorly understood.

Received 3 December 2012. Accepted 4 June 2013

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| Table 1. | Frequency | of multiple | paternity in | ı freshwater and | marine turtles. |
|----------|-----------|-------------|--------------|------------------|-----------------|
|----------|-----------|-------------|--------------|------------------|-----------------|

| Species | Technique | No. of loci | No. of clutches | Frequency of multiple paternity (%) | Reference |
|--|-----------|----------------|--------------------|---|-------------------------|
| Freshwater turtles | | | | | |
| Painted Turtle, Chrysemys picta | Μ | 2 or 3 | 227 | 30 | Pearse et al. 2001 |
| Blanding's Turtle, <i>Emys blandingii</i> (Holbrook, 1838) = <i>Emydoidea blandingii</i> (Holbrook, 1838) | М | 4 | 16 | 56 | Refsnider 2009 |
| Wood Turtle, <i>Clemmys insculpta</i> (LeConte, 1830) = <i>Glyptemys insculpta</i> (LeConte, 1830) | F | na | 10 | 50 | Galbraith 1991 |
| Chrysemys picta | Μ | 3 | 20 | 0 | McTaggart 2000 |
| Marine turtles | | | | | |
| Kemp's Ridley Sea Turtle, Lepidochelys kempii (Garman, 1880) | М | 3 | 26 | 58 | Kichler et al. 1999 |
| Loggerhead Sea Turtle, Caretta caretta | М | 4 | 21 | 95 | Zbinden et al. 2007 |
| Green Sea Turtle, Chelonia mydas | М | 5 | 18 | 61 | Lee and Hays 2004 |
| Olive Ridley Sea Turtle, Lepidochelys olivacea (Eschscholtz, 1829) | М | 2 | 10 | 20 | Hoekert et al. 2002 |
| Chelonia mydas | Μ | 5 | 22 | 1 | Fitzsimmons 1998 |
| Flatback Sea Turtle, Natator depressus (Garman, 1880) | М | 4 | 16 | 69 | Theissinger et al. 2009 |

Note: Genetic markers include microsatellites (M) or DNA fingerprinting (F) techniques. na, not available.

To account for the observed high rates of female promiscuity, promiscuous females should gain benefits that are inaccessible to monogamous females (excluding instances in which mating is forced). Possible benefits to females from multiple mating fall into two broad categories: the direct or material benefit hypothesis suggests that females mate multiply to acquire resources such as food or sperm that directly enhance their fitness, whereas the indirect or genetic benefits hypothesis supposes that females gain good genes for their offspring, improving their fitness through increased offspring viability (Zeh and Zeh 2001). Direct benefits to females accrue with the number of copulation events, either with a single male or with multiple males. When a male has mated with a female, he has an increased stake in the paternity of her offspring, and thus is more likely to invest in that female. By engaging in multiple mating, females of many species can improve their own fitness directly through added male investment, for instance through the acquisition of nuptial gifts (Lamunyon 1997; Karlsson 1998), increased paternal investment (Stacey 1982), the avoidance of infanticide (Hrdy 1979), or the assurance of insemination (Pai et al. 2005).

Although convincing evidence supports the many direct benefits of polyandry, the indirect benefits of multiple mating remain controversial. There are several mechanisms by which multiple mating can indirectly enhance a female's fitness through the acquisition of good genes for her offspring: by way of genetic trade up, higher genetic diversity of sperm, or postcopulatory sperm selection for superior or more compatible genes (Jennions and Petrie 2000). Alternatively, females may mate multiply to "hedge their bets" with different alleles, improving the average fitness of each generation of offspring (Fox and Rauter 2003). Finally, because male genetic quality may sometimes be difficult to assess, females may mate multiply to "hedge their bets" with regards to sire genetic quality.

The Northern Map Turtle (*Graptemys geographica* (LeSueur, 1817)) is a good species to study the genetic benefits of female promiscuity. As in other reptiles, female map turtles receive no nuptial gifts or parental care from males, so if female map turtles mate with more than one male, they should derive genetic benefits for their offspring, unless there are strong sexually antagonistic genes that would favour paternal fitness to the detriment of maternal fitness (Pischedda and Chippindale 2006). Females of many turtle species mate multiply (Table 1), and map turtles have large enough clutches to detect multiple sires (between 2 and 18 eggs; Bulté et al. 2008*a*). Because female map turtles are so much larger than males (Bulté et al. 2008*b*) and because males do not appear to be aggressive (according to our observations of mating in nature; G. Bulté, personal observation), multiple mating by female map turtles is not likely a side effect of male harassment. We must, however, mention two important potential confounding factors of any field study of the apparent benefits of female promiscuity. First, females could use the sperm of only one of her multiple suitors to fertilize her ova (cryptic female choice: Olsson et al. 1996). Second, in an extreme case of good genes, a single highquality male could sire all offspring despite multiple mating by the female. In both cases, the relationship between multiple mating and the number of genetic sires becomes obscured. This is why some researchers experimentally assign potential mates to females (Fisher et al. 2006), but this comes with its own potential issues such as unnaturally high density of mates, arbitrary assignment of mates to females, or the inclusion of inferior mates that would not have had a chance of mating in a natural setting.

Our first objective is to examine the genetic mating system of map turtles because little is known about mating in this species. Courtship and mating have been observed during spring and autumn when turtles aggregate at communal hibernacula (Vogt 1980) and the chance of encountering a potential mate is high. We use microsatellite genotypes to determine the incidence of multiple paternity in an Eastern Ontario population of map turtles.

The second objective of this study is to investigate the role of sexual selection in map turtle mating system. In polygynous mating systems, it is common for males to mate promiscuously and for females to mate selectively. Here, we consider whether in polyandrous mating systems, males are more selective about mates when females are mating promiscuously. Although sperm are relatively cheap to produce compared with eggs, various studies have shown that the cost of sperm production cannot be completely discounted; significant costs to males associated with spermatogenesis have been documented in many species (e.g., European Adders (Vipera berus (L., 1758)): Olsson et al. 1997; nematodes: Van Voorhies 1992; sheep: Preston et al. 2001). Moreover, spermatogenesis in freshwater turtles is episodic, occurring once annually in the summer (Licht 1984), with sperm stored in the male epididymis for up to a year (Gist et al. 2002). Assuming that the cost of sperm production is not negligible and that the quantity of sperm is fixed, the reproductive success of male map turtles would benefit from judicious allocation of sperm among receptive females. Furthermore, the size of offspring turtles is directly constrained by female body size (Congdon and Gibbons 1987; Ryan and Lindeman 2007), and body size is important for survival in hatchling turtles (Janzen et al. 2000). Large females, therefore, have a higher potential reproductive output and success (Bulté et al. 2008a). If sperm is indeed limited, then males should mate selectively with large females, and there should be more sires within the clutches of larger females. We test whether there is a

relationship between female body size and number of sires within clutches.

Our final objective is to investigate the role of genetic benefits in map turtle mating systems. We test the prediction that female fitness should increase with the number of sires in the clutch. We look at three aspects of female fitness: clutch survival, hatchling morphology, and hatchling locomotor performance.

Materials and methods

Study species and study site

We collected 37 gravid female Northern Map Turtles between May and June 2010 in Lake Opinicon (44°34'N, 76°19'W) at the Queen's University Biological Station (QUBS) approximately 100 km southwest of Ottawa, Ontario, Canada. Animals were cared for in accordance with the *Guide to the Care and Use of Experimental Animals* of the Canadian Council on Animal Care. We captured all females by hand at the same nesting site on a small island during peak nesting times. Using a forestry caliper, we measured plastron length to the nearest 1 mm, and with an electronic scale, we measured mass to the nearest 1 g. We marked females individually by drilling unique combinations of small holes in their marginal scutes. We took a small (2–3 mm²) clipping of skin from the lateral edge of one of the forefeet of each female using flamesterilized surgical scissors and preserved it in 70% ethanol for use in genetic parentage analyses.

Induction of oviposition and care of eggs

We housed all gravid females individually in clean plastic containers (120 cm \times 80 cm \times 50 cm) shallowly filled with lake water at ambient temperature. We induced oviposition in females by injecting them intramuscularly in the hind leg with oxytocin (20 IU/mL, 0.5 mL/kg gravid body mass; Ewert and Legler 1978). We removed all eggs and recorded their individual masses as they were laid. We grouped eggs by clutch in plastic containers (15 cm \times 15 cm \times 6 cm) with perforated lids and half-buried them in moist vermiculite (1:1 ratio by mass of water and vermiculite).

We incubated the eggs at 29 °C until hatching, a temperature within the natural range for nests. We weighed the containers with the eggs and vermiculite at the onset of incubation; to account for evaporation, we added sufficient water every 3 days to maintain a constant total mass. During these manipulations, we shuffled the position of the containers within the incubator to minimize potential position effects.

Hatchling collection and phenotypic measurements

Eggs (N = 418) began hatching after 55 days of incubation. The mean clutch size was 11 eggs (range 6–14); eggs averaged 11.8 g (range 7.7–16.0 g) and entire clutches averaged 133.9 g (range 73.4–190.4 g). As they emerged, we transferred each hatchling to a plastic pill bottle (12 cm × 5 cm diameter) containing moist vermiculite (2:1 by mass with water; Costanzo et al. 2000) to be housed individually in the incubators until each had been marked. We took small blood samples (30–50 μ L) from all emerged hatchlings (N = 242) from the coccygeal vein using a 0.5 mL insulin syringe fitted with a 28 gauge (1 cm) needle (Bulté et al. 2006) and stored samples in 70% ethanol at 4 °C.

We marked all hatchlings individually using nontoxic waterbased permanent paint, and placed them in randomly selected groups of 10 in clean plastic containers (30 cm × 20 cm × 10 cm) shallowly filled with damp sand to be housed over winter in an darkened environmental chamber (12 h light : 12 h dark) and denied them access to food and free water, following Costanzo et al. (2000). We lowered the temperature of the chambers gradually from 29 to 20 °C in September, to 15 °C in October, to 10 °C in November, and down to 4 °C in December 2010. The temperature was then held constant at 4 °C during the winter. We released all hatchlings in late April 2011 in Lake Opinicon.

Table 2. Characteristics of six microsatellite loci used to infer paternity in Northern Map Turtles (*Graptemys geographica*) in Lake Opinicon, Ontario, Canada.

| Locus | Ν | k | Ho | $H_{\rm E}$ | PE |
|----------|----|----|-------|-------------|-------|
| GmuB08 | 53 | 4 | 0.717 | 0.680 | 0.388 |
| GmuA18 | 53 | 7 | 0.264 | 0.547 | 0.311 |
| GmuD87 | 51 | 4 | 0.549 | 0.586 | 0.292 |
| TerpSH7 | 55 | 5 | 0.818 | 0.688 | 0.438 |
| GmuD51 | 36 | 12 | 0.972 | 0.813 | 0.637 |
| GmuD90 | 55 | 6 | 0.800 | 0.586 | 0.296 |
| All loci | | | | | 0.957 |

Note: *k*, the number of alleles detected; *N*, the number of individuals genotyped for each locus; H_0 , observed heterozygosity; H_F , expected heterozygosity; PE, probability of exclusion.

DNA extraction, PCR, and microsatellite loci

We extracted DNA from the tissue samples of each mother and her offspring using a spin-column DNA extraction protocol modified from a glass-fiber protocol (Ivanova et al. 2006). We were unable to extract good-quality DNA from unhatched eggs to successfully run genetic analyses. Because we were much less likely to detect multiple paternity in clutches with very few offspring, we elected to analyze only clutches in which a minimum of eight hatchlings successfully emerged; this number optimized both the number of clutches available for analysis and the likelihood of detecting multiple paternity. Because of low hatching success, only 14 clutches met this criterion. To ensure that the likelihood of detecting multiple paternity for all 14 clutches was the same, we randomly selected eight offspring from each clutch for genotyping.

For paternity analysis, we used polymerase chain reactions (PCR) to amplify six microsatellite loci previously characterized for use in closely related species: *GmuA18*, *GmuB08*, *GmuD87*, *GmuD51*, *GmuD90* (King and Julian 2004), and *TerpSH7* (Hauswaldt and Glenn 2003). We carried out all PCR reactions in 10 μ L volumes in 200 μ L microtubes. Details of the PCR cycling parameters and reagent concentrations can be found in Banger (2012). All PCR products were separated on a Beckman Coulter CEQ8000 capillary automated sequencer and sized using the CEQTM 8000 Genetic Analysis System.

We also obtained multilocus genotypes for 23 females caught in 2010 and 42 adult males and females caught in 2006 as part of another study (Bulté et al. 2008*a*) to provide an estimate of allele frequencies of the population to be used in our paternity analyses. We assessed population allele frequencies of each locus using the program CERVUS version 3.0 (Kalinowski et al. 2007). There was no significant deviation from Hardy–Weinberg equilibrium at four out of the six loci. The combined probability of exclusion (given a known maternal genotype) across all loci was 0.957, the mean expected heterozygosity across all loci was 0.68 (Table 2).

Paternity analysis

We used three approaches to assess paternity. We counted alleles at each locus to estimate the minimum number of fathers for each clutch. We determined maternal genotypes directly and then deduced paternal alleles from offspring genotypes by subtracting the known maternal alleles. We assumed that all fathers were heterozygous for each of the six loci as a conservative estimate of the number of contributing sires. We inferred the minimum number of fathers to be the smallest whole number greater than or equal to one half the number of deduced paternal alleles. For cases in which an offspring had the same heterozygous genotype as its mother, we counted the heterozygous pairing of alleles to be a single allelic contribution, and only counted this pair if neither of the contributing alleles was detected as a paternal allele in any other offspring from that clutch. As a conservative estimate (to account for mutations that may lead to an overestimation of the number of paternal alleles), we inferred multiple sires only when more than two paternal alleles were detected at more than one locus. We also used the programs DADSHARE version 4 (available from http://www.zoo.cam.ac.uk/zoostaff/meg/amos.htm, accessed 15 February 2011) and GERUD2.0 (Jones 2005) to estimate the number of sires, but because the results were qualitatively similar (having more sires appear to increase hatching success and survival), we only present the statistical analyses based on the more conservative allele counting.

Clutch survival

We measured hatching success as the percentage of hatchlings that successfully emerged out of the total number of eggs laid in each clutch. To measure first-year survival of each clutch, we recorded the proportion of offspring from each clutch that survived the first winter.

Hatchling morphology

We measured hatchling plastron length to the nearest 0.1 mm with digital calipers and body mass to the nearest 0.01 g with an electronic scale within a week of hatching. We estimated body condition (BC) of hatchlings as the residuals of an ordinary least square regression with log_{10} (plastron length) as the independent variable and log_{10} (mass) as the dependent variable (Jakob et al. 1996). We took photographs of the carapace of each turtle and used them to record the numbers of supranormal scutes. We calculated the mean plastron length, body condition, and number of scute anomalies for hatchlings in each clutch, and used mean clutch values to compare clutches in analyses.

Hatchling performance

In spring, we recorded swimming speed. We placed hatchings individually in a 2 m long plastic trough filled with water at 22 °C and encouraged them to swim at maximum speed by gently tapping their tails. We recorded the length of time it took each hatchling to swim at its maximum speed for 1 m; we measured speed in this way four times for each hatchling, once a day for 4 days. We used the fastest trial from each hatchling in analyses.

In spring, we also measured the righting response of the hatchlings. We placed hatchlings on a flat surface lined with felt and turned them upside-down and recorded the latency period (i.e., the length of time until the hatchling's first attempt at righting itself) and the righting response (i.e., the length of time from the hatchling's first righting attempt to successful righting). We took two measures of righting response for each hatchling, the second trial 1 day following the first trial. We used the fastest trial of each measure for our analyses. We calculated the mean swimming speed, latency period, and righting response from hatchlings of each clutch, and used mean clutch values in the analyses.

Statistical analyses

We analyzed the relationship between female size and minimum number of sires using ordinal logistic regression, with the minimum number of sires responsible for a clutch as the dependent variable and female plastron length as the independent variable. We ran one MANOVA for survival (with hatching success and first-year survival as dependent variables), one MANOVA for morphology (with mean plastron length, mean body condition, and mean scute anomalies as dependent variables), and one MANOVA for performance (with mean swimming speed, mean latency period, and mean righting response as dependent variables), testing the prediction that each measure of fitness would be affected by the minimum number of sires detected in each clutch. We inspected distributions and residual plots to verify assumptions of normality and homogeneity of variance. For all statistical analyses, we used JMP version 10 (SAS Institute Inc., Cary, North **Fig. 1.** Number of sires per clutch estimated via allele counting, program DADSHARE, and program GERUD, indicating that most clutches are multiply sired in Northern Map Turtles (*Graptemys geographica*) in Lake Opinicon, Ontario, Canada.



Carolina, USA). We considered statistical results to be significant at P < 0.05.

Results

Based on allele counting, we detected a single sire in 4 out of the 14 clutches (28.6%), at least two sires in 8 clutches (57.1%), and at least three sires in the remaining 2 clutches (14.3%) (Fig. 1). This indicated that 71.4% of clutches were multiply sired. Multiple paternity was supported by at least three paternal alleles at two loci for 10 females and at three loci for 6 females, indicating that mutation is unlikely to explain many of the multiple paternal alleles observed in this study. We obtained comparable results when using the programs DADSHARE and GERUD, except that the analyses of paternity with GERUD indicated that all clutches were multiply sired. (Fig. 1). The number of sires did not increase with female plastron length (ordinal logistic regression: Wald $\chi^2 = 0.097$, N = 14, P = 0.76; Fig. 2).

Hatching success and first-year survival tended to increase with the minimum number of sires, but this was not statistically significant (MANOVA: Wilks' $\lambda = 0.448$, $F_{[2,11]} = 2.47$, P = 0.077). Clutches sired by at least three males tended to have a higher percentage of hatchlings emerge (96.7%) than did clutches sired by one (81.8%) or two (82.9%) males, but this difference was again not statistically significant (ANOVA: $R^2 = 0.369$, $F_{[2,11]} = 3.22$, P = 0.080). Clutches sired by at least three males tended to have higher off-spring survival (86.8%) than clutches sired by only one (57.7%) or two (68.8%) males, but this difference was again not statistically significant (ANOVA: $R^2 = 0.402$, $F_{[2,11]} = 3.69$, P = 0.059; Figs. 3A–3B).

There was no significant relationship between the number of sires and hatchling morphology (MANOVA: Wilks' $\lambda = 0.586$, $F_{[2,11]} = 0.919$, P = 0.504; Figs. 4A–4C) or hatchling performance (MANOVA: Wilks' $\lambda = 0.796$, $F_{[2,11]} = 0.362$, P = 0.894; Figs. 5A–5C). Repeating these analyses using clutch size as a covariate gave qualitatively the same results.

Discussion

Based on the multilocus genotypes from the six microsatellite loci used, at least 71% of the clutches were sired by multiple males.

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Minimum number of sires

Fig. 2. The number of sires does not increase as a function of female size in Northern Map Turtles (*Graptemys geographica*) in Lake Opinicon, Ontario, Canada.

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230

Female plastron length (mm)

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240

250

260

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220

Although multiple paternity is common in many species of turtles that have been evaluated (Pearse and Avise 2001), this is the first study in which multiple paternity has been found in *G. geographica*, and this is one of the highest frequencies of multiple paternity documented in turtles (Table 1). Notably, the minimum number of sires is likely to be underestimated because not all offspring from each clutch were analyzed and different males may have contributed the same allele to certain offspring in a clutch; thus, some males may have gone undetected in analyses of minimum number of sires. The analyses of paternity using GERUD indicated that all clutches were multiply sired.

Contrary to our prediction, we found no correlation between female body size and number of sires, suggesting that male map turtles may not mate selectively with larger females, despite their higher fecundity, or that we lacked the power to detect a correlation. This is similar to what Blouin-Demers et al. (2005) found in Black Ratsnakes (Elaphe obsoleta (Say in James, 1823)). In contrast, Zbinden et al. (2007) found a positive relationship between the number of sires and female size in Loggerhead Sea Turtles (Caretta caretta (L., 1758)), and Lee and Hays (2004) found that females laying multiply sired clutches were larger than those laying singly sired clutches, although their results were not statistically significant. Similar evidence of male preference for larger females has been documented in many species of fish and other species of reptiles in which female fecundity is related to size, including Painted Turtles (Chrysemys picta (Schneider, 1783)) (Pearse et al. 2002), Terrestrial Gartersnakes (Thamnophis elegans (Baird and Girard, 1853)) (Garner and Larsen 2005), Sand Lizards (Lacerta agilis L., 1758) (Olsson 1993), guppies (Poecilia reticulata Peters, 1859) (Dosen and Montgomerie 2004), and redlip blennies (Ophioblennius atlanticus (Valenciennes in Cuvier and Valenciennes, 1836)) (Côté and Hunte 1989)

Our hypothesis that males should mate selectively with larger females because of their higher potential reproductive output was based on the assumption that sperm may be a limiting factor in male reproductive success, as evidenced in other species (Van Voorhies 1992; Olsson et al. 1997; Preston et al. 2001); however, this assumption was not tested and no studies on sperm production in male map turtles have been performed. In addition, the link





between multiple paternity and multiple mating is unclear, as copulations do not necessarily lead to fertilizations, and paternity patterns are thus not necessarily accurate reflections of mating patterns.

Keeping in mind the caveat that multiple paternity and multiple mating can be decoupled, we found that hatching success and first-year survival tended to increase with the number of sires, although our results were not statistically significant. Contrary to our predictions, however, we found no significant relationship between either the morphology or the performance of hatchlings and the number of sires. These results suggest that promiscuous females may benefit from increased reproductive success in terms of the number of surviving offspring produced. Greater offspring survival associated with more sires has been found in many other reptiles in which females are assumed to receive no direct benefits, including Leopard Geckos (*Eublepharis macularius* (Blyth, 1854))

Fig. 4. Plastron length (A), body condition (B), and the frequency of scute anomalies (C) are not affected by the number of sires in the clutch in Northern Map Turtles (Graptemys geographica) in Lake Opinicon, Ontario, Canada.

Fig. 5. Swimming speed (A), latency before righting (B), and righting time (C) are not affected by the number of sires in the clutch in Northern Map turtles (Graptemys geographica) in Lake Opinicon, Ontario, Canada.

Number of sires







(LaDage et al. 2008), Black Ratsnakes (Blouin-Demers et al. 2005), and Common Lizards (*Lacerta vivipara* Jacquin, 1787) (Uller and Olsson 2005), although in similar studies on Green Sea Turtles (*Chelonia mydas* (L., 1758)) (Lee and Hays 2004) and Painted Turtles (Pearse et al. 2002), there were no differences in the success of multiply sired versus singly sired clutches.

The potential association between the number of sires and reproductive success in this population of map turtles supports the hypothesis that female promiscuity may be associated with genetic benefits. However, our data do not allow us to rule out the possibility that females may also mate with multiple males simply because of intense male harassment or that survival benefits arise through maternal effects. Although genetic benefits potentially explain the high rates of polyandry in map turtles, the mechanism by which these benefits arise is less clear. Genetic benefits could come in the form of superior or more compatible genotypes or increased genetic diversity, allowing females to "hedge their bets"; however, our results do not directly support or refute any of these hypotheses.

Unfortunately, we were unable to extract good-quality DNA from unhatched eggs to successfully run genetic analyses. The implications of this are twofold: first, we evaluated fewer hatchlings per clutch and our subsequent analyses of fitness indicators from clutches sired by one, two, or three males were comparing smaller groups than would otherwise be possible if we had genotypes from all eggs, not just those that hatched. Second, the clutches used in our analyses were those with high hatching success, and the clutches in which very few eggs hatched were excluded. If there were differences in paternity between "successful" and "unsuccessful" clutches, then these differences were undetected. Increasing the number of families studied and constructing the multilocus genotypes of unhatched eggs could improve the power and resolution of future studies.

An unavoidable limitation of this study is our inability to evaluate the link between multiple mating and multiple paternity. Observational studies on mating frequency of female map turtles together with analyses of multiple paternity would give a clearer picture of the mating system, but are logistically very challenging. In this study we looked at multiple paternity from the female perspective; a comprehensive understanding of the map turtle mating system would require the male perspective to be included: are certain males more successful, and if so, do they display specific characteristics? Are multiply sired clutches biased towards one father, or are they equally distributed among males? Addressing such questions through observational and experimental studies will lead to a greater understanding of the causes, mechanisms, and effects of mating systems and the importance of multiple mating and multiple paternity in natural populations.

Acknowledgements

We are very grateful to D. Hanna for help with fieldwork, and to P. Pelletier, B. Fletcher, Z. Sun, B. Acker, L. Campagna, L. Caldéron, T. Wu, and A. Herod for their help with laboratory work. We thank Queen's University Biological Station for logistical support. Funding for this research came from the Natural Sciences and Engineering Research Council of Canada, the University of Ottawa, Queen's University, and the Ontario Graduate Scholarship program. We obtained research permits from the Ontario Ministry of Natural Resources and our protocols were approved by the University of Ottawa Animal Care Committee.

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