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Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature

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Abstract When females receive no direct benefits from multiple matings, concurrent multiple paternity is often explained by indirect genetic benefits to offspring. To examine such possibilities, we analyzed genetic paternity for 1,272 hatchlings, representing 227 clutches, from a nesting population of painted turtles (*Chrysemys picta*) on the Mississippi River. Goals were to quantify the incidence and distribution of concurrent multiple paternity across clutches, examine temporal patterns of sperm storage by females, and deduce the extent to which indirect benefits result from polyandrous female behaviors. Blood samples from adult males also allowed us to genetically identify the sires of surveyed clutches and to assess phenotypic variation associated with male fitness. From the genetic data, female and male reproductive success were deduced and then interpreted together with field data to evaluate possible effects of female mating behaviors and sire identity on offspring fitness. We document that more than 30% of the clutches were likely fathered by multiple males, and that presence of multiple paternity was positively correlated with clutch size. Furthermore, the data indicate that the second male to mate typically had high paternity precedence over the first.

Keywords Microsatellites · Paternal effects · Mate choice · Sperm competition · Mating systems

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Introduction

Numerous genetic studies have shown that multiple paternity within a single reproductive bout (e.g., clutch, litter, nest) is common in species representing a diverse array of animal taxa (Gowaty 1985; Avise 2001). In turtles, multiple paternity has been reported in nearly every species studied to date (reviewed by Pearse and Avise 2001). However, the classical sexual selection framework of Bateman (1948), in which males (more so than females in most species) improve their reproductive success by obtaining multiple mates, does not easily explain these findings. Because a single copulation is usually sufficient to fertilize all eggs a female turtle can produce, females might not be expected to solicit additional mates. The high frequency of multiple paternity in turtles is especially puzzling because, unlike birds or social insects for example, turtles do not have strong social interactions that might encourage multiple mating, nor do females benefit directly from mating events via nuptial gifts (such as courtship feeding) or territorial access (Pearse and Avise 2001).

Among the various hypotheses advanced to explain the frequent occurrence of multiple paternity in nature (Andersson 1994), 'indirect' genetic benefits to offspring may best explain polyandrous behaviors in species without social constraints or direct female benefits (Thornhill and Alcock 1983). Several studies have suggested that genetic variation in multiply sired broods increases the reproductive success of polyandrous females (Madsen et al. 1992; Kempnaers et al. 1999; Jennions and Petrie 2000; but see Byrne and Roberts 2000). In addition, the genetic quality of mates may differ depending on specific compatibilities between male and female genotypes, leading to situations where females with multiple mates might often achieve higher reproductive success (Zeh and Zeh 1996; Tregenza and Wedell 2000).

Multiple paternity within a clutch also provides an opportunity for sperm competition, which may increase offspring fitness if sperm quality and genetic quality are related (Smith 1984; Madsen et al. 1992). Sperm competition (and precedence) have been considered in both theo-

retical and laboratory settings (Parker 1970; Olsson et al. 1994; Clark et al. 1999) but seldom have they been addressed by genetic examination of progeny produced under natural circumstances. Patterns of sperm usage in sequentially laid clutches from specific females can provide information on female remating behavior and the potential for sperm competition. When the mating order of sires is deduced, paternity analyses can also yield data on sperm precedence and/or mixing within a female's sperm storage tubules. This information can also be used to classify multiply sired clutches based on their mode of production, either multi-year sperm storage or within-season double (or multiple) mating by the female (Pearse et al. 2001).

Female turtles are able to store viable sperm for up to 4 years (Birkhead and Møller 1993; Pearse et al. 2001). All else being equal, long-term sperm storage enhances the opportunity for multiple paternity and increases the potential for significant interactions among the sperm of competing males (Olsson et al. 1994; Ross 2001). A female's ability to store sperm might also increase her control over the paternity of a clutch. For example, by storing sperm, a female can 'remate' with a desirable partner to produce additional clutches, even if he is deceased or otherwise unavailable (Zamudio and Sinervo 2000; Pearse and Avise 2001). Furthermore, because forced copulation is unlikely in open-water aquatic turtles (Berry and Shine 1980), mating patterns are likely to be representative of female choice (Gowaty 1997). Thus, if a female can detect variation in male genetic quality or compatibility and adjust her sperm storage or mating behaviors accordingly, she can play an active role in enhancing her overall genetic fitness.

In studies of turtle mating systems, few data are available on the male side of the reproductive equation. However, observations of size-based male dominance hierarchies in some turtle species (Kauffmann 1992), and genetic evidence for a high variance in male reproductive success (McTaggart 2000) demonstrate the importance of including males in analyses of reproductive patterns. Thus, to extend our clutch-based analyses of genetic parentage in painted turtles (*Chrysemys picta*), we attempted to match the deduced paternal genotypes, as reconstructed from the progeny arrays of known females, with those of particular males trapped in the surrounding area. The identification of actual sires provides additional information on the residency times of reproductive males in the population and on physical factors that might affect variation in male fitness.

Methods

Samples of adult females and their clutches (mean clutch size 10.9 eggs, range 4–17) were taken from May to July 1995–1998, from a nesting population at South Potter's Marsh on the Mississippi River near Thomson, Ill. The specimens used here are a subset of those originally collected for a long-term study on temperature-dependent sex determination in this species (Janzen 1994; Valenzuela and Janzen, in press). Upon first capture, each female was notched with a unique marginal scute pattern for subsequent identification, and a blood sample was drawn and stored in lysis buffer (Seutin et al. 1991). For each observed nest laid by a marked female, the

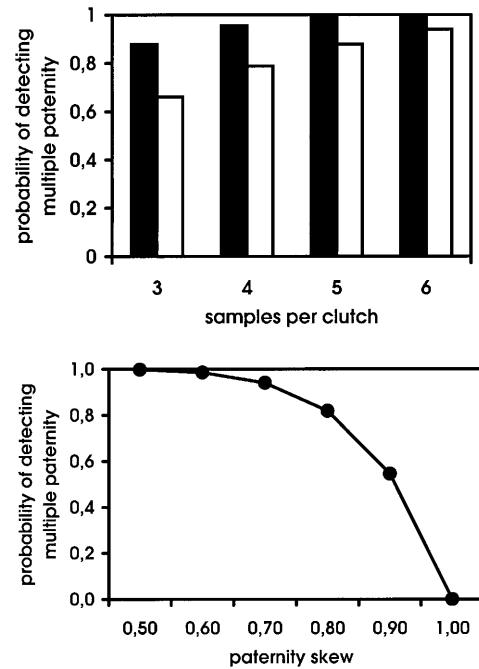


Fig. 1a, b Probabilities of detecting multiple paternity when present, based on a hypergeometric sampling distribution (with total clutch size=11) and an assumption of no alleles shared among males. **a** As a function of the number of offspring sampled per clutch. *Solid bars* assume equal paternal contributions; *open bars* are probabilities calculated using the observed mean paternity skew (0.76). **b** As a function of paternity skew (proportion of offspring sired by more successful male) with a sample of six hatchlings

percentage of eggs that hatched was determined by excavating the nest at the end of the season, prior to the overwintering period (during which hatchlings normally remain in the nest). A random subset (usually six) of the surviving hatchlings was then preserved in 95% ethanol (Janzen 1994). DNA was extracted from blood following standard organic protocols, and from the preserved liver tissue of hatchlings using Chelex (Bio-Rad). Each individual was genotyped using two or three hypervariable microsatellite loci developed for *C. picta* (Pearse et al. 2001).

Genetic sire(s) for each clutch were deduced by subtracting the known maternal contribution from the multilocus genotype of each offspring, following the procedure of Pearse et al. (2001). For a few clutches ($n=34$), no blood sample was available from the mother (and thus no maternal genotype) so paternity was deduced using criteria derived from Valenzuela 2000) as follows. A clutch was considered to show evidence of multiple paternity if at least two loci displayed five or more alleles in heterozygous hatchlings, four alleles and *one* homozygous hatchling, or three alleles and *both* homozygous genotypes. However, individual sire genotypes could not be reconstructed from these clutches. For all clutches, single paternity was considered the null hypothesis, and multiple paternity was determined only when there was concordant support from multiple loci. Similarly, female remating between clutches was deemed the null hypothesis, and sperm storage was considered documented only when multiple loci indicated the identical sire in consecutive clutches (see Pearse et al. 2001).

Clutches with only one or two sampled hatchlings provide no information with respect to single versus multiple sires, so they were excluded from all analyses related to paternity. However, any finite sample of hatchlings provides reduced power to detect multiple paternity relative to exhaustive sampling, and such power is further reduced if sire contributions to a clutch are highly skewed (Fig. 1; Ross 1998). Therefore, we corrected estimates of the overall frequency of multiple paternity in two ways.

Table 1 Frequencies of multiple paternity in groups of painted turtle clutches differing in the number of hatchlings sampled

Hatchlings sampled per clutch (mean)	Number of clutches	Number of detected multiply sired clutches	Observed frequency of multiply sired clutches	Likely number of undetected multiply sired clutches ^a
≥3 (5.8)	215	23	10.7	42
=6 (6.0)	148	17	11.5	28
≥6 (6.2)	160	21	13.1	27
>6 (9.3)	12	4	33.3	0

^a Based on the maximum-likelihood estimate of the true rate of multiple paternity

First, we estimated the occurrence of multiple paternity using subsets of the assayed clutches, grouped according to how many hatchlings were sampled (Table 1). This method provides an empirical evaluation of the proportion of multiple-sire clutches documented as a function of sample size. Second, the program LAMP (likelihood analysis of multiple paternity, version 3.0; Kichler et al. 1999) was used to obtain a maximum-likelihood estimate of the true proportion of multiply sired clutches, as well as the average paternity skew in clutches with multiple paternity. The program simultaneously estimates three parameters from the data: m , the probability that a female mates with two males; r , the proportion of a clutch sired by the more successful male (paternity skew); and μ , the marker mutation rate. It then calculates a likelihood value for that set of parameters (Kichler et al. 1999). The maximum-likelihood value obtained can be compared to likelihood values calculated when one or more of the parameters are constrained (i.e., $m=0$ to test the hypothesis of no multiple paternity, or $r=0.5$ to test for equal paternity sharing among males).

These statistical methods were employed to correct the population estimate of the proportion of multiply sired clutches, but sampling problems are unavoidable in classifying individual clutches as singly or multiply sired (Akin et al. 1984). Thus, in our analyses of the relationship of multiple paternity to total clutch size and hatching success, we equalized our power to detect multiple paternity across clutches by comparing only those clutches from which we had sampled exactly six hatchlings (by far the most common category).

For comparisons among groups of clutches and for adult morphological measurements, standard parametric statistical tests were performed using Excel. Data on percent hatching success are not normally distributed, so in some analyses, the data were first arcsine square-root transformed. In another statistical approach, a Visual Basic randomization program (which makes no assumptions about the underlying distribution) was created to calculate the probability that subsets of the data, drawn randomly from the pool of clutches, differed by more than the observed samples (Manly 1997). A modified version of this randomization program was used to compare the mean difference between first- and second-year hatching success among females that did or did not remate between years. For this latter analysis only, if a female laid two clutches in a year, her average hatching success in that year was the value recorded.

Results

The three microsatellite loci displayed a mean of 24 alleles per locus. Based on allele frequencies in the adult population sample, expected mean heterozygosity was 0.85 and the combined paternity exclusion probability was 0.997. Data from a total of 227 clutches were analyzed for the present study (including 113 clutches originally described in an earlier microsatellite survey that provided the first genetic documentation of long-term sperm storage by female turtles in nature; Pearse et al. 2001). Females laid a mean of 10.9 eggs per nest but

hatching success was occasionally as low as 9%. Thus, the mean number of offspring genotyped per nest was 5.6 (range 1–14). Altogether, 1,517 individuals were genotyped, including 98 mothers, 147 adult males, and 1,272 hatchlings.

In three instances, the genotypes of some or all of the offspring in a clutch did not match the putative mother's genotype. These apparently reflected sample mix-ups, and were subsequently treated as clutches with unknown maternal genotypes. In each of these clutches, the genotypic data nonetheless indicated that all of the offspring shared the same sire.

When an 'extra' allele in a clutch was observed at a single locus only, it was provisionally considered to be the result of de novo mutation (rather than an additional sire). Six such putative mutations were observed among the 1,272 hatchlings ($\mu=0.0008$ per gamete per locus). Based on known maternal genotypes, three of these apparently arose in the paternal germ line, two in the maternal germ line, and one was ambiguous with respect to the sex of origin.

Paternity analysis

The overall observed incidence of multiple paternity was 10.7% (23 of 215 clutches; Table 1), but this is clearly a minimum estimate because some sire contributions will have been missed in clutches sampled incompletely. Limiting the analysis to clutches sampled more extensively (more than six hatchlings per nest), the estimated frequency of multiple paternity increased to 33.3% (Table 1). Thus, this latter estimate is probably a closer approximation to the true fraction of multiply sired clutches in this population.

This contention is supported by the maximum-likelihood estimate of multiple paternity obtained from the computer program LAMP. In an analysis of the 190 clutches for which we had appropriate mother and offspring samples, LAMP found that the maximum-likelihood value was obtained with the parameter values of $m=0.301$ (30.1% multiple paternity), $r=0.854$ (mean proportion of a clutch sired by the more successful male), and $\mu=0$. The observed mean (\pm SE) paternity skew in multiply sired clutches was 0.76 ± 0.26 (Fig. 2). Note that this value is about 10% lower than the LAMP-based estimate of skew, a result which may reflect our reduced ability to detect multiple paternity in highly skewed clutches (Fig. 1b).

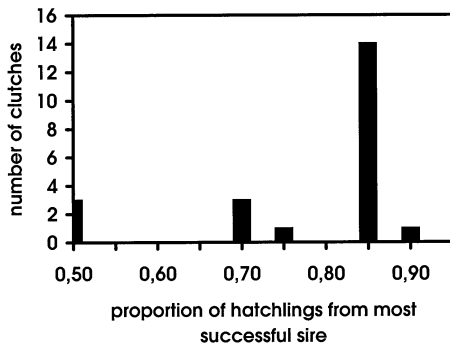


Fig. 2 Observed distribution of paternity skew for painted turtle clutches in which multiple paternity was detected. The observed mean skew was 0.76, the maximum-likelihood estimate of skew was 0.85

As described by Pearse et al. (2001), multiply sired clutches could be divided into two distinct groups based on the pattern of mating from which the multiple paternity resulted. Using paternity data from earlier clutches laid by the same mother, the mode of production could be determined for 13 of the 23 clutches genetically documented to have resulted from multiple paternity. Eight of these were apparent cases of multi-year sperm use, and 5 resulted from double mating by a female within a single season.

In cases of multiple paternity resulting in part from cross-year sperm storage, the apparent second mate invariably sired the majority of the offspring in the clutch: $P_2=0.784\pm 0.27$. However, this estimate is not directly comparable to most laboratory-derived values of second male precedence (Harshman and Clark 1998) because we have no information on the number of cases in which a female remated and the second-year mate fathered all ($P_2=1$) or none ($P_2=0$) of the offspring. Similarly, we have few data on the actual frequency of multiple within-year mating by females, beyond the observation that this behavior leads to multiple paternity at least occasionally.

Correlates of clutch size

Clutches in which we detected multiple paternity contained significantly more eggs at the time of laying (12.3) than those sired by only one male (10.8; $t=-2.75$, $P<0.01$ in a two-tailed t -test assuming equal variances). As noted above, however, small clutch size combined with low hatching success meant that sometimes fewer than five offspring per nest were available for genetic assay. Thus, to equalize the probability of detecting multiple paternity across nests, we restricted the analysis to include only the 99 clutches with six sampled offspring each. Even with these truncated data, multiply sired clutches contained significantly more eggs (12.4) than those with only one sire (10.9; $t=-2.40$, $P<0.01$).

Considering the distribution of multiple paternity, the 22 largest clutches in our sample (those with 14 or more

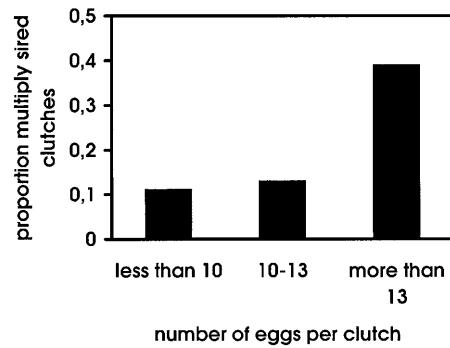


Fig. 3 Distribution of multiple paternity in clutches that were small ($n<10$), medium ($10\leq n\leq 13$), and large ($n>13$). This observed distribution differs significantly from that expected if all clutch size classes had the same mean proportion (11.5%) of multiple paternity (for clutches with exactly $n=6$ hatchlings, $\chi^2=11.54$, $P<0.005$)

eggs) showed a threefold higher rate of multiple paternity than those with smaller egg numbers (Fig. 3). LAMP analyses support this trend. For these largest clutches, the maximum-likelihood value occurred when the frequency of multiple paternity was 0.482, a value significantly larger than the comparable estimate for smaller clutches ($m=0.286$) when skew is held constant (likelihood ratio test using a χ^2 -distribution, $df=2$, $P<0.05$).

We also examined the relationship between multiple paternity and female body size (which is positively associated with clutch size in turtles). Female carapace length was not significantly related to single versus multiple paternity of clutches. However, females that laid at least one multiply sired clutch were on average larger (170.7 mm) than females that laid only single-paternity clutches (167.9 mm), and in turn were larger than females that stored sperm for at least 1 year (165.6 mm). The magnitude and rank order of these differences suggest a significant male preference for large females as mates (ANOVA, $F=1.19$, $P=0.309$, $r_s P_c \approx 0.05$; Rice and Gaines 1994).

If male preference for large females affects the distribution of multiply sired clutches among females, it may also affect the frequency of sperm storage. To address this question, we asked if females who produced at least one multiply sired clutch were also more likely than other females to remate between years (rather than utilize stored sperm). In our sample, females that mated with multiple males to produce a clutch in one year also always remated between clutches in successive years (15/15, 100%). In contrast, females that laid only single-sire clutches had a significantly lower remating frequency between years (39/50, 78.0%; z -test of binomial proportions, $P=0.0228$). Thus, females that produce multiple-paternity clutches are also more likely than others to remate annually.

Correlates of clutch hatching success

The mean percentages of eggs that hatched in multiple- and single-paternity clutches were 90.3% and 86.2%, re-

Table 2 Expected frequencies of the nine perfect multi-locus genotypic matches between the deduced sire of a clutch (based on paternity analysis) and an adult male that was physically captured

Genetic match	Year that the male sired a clutch	Year that the male was trapped	Expected genotype frequency
1	1998	1999	3.89×10^{-7}
2	1995	2000	2.51×10^{-6}
3	1998	1999	1.16×10^{-5}
4	1997	2000	2.17×10^{-5}
5	1996	2000	8.36×10^{-8}
6	1996	1999	6.63×10^{-7}
7	1998	2000	1.06×10^{-5}
8	1997	2000	3.43×10^{-9}
9	1997 ^a	1999	2.46×10^{-6}

^a The sire's genotype at one locus was ambiguous, but most likely matches trapped male 9

spectively. This difference was not significant based on a randomization test ($P=0.14$). Furthermore, in an analysis limited to clutches from which exactly six hatchlings were sampled, no difference in hatching success existed (88.5 versus 88.7%). Considering the clutches of individual females that laid at least one multiple-sire clutch and one or more apparently single-sire clutches, there was likewise no difference in hatching success between the two categories of nest (89.1 versus 87.6%; $t=-0.29$, $P=0.77$).

With regard to female remating patterns, there was no evidence that females altered their behavior based on hatching success in past clutches. Mean hatching success in clutches laid by females who later remated (83.5%) did not differ significantly from that of clutches laid by females who used stored sperm from the same male in the subsequent nesting season (84.0%). Finally, we found no evidence that successful matings with 'preferred' males conferred higher clutch hatching success. Nine males were genetically documented to have sired assayed clutches laid by more than one female, but the mean hatching success of their offspring [82.0 ± 4.0 (SE)] did not differ significantly from that of males who sired only one assayed clutch (84.2 ± 2.0). Furthermore, based on the lack of a significant correlation in hatching success across clutches of different females mated to the same male (data not shown), sire quality did not appear to be consistent across females.

Male mating success

Among the 227 clutches assayed, we were able to fully reconstruct 133 paternal genotypes from the progeny genotypes. We compared these to the multi-locus genotypes of the 147 captured males. Nine genotypes matched exactly, and their expected frequencies in the population (based on Hardy-Weinberg equilibrium) were sufficiently low to indicate that the actual sire in most or all cases had indeed been identified (Table 2). Mean carapace length did not differ between 'match' and 'non-match' males (145.1 versus 141.5; $t=-1.28$, $P=0.22$), suggesting no obvious size differences between successful and apparently unsuccessful males.

In cases where a male's genotype was detected in clutches laid by more than one female, variation in male

reproductive success can be qualitatively assessed. Among our 133 reconstructed paternal genotypes, nine males were implicated as the sire of at least two clutches. Furthermore, three of these males sired the clutches of two different females in a single year, and two of them apparently sired clutches laid by three different females (Pearse et al., in press).

Discussion

A growing number of genetic parentage studies in birds (e.g., Lifjeld and Slagsvold 1988; Houtman 1992) and other animals (Loughry et al. 1998; Madsen et al. 1992; DeWoody et al. 2000; Jones et al. 2000) have gone beyond the mere estimation of mate numbers to examine the consequences of multiple maternity and paternity with respect to mating system parameters, variations in genetic fitness as a function of alternative behaviors, or quantitative genetic issues. For example, Olsson et al. (1996) assigned paternity in sand lizards using DNA fingerprinting and showed that paternal genotype influenced various offspring traits; Kvarnemo et al. (2000) showed that male-pregnant seahorses who retained the same mate over multiple reproductive cycles had shorter inter-brood intervals and traveled less than males who switched mates, suggesting substantial benefits to monogamy; and King et al. (2001) used microsatellite parentage analyses to discriminate between maternal and genetic effects on scalation and behavior in multiply sired litters of garter snakes.

Such genetic studies of parentage in nature can reveal environmental and genetic factors that affect variation in male and female reproductive success, and they enable analyses that formerly were possible only for captive populations in artificial settings. Here, we have carried out the most complete genetic characterization of reproduction in a wild turtle population to date, in order to assess fitness effects of various reproductive tactics by the two sexes.

Multiple paternity and clutch size

In this population, at least 10% of the clutches had multiple sires, and statistical analyses suggest that the true in-

idence of multiple paternity is probably closer to 30% (a value similar to the reported mean in 14 other studies of turtle species; reviewed in Pearse and Avise 2001). Furthermore, multiple paternity was not evenly distributed across clutches: clutches sired by multiple males contained significantly more eggs on average than those in which we did not detect multiple paternity. Although the exact cause-effect relationship remains uncertain, at least two scenarios are possible.

First, females might adjust their clutch sizes upward in response to multiple mating. In laboratory experiments with bruchid beetles, females mated to three different males laid more eggs than those mated three times to the same male (Eady et al. 2000). Although these insects gain nutritional benefits from mating, this effect was controlled for experimentally, leaving mating regime per se as the apparent sole difference between treatments. The authors suggested that subtly different chemical cues from multiple males may stimulate increased oviposition in polyandrous females. Similar phenomena have been described in newts (Osikowski and Rafinski 2001), and conceivably might also apply to turtles.

Alternatively, larger turtle females might be preferred as mates (as our data for painted turtles suggest, and as has been found in some lizards; Whiting and Bateman 1999). In turtles, larger females are more fecund (McTaggart 2000) and, thus, might be more attractive to males seeking to maximize genetic fitness per mating. Like many aquatic turtles, painted turtles engage in an elaborate, time-consuming courtship (Ernst et al. 1994) that may limit the number of females a male can court in a given season. Furthermore, as has been shown for adder snakes (Olsson et al. 1997), sperm production may entail a major energetic cost for a male, further reducing the number of times he can mate successfully. Another reproductive consequence might follow: if larger, more fecund females attract more mates, and especially if males are sperm limited, then small females may utilize stored sperm more often simply because they have no other viable option.

In contrast to some previous studies (Sakaluk and Cade 1980; Madsen et al. 1992; Tregenza and Wedell 1998; Osikowski and Rafinski 2001), our genetic data provide no evidence that multiple paternity increases hatching success (although multiple *mating* by females would go undetected with our methods if the eggs were fertilized by only one male, and this could also affect hatching success). This conclusion is not entirely satisfactory, however, because the exclusion of clutches with small sample sizes inevitably lowers the power to detect any differences in hatching success that might exist. In the future, this sampling problem might be ameliorated by including genetic paternity data from both live and unhatched offspring.

In summary, we found evidence for increased egg production in multiply as opposed to singly sired clutches of the painted turtle, but no documented difference in mean hatching success. All else being equal, the net effect of the two factors is that multiply sired clutch-

es produce more offspring on average than those that are fathered by only one male.

Specific mate pair effects

Previous studies of turtles have noted that successful males were either larger (wood turtles: Kauffmann 1992) or smaller (painted turtles: McTaggart 2000) than average in mean body size. In contrast, we found no evidence of a size difference between 'successful' and 'unsuccessful' males. However, the population at South Potter's marsh is large and open (Pearse et al., in press), so many apparently 'unsuccessful' males may have sired unsampled clutches. Thus, our data on the relationship between male body size and mating success are inconclusive.

McTaggart (2000) also found evidence that clutches fathered by 'preferred' males (those who sired clutches with at least two females) had higher mean hatching success than those sired by males who produced only one assayed clutch. The current study provided no evidence for this pattern. We also detected no significant correlation in hatching success between pairs of clutches from different females who mated with the same male (data not shown), suggesting that these 'preferred' males are not universally better sires.

This latter conclusion is consistent with data from recent experiments on crickets and house mice. In a laboratory experiment in which female crickets were mated an equal number of times to either one or multiple males, no repeatable differences were detected between males in the hatching success of their mate's eggs (Tregenza and Wedell 1998). In laboratory house mice, Drickamer et al. (2000) reported increased viability and performance among offspring of females mated to males of their choosing. Thus, data in the current turtle study, as well as those from previous experimental studies on some other species, are consistent with the hypothesis that mating pairs have different genetic compatibilities with respect to offspring viability (Zeh and Zeh 1996).

Conclusions

Whereas descriptive or experimental studies of animal mating systems can be informative with regard to the potential forces of sexual selection, studies that examine the fitness consequences of various reproductive tactics in nature are likely to produce additional key ecological or evolutionary insights. In the current case, genetic parentage analyses on a population of painted turtles have shown that multiple paternity (1) probably occurs in more than 30% of the clutches, (2) can be produced by at least two different patterns of female mating behavior, and that (3) the second male to mate often has high paternity precedence over the first. Furthermore, multiple paternity is not evenly distributed across clutches, with larger clutches showing a higher frequency of multiple paternity than small clutches. Future studies of genetic

mating systems in turtle populations from nature will profit from applying parentage data to specific ecological or life history questions.

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