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Original Article

No benefits of polyandry to female green turtles

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Multiple paternity is extremely common in natural populations of almost all reptiles studied to date, suggesting that pay-offs from polyandrous mating systems are important in these taxonomic groups. However, strong evidence in support of direct or indirect benefits to females is scarce. We examined the relationship between polyandry and components of female reproductive success and offspring fitness in the promiscuous green turtle (*Chelonia mydas*), a species that exhibits highly variable levels of multiple paternity. We did not detect any clear fitness benefits to polyandrous females in this study, and we discuss the potential of sexual conflict to influence female mating patterns in marine turtles. We show that polyandrous females produce significantly smaller clutches than monandrous females, highlighting a potential cost to polyandry in green turtles. Furthermore, multiple paternity was more common in returning females (recorded breeding in a previous season) than in females nesting for the first time at our study site, possibly reflecting increased encounter rates with males or sperm storage across breeding seasons. Our results reveal potentially complex influences of female traits, environment, and mating strategy on components of reproductive success, and we discuss the challenges associated with unraveling the costs and benefits of multiple mating in natural populations.

Key words: ***Chelonia mydas,* Mediterranean, microsatellites, multiple paternity, polyandry.** *[Behav Ecol]*

INTRODUCTION

Female promiscuity is common in natural populations across almost all taxa studied (Birkhead and Møller 1998) but, despite substantial evidence that polyandry can increase female fitness (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Simmons 2005), its adaptive significance in the wild remains controversial (Jennions and Petrie 2000; Arnqvist and Kirkpatrick 2005; Akçay and Roughgarden 2007; Uller and Olsson 2008). Mating can carry significant costs to females, such as exposure to disease (Thrall et al. 2000), increased predation risk (Rowe 1994), time and energy costs (Watson et al. 1998), as well as the risk of physical harm (Crudgington and Siva-Jothy 2000); hence, the persistence of this behavior in natural populations suggests that these costs should be offset by net benefits.

In many cases, females can receive direct benefits from mating with multiple partners, which include all material benefits ranging from fertilization assurance to provision of or access to resources and parental care of offspring (reviewed in Arnqvist and Nilsson 2000; Hosken and Stockley 2003). Polyandrous females may also gain indirect genetic benefits, via the processes of sperm competition and/or cryptic female choice, if it results in the production of more viable offspring (reviewed in Jennions and Petrie 2000; Hosken and Stockley 2003; Simmons 2005). Indirect benefits can potentially arise through the increased genetic quality (Yasui 1997; Fisher et al. 2006; Hosken et al. 2003), sperm competitive ability (Keller and Reeve 1995), genetic compatibility (Zeh and Zeh 1996, 1997; Tregenza and Wedell 2000, 2002), or genetic diversity (Yasui 1998) of offspring. However, unequivocally demonstrating that polyandrous females gain indirect benefits in the wild is challenging, and evidence in support of these hypotheses is largely restricted to laboratory experiments (Simmons 2005; but see Foerster et al. 2003; Garant et al. 2004; Fisher et al. 2006)). Multiple mating by females may also arise in the absence of benefits to females, as a consequence of male coercion (Clutton-Brock and Parker 1995) or to avoid the costs of male harassment ("convenience polyandry"; Thornhill and Alcock 1983). In this situation, multiple mating is driven by strong selection on males to increase their reproductive success, and multiple paternity results from conflict between the sexes over mating rate, with females potentially paying a net cost (Arnqvist and Rowe 2005; Hosken and Stockley 2005; Maklakov et al. 2005).

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In the vast majority of reptiles, parental care of offspring is absent, and there is no evidence that males provide any resources to females other than sperm, yet multiple paternity of clutches is widespread, occurring in all species that have been studied to date (Uller and Olsson 2008). Furthermore, it can be extremely common within natural populations; high levels of multiple paternity have been reported in numerous species of snakes and lizards, as well as in some marine turtles (Jensen et al. 2006; Zbinden et al. 2007; Uller and Olsson 2008). Early work in adders (*Vipera bergus*) and sand lizards (*Lacerta agilis*) suggested that polyandrous females suffered lower offspring mortality (Madsen et al. 1992; Olsson et al. 1994), possibly through female selection of more compatible sperm (Olsson et al. 1996), and subsequent studies have found correlations between multiple paternity of clutches and increased clutch size, hatching success or offspring viability (e.g., Pearse et al. 2002; Blouin-Demers et al. 2005; Madsen et al. 2005; Uller and Olsson 2005; Eizaguirre et al. 2007; Zbinden et al. 2007; Olsson et al. 2011) . However, overall, there is very little clear evidence that multiple paternity is associated with indirect genetic benefits in reptiles (Uller and Olsson 2008; but see Madsen 2008).

Multiple paternity has been documented in all 7 marine turtle species (reviewed in Bowen and Karl 2007; Joseph and Shaw 2010), but few studies have attempted to assess potential fitness gains of polyandry, and to date, none have been clearly demonstrated. Lee and Hays (2004) found no benefits of multiple paternity in green turtles (*Chelonia mydas*) nesting on Ascension Island, leading to the suggestion that female turtles "make the best of a bad job" and mate multiply in response to male harassment (Lee and Hays 2004). Male marine turtles are aggressive in their pursuit of receptive mates (Booth and Peters 1972; Miller 1997; Bowen and Karl 2007), and females undoubtedly pay energetic costs in order to avoid their advances. On the other hand, mating is also likely to incur substantial costs to females (e.g., physical injury; Miller 1997). Female marine turtles are able to refuse copulations (Booth and Peters 1972), suggesting that female choice can influence mating patterns. In this study, we build on previous work, by conducting a more extensive analysis of the potential effects of polyandry on components of reproductive success and offspring fitness in female turtles. We previously reported that 24% (19 out of 78) of female green turtles nesting at our study site in northern Cyprus produced clutches with multiple paternity (Wright et al. 2012). Here, using a large data set, collected over 3 consecutive years and incorporating a large proportion of the nesting population, we specifically ask 1) Is polyandry more common in larger or more experienced females? 2) Do polyandrous females benefit from increased clutch size, fertilization success, hatching success, or offspring size? and 3) Do males that achieve a high paternity share in polyandrous clutches sire larger or more viable offspring than males that achieve lower siring success?

MATERIALS AND METHODS

Field methods and sample collection

Samples were collected from nesting female turtles and their offspring at Alagadi beach, northern Cyprus, during 3 breeding seasons; May to October, 2008–2010. Tissue samples were taken from nesting females of known identity from the trailing edge of the foreflipper. Female size was recorded (curved carapace length [CCL]), and nest locations were marked. On hatching, tissue biopsies were taken from a random sample of hatchlings in each clutch from the trailing edge of the carapace. Hatchlings were measured (straight carapace length [SCL], straight carapace width [SCW] and weight) before being released. Nests were allowed to hatch naturally and were excavated when no further hatchlings had emerged for 48 h, or after 5 days since the first hatchling emerged, whichever was sooner. Live and dead hatchlings found inside the nest, as well as dead embryos from unhatched eggs, were also randomly sampled. In 2008, tissue samples were stored in Queen's lysis buffer (Seutin et al. 1991), and in subsequent years, tissue samples were stored in 96% alcohol. Clutch size, fertilization success, hatching success, and emergence success (the number of hatchlings that survived to leave the nest, including those found alive during excavation) were recorded for each clutch, through excavation of clutch contents once hatching was complete. Unhatched eggs were classified as unfertilized if there were no gross signs of embryo presence (although some of these eggs could potentially represent very early embryo mortalities).

In 2008, 2 clutches were sampled from a subset (16 out of 20) of females. Inferred paternity was highly correlated in the successive clutches of these females (see RESULTS), so in further years, we reduced our sampling effort to a single clutch per female. In total, 94 clutches from 78 females were sampled for microsatellite analysis of paternity, representing 87% of females that nested at the site during the study period. If there was evidence of multiple sires in at least 1 genotyped clutch from a particular female, then that female was considered to be polyandrous and all of her subsequent ungenotyped clutches were also classified as polyandrous clutches. The full data set consisted of 219 clutches from 78 females (13 clutches failed due to inundation so were excluded from the analysis).

Genotyping and parentage analysis

Mothers and offspring were genotyped at 13 microsatellite loci, as previously described (Wright et al. 2012). All loci were designed for use in marine turtles, were variable in our population, conformed to expectations of Hardy–Weinberg equilibrium, showed low probability of null alleles, and showed no evidence of linkage disequilibrium (Wright et al. 2012). Green turtle clutches are large and could not be sampled to completion. Between 18 and 23 offspring were genotyped per clutch (mean ± standard deviation, 21.7 ± 1.2), representing 12–52% of the total clutch size (mean 21%). In total, 2042 offspring and 78 mothers were successfully genotyped at a minimum of 10 loci, but genotypes were complete at all 13 loci for 97% of individuals. Genotyping error, assessed by retyping approximately 5% of samples in a blind fashion, was <1% at all loci (for details, see Wright et al. 2012). Sibship reconstruction and parentage inference were carried out using the program COLONY v2.0 (Wang 2004; Wang and Santure 2009) as previously described (Wright et al. 2012). In brief, 3 replicate runs of "long" length were conducted on the same data set assuming an error rate of 0.004 for allelic dropout and 0.008 for genotyping error. Each of the replicate runs used different random number seeds to initiate the simulated annealing processes. We only concluded that a female had mated with more than 1 male when all the 3 runs gave consistent results. Probability of detecting multiple paternity (assessed using PrDM software [Neff and Pitcher 2002; downloaded November 2011]) with 20 offspring sampled per clutch was 0.874 when assuming 2 fathers and skewed paternal contributions (10:90%) and 0.999 when assuming 3 fathers and skewed paternal contributions (10:20:70%). Within polyandrous clutches, males that sired the highest number of genotyped offspring were classified as primary fathers, whereas additional males that sired smaller proportions of sampled offspring were classified as secondary fathers. As with all situations where a sample is taken, we are assuming that our sample is representative of the population as a whole and hence that paternal contributions detected in our random samples of hatchlings are representative of the true

paternity skew within clutches. We attempted to standardize the number of hatchlings sampled per clutch, but, due to variation in clutch size (range = 40–182 eggs), the proportion of each clutch sampled varied considerably. However, there was no correlation in our data set between the proportion of a clutch sampled and the number of sires detected (Spearman rank correlation coefficient = 0.57, df = 92, $P = 0.569$), suggesting that our sampling regime did not introduce significant bias into our estimates of paternity skew. The estimates of the power of our study based on our sampling sizes provided by PrDM (above) and the fact that we sampled a significant proportion randomly from each clutch suggest that we will only have incorrectly classified a few polyandrous females as monandrous and that our overall estimate of the relative contributions of multiple fathers to paternity is robust.

Statistical analyses

All statistical analyses were run in GenStat release 13.2 (GenStat 2010) using restricted maximum likelihood (REML) and generalized linear mixed models (GLMM) with a binomial error structure and a logit link function. REMLs and GLMMs allow both fixed and random factors, as well as covariates, to be fitted, and the random terms control for the use of repeated measurements (Schall 1991<cb>. The significance of fixed terms in linear mixed models is calculated using maximum likelihoods and is assessed by their Wald statistics, which are distributed as χ^2 for each term fitted last in the model. Full models contained higher order interactions, and final models were selected using stepwise elimination of nonsignificant terms ($P > 0.05$; for discussion of the use of stepwise modeling, see Whittingham et al. 2006 and Murtaugh 2009). Residuals were checked for normality and homoscedasticity after each deletion step.

We investigated the effect of polyandry on estimators of female reproductive success, including clutch size (total number of eggs including unfertilized eggs), fertilization success (proportion of the clutch that showed signs of embryo development), hatching success (proportion of the clutch that hatched), and emergence success (proportion of the clutch that survived to leave the nest). We also examined the effect of polyandry on mean hatchling size (SCL × SCW) as a proxy for hatchling fitness; large hatchling size is associated with increased swimming ability (Booth and Evans 2011) and lower mortality rates during early offshore migration (Gyuris 2000). Polyandry (binary variable: polyandrous vs. monandrous clutches) was included in all models as a fixed factor, and female identity (ID) was included as a random term to control for the inclusion of repeat clutches from the same females. Covariates included female size (CCL), lay date (number of days since 1 January), year, incubation duration, and clutch size.

Hypotheses relating to indirect benefits from good genes and cryptic female choice predict that males achieving a high paternity share in multiply sired clutches should produce "better" offspring (Yasui 1997; Zeh and Zeh 1997). Therefore, considering only hatched offspring from polyandrous clutches that were consistently assigned to specific fathers ($n = 452$), we investigated whether primary fathers sired larger offspring than secondary fathers, using the same covariates as previous models, with primary or secondary father included as a fixed factor, and female ID and clutch ID included as random terms. Considering only hatched offspring sired by primary fathers in polyandrous clutches and hatched offspring from monandrous clutches ($n = 1687$), we also asked whether primary fathers from polyandrous clutches sired larger offspring than fathers from monandrous clutches. Results are presented as means ± standard error (SE) unless otherwise stated.

RESULTS

Paternal contributions to clutches and patterns of paternity in successive clutches

Parentage analysis was successfully carried out for 94 clutches from 78 females. Multiple paternity was detected in 22 clutches from 19 (24%) females (subsequently referred to as "polyandrous clutches"), and the minimum number of inferred fathers per clutch ranged from 1 to 3 (Wright et al. 2012). The proportion of females that was polyandrous did not differ significantly across the 3 breeding seasons (goodness-of-fit test; $\chi^2 = 3.35$, df = 2, $P = 0.187$). Within polyandrous clutches, primary males gained between 48% and 96% of the paternity share (mean 77%). In 17 out of 21 polyandrous clutches, paternal contributions were significantly skewed from equal contributions (χ^2 all $P < 0.05$; 1 clutch was not analyzed because offspring could not be consistently assigned to specific sires, due to extensive allele sharing). Identical paternal alleles were found in the successive clutches of 13 out of 16 females, suggesting that females did not generally remate between clutches. Paternal contributions to successive clutches were very similar, and in polyandrous clutches, the primary male in the first clutch was also the primary male in the second clutch in all cases.

Polyandry and female size/reproductive history

Female mating strategy (monandry vs. polyandry) was strongly influenced by whether the female was a remigrant (had nested at our study site in previous years) or a first-time nester: polyandry was significantly more common in remigrants (Table 1, Figure 1). However, although remigrant females were larger than first-time nesters in this study (*t*-test, $t = 6.53$, df = 75, $P < 0.001$), mating strategy was not affected by female size (CCL) or by an interaction between female size and remigration status (Table 1).

Effects of polyandry on fitness-related traits

Clutch size was largely predicted by female size (larger females produced larger clutches) and a quadratic effect of lay date, as expected based on previous studies (e.g., Broderick et al. 2003), but there was an additional negative effect of polyandry (Table 2): clutches from polyandrous females were significantly smaller than clutches from monandrous females (107.17 ± 2.55 and 112.75 ± 1.42, respectively; Figure 2). Polyandry was unrelated to fertilization success, hatching success, emergence success, or mean hatchling size (Table 2), whereas lay date, incubation duration, female size, and interactions between these variables had much stronger influences on these fitness measures (Table 2).

Relationship between relative male siring

Table 1

Factors affecting female mating strategy (GLMM)

Explanatory terms	Estimated effect (SE)	F_{df}	*P*
Remigration status			
First-time nester	0.0 (0.0)	$17.24_{1,76}$	**<0.001**
Remigrant	5.17 (1.25)		
Female size	-0.05 (0.06)	$0.87_{1,74}$	0.354
Remigrant × female size	-0.45 (0.30)	$2.32_{1,73}$	0.132
Year	-0.06 (0.37)	$0.03_{1,75}$	0.872
Constant	-6.48 (0.82)		

Significant terms are shown in bold.

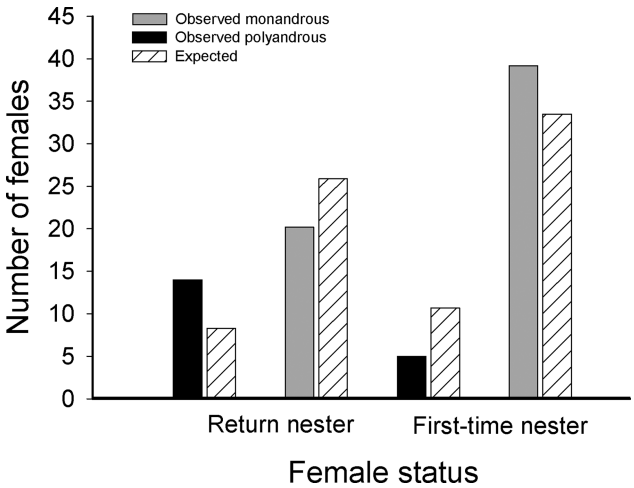

Figure 1
Numbers of polyandrous (black bars) and monandrous females (gray bars) that were remigrants (had previously nested at our study site) compared with first-time nesters. Solid bars show the observed values, and hashed bars show the expected values (based on the null hypothesis of no association between polyandry and remigration status).

success and offspring quality

Within polyandrous clutches, hatchling size was not affected by sire rank (primary or secondary), nor were offspring sired by primary fathers in polyandrous clutches different in size than offspring from monandrous clutches (Table 3). Furthermore, primary fathers did not have a higher proportion of offspring surviving to leave the nest than secondary fathers (Fisher's Exact test; $P = 0.143$), suggesting that males which achieved a high paternity share ("preferred" males) did not sire larger or more viable offspring. Hatchling size was strongly positively affected by incubation duration, in line with previous work (Glen et al. 2003), with a smaller quadratic effect of lay date and smaller effects of clutch size and year (alone and as interactions, Table 3).

DISCUSSION

We found no evidence in this study to suggest that polyandry confers direct or indirect benefits to female green turtles. Our analysis revealed no differences in fertilization success, hatching success, emergence success, or mean hatchling size between polyandrous and monandrous females. Furthermore, there was no evidence that males which sired a large proportion of polyandrous clutches

Table 2
Factors affecting estimators of reproductive success and hatchling size

Explanatory terms	Estimated effect (SE)	F_{df}	P
Clutch size (REML)			
Female size	2.62 (0.30)	$74.51_{1,68}$	**<0.001**
Lay date^2	-0.02 (0.005)	$17.61_{1,172}$	**<0.001**
Mating strategy			
Monandry	0.0 (0.0)	$7.20_{1,68}$	**0.009**
Polyandry	-11.05 (4.12)		
Year	0.11 (2.50)	$<0.01_{1,75}$	0.966
Constant	113.8 (2.10)		
Fertilization success (GLMM)			
Lay date^2	-0.001 (0.0002)	$15.74_{1,184}$	**<0.001**
Mating strategy			
Monandry	0.0 (0.0)	$1.58_{1,85}$	0.212
Polyandry	0.34 (0.27)		
Female size	-0.02 (0.02)	$1.13_{1,70}$	0.291
Year	-0.16 (0.15)	$1.07_{1,76}$	0.304
Constant	1.89 (0.11)		
Hatching success (GLMM)			
Female size × incubation duration	-0.01 (0.004)	$7.69_{1,209}$	**0.006**
Female size × lay date^2	-0.00001 (0.000002)	$5.55_{1,197}$	**0.019**
Incubation duration × year	0.07 (0.04)	$4.03_{1,209}$	**0.046**
Mating strategy			
Monandry	0.0 (0.0)	$0.09_{1,69}$	0.762
Polyandry	0.07 (0.23)		
Constant	1.14 (0.10)		
Emergence success (GLMM)			
Female size × incubation duration	-0.009 (0.003)	$6.32_{1,197}$	**0.013**
Female size × lay date^2	-0.000005 (0.000002)	$5.89_{1,183}$	**0.016**
Mating strategy			
Monandry	0.0 (0.0)	$1.71_{1,69}$	0.195
Polyandry	0.29 (0.22)		
Constant	0.68 (0.10)		
Mean hatchling size (REML)			
Female size × incubation duration	-0.73 (0.27)	$7.51_{1,147}$	**0.007**
Female size × clutch size × lay date^2	-0.00001 (0.000004)	$5.69_{1,129}$	**0.019**
Year	25.98 (13.36)	$3.78_{1,70}$	0.056
Mating strategy			
Monandry	0.0 (0.0)	$1.10_{1,66}$	0.298
Polyandry	-24.16 (23.01)		
Constant	1636.0 (10.4)		

Main effects and significant interactions are presented. Significant terms are shown in bold.

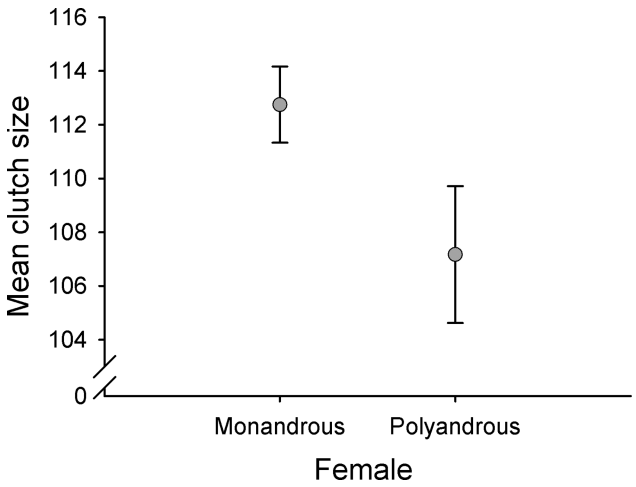

Figure 2
Effect of polyandry on total clutch size. Data points show fitted values of the models, which incorporate all predictor variables (see Table 2). Values are means ± 1 SE.

produced better quality offspring than males which achieved a smaller paternity share, or than sires of monandrous clutches. In contrast, we show that polyandrous females produce significantly smaller clutches than monandrous females, highlighting a potential cost to polyandry in green turtles.

Despite considerable evidence that polyandry affords indirect genetic benefits to females across a range of taxa (Jennions and Petrie 2000; Hosken and Stockley 2003; see INTRODUCTION), the lack of support for indirect benefits of polyandry in this study is consistent with previous work in green turtles, which found no relationship between multiple paternity and estimators of female reproductive success (Lee and Hays 2004). Similarly, Pearse et al. (2002) report no difference in hatching success between singly and multiply sired clutches of the freshwater turtle (*Chrysemys picta*), although, Zbinden et al. (2007) showed a weak, but significant, positive correlation between hatching success and number of sires in loggerhead turtles (*Caretta caretta*). Gaining meaningful measures of female and offspring fitness is difficult in marine turtles due to their long lifespan and complex life history. Hatching success and offspring size are strongly associated with posthatching offspring survival in pythons and freshwater turtles (Madsen and Shine 1998; Janzen et al. 2000) and are likely to be important fitness components; however, any benefits of polyandry in this species may only become evident if the reproductive success of females and their offspring are estimated over much longer timescales.

In more than 80% of polyandrous clutches in this study, paternal contributions were significantly skewed toward 1 or more males, raising questions about potential postcopulatory processes. However, this pattern may be a consequence of sperm precedence rather than any female-driven process, particularly considering that "preferred" males did not sire more viable offspring than secondary fathers. It has been hypothesized that first-male sperm precedence is likely to influence paternity patterns in marine turtles, due to the location of sperm storage tubules high in the female reproductive tract, and the behavior of male turtles, which leave breeding grounds early in the nesting season rather than continuing to mate with females as they ovulate new eggs (Fitzsimmons 1998).

Recent reviews of multiple paternity in birds and reptiles have questioned the importance of indirect benefits to females, and instead, these reviews have highlighted the roles of sexual conflict and ecological factors, such as population density and sex ratio, in driving multiple mating by females (Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005; Akçay and Roughgarden 2007; Uller and Olsson 2008). In the absence of positive fitness effects, costs of mating and of resistance to mating may be of central importance to female mating strategies, and levels of polyandry are likely to be influenced by mate encounter rates and levels of competition for access to mates. Sexual harassment by courting males can be extremely costly to females (Clutton-Brock and Parker 1995; Arnqvist and Rowe 2005; Sakurai and Kasuya 2008; Gay et al. 2009) and may be an expensive drain on energy resources, especially for capital breeders such as marine turtles (Bonnet et al. 1998), which rely largely on stored energy during the breeding period. Cost minimization could explain polyandry

Table 3
Factors affecting hatchling size (REML)

Explanatory terms	Estimated effect (SE)	F_{df}	*P*
Primary vs. secondary males (polyandrous clutches only)			
Incubation duration	15.94 (4.09)	$15.18_{1,11}$	**0.002**
Clutch size × lay date^2	-0.0003 (0.0001)	$5.59_{1,10}$	**0.041**
Female size	6.39 (2.75)	$5.41_{1,4}$	0.080
Sire status			
Primary	0.0 (0.0)	$2.07_{1,438}$	0.151
Secondary	-15.39 (10.70)		
Year	-1.41 (22.41)	$<0.01_{1,14}$	0.951
Constant	1636.0 (12.4)		
Primary males (polyandrous vs. monandrous clutches)			
Incubation duration	25.51 (3.71)	$47.42_{1,83}$	<0.001
Year	32.74 (12.04)	$7.40_{1,56}$	0.009
Lay date^2	-0.11 (0.05)	$4.00_{1,81}$	0.049
Primary sires from			
Monandrous clutches	0.0 (0.0)	$0.64_{1,58}$	0.426
Polyandrous clutches	-17.74 (22.14)		
Female size	1.26 (1.67)	$0.56_{1,69}$	0.455
Clutch size	-0.24 (0.41)	$0.35_{1,85}$	0.558
Constant	1624.0 (9.3)		

Main effects and significant interactions are presented. Significant terms are shown in bold.

in this scenario, if the cost of resistance to mating exceeds that of accepting additional mates. Lee and Hays (2004) suggested that convenience polyandry was the most likely explanation for multiple paternity in their study of green turtles, in which no fitness benefits were detected. This idea is supported by work that demonstrates higher levels of multiple paternity in high-density marine turtle populations, presumably due to increased encounter rates with males (Jensen et al. 2006). Male turtles exhibit very aggressive mating behavior (see Miller 1997; Bowen and Karl 2007 and references therein); females are often pursued by multiple males simultaneously, and mating pairs can be harassed and bitten by attendant males (Booth and Peters 1972; Miller 1997). There is evidence from a number of species that females will accept copulations from harassing males if they cannot avoid them (Clutton-Brock and Parker 1995), particularly when females are persistently courted by more than 1 male concurrently, but experimental support for convenience polyandry is sparse, particularly in vertebrates (Huchard et al. 2012; but see Rowe 1992; Cordero and Andrés 2002; Arnqvist and Rowe 2005 for examples in invertebrates).

The costs of mating and of resistance to mating are difficult to quantify in natural populations (but see Fitze et al. 2005; Maklakov et al. 2005), particularly for long-lived organisms, and no data exist from marine turtles. We showed that polyandrous green turtles produced significantly smaller clutches than monandrous females, but whether this represents a cost to females of polyandry is unclear. Uller and Olsson (2005) found that promiscuous female common lizards produced significantly smaller clutches but heavier offspring and speculated that multiple mating might result in higher quality offspring that benefit from more efficient yolk utilization. Female turtles might also increase their investment into eggs in response to polyandry, or mate quality, as has been demonstrated in other taxa (Cunningham and Russell 2000; Sheldon 2000). However, we found no effect of polyandry on mean hatchling size in this study, as might be expected if polyandrous females were adjusting their nutrient allocation to offspring in relation to multiple mating or male traits. Furthermore, evidence indicates that yolk formation is completed prior to courtship in marine turtles (Hamann et al. 2003), suggesting that the potential for postcopulatory nutrient adjustment to eggs is low. An alternative interpretation of our results is that lower quality females produce smaller clutches and are also more likely to be polyandrous because they are more vulnerable to multiple mating by coercive males, or are themselves less choosy.

Other studies have found multiple paternity to be positively correlated with clutch size and female size in turtles (Pearse et al. 2002; Zbinden et al. 2007) and other reptiles (Eizaguirre et al. 2007; Lance et al. 2009), suggesting a male preference for larger (and more fecund) females. In contrast, we found no difference in size between polyandrous and monandrous females. We show that polyandry was significantly more common in remigrant females (that have previously nested at our study site) compared with first-time nesters (potential new recruits, although it is possible that they have nested previously elsewhere), which could result from more intense courting of older and more experienced females, or perhaps experienced females actively seek additional mates. Remigrant females might also arrive at mating grounds earlier and encounter more males than first-time breeders (Hamann et al. 2003). Alternatively, this result may reflect sperm storage across breeding seasons, with returning females utilizing residual sperm from a previous breeding season. Long-term sperm storage has been reported in freshwater turtles (up to 4 years; Ewing 1943), as well as multiple paternity resulting from the use of sperm stored from previous seasons (Pearse et al. 2002). Whether sperm stored over several years is of any value in marine turtles is not clear and will depend on breeding intervals of particular females. Finally, it is possible that both males and females employ different mating strategies, influenced by age, body size, condition, and mating history. For example, larger females may mate multiple times because they are preferred by males and have a choice of high-quality mates, whereas smaller females may show high levels of multiple paternity because they are more easily manipulated by both small and large males, potentially masking any link between polyandry and fitness in this study.

Due to the difficulty of reliably observing mating behavior of marine turtles at sea, molecular parentage analysis has been fruitfully employed to gain insights into their mating strategies (reviewed in Bowen and Karl 2007; Lee 2008). Here, we used microsatellites to detect multiple paternity of clutches and infer polyandry, as do many other studies of mating systems in natural populations (e.g., Lee and Hays, 2004; Eizaguirre et al. 2007; DiBattista et al. 2008). However, using this approach to examine potential benefits of polyandry is not straightforward because, under scenarios of cryptic female choice or strong sperm competition, multiple mating might not result in multiple paternity (Birkhead and Møller 1998). Behavioral observations or experimental manipulations are required in conjunction with the use of molecular techniques, in order to gain a more complete understanding of the fitness consequences of multiple mating in marine turtles.

CONCLUSIONS

The ubiquity of multiple paternity in marine turtle populations, albeit at widely varying levels, suggests that female promiscuity is common in these species and that pay-offs from polyandrous behavior are important. We were unable to demonstrate any direct or indirect benefits to polyandrous females in this study, suggesting that alternative explanations for the high frequency of multiple paternity should be explored. The costs of mating and of resistance to mating, possibly influenced by population density and sex ratio, may well be key determinates of polyandry in marine turtles, although quantifying these costs in natural populations would be difficult. Our results highlight the apparently complex influences of female traits, environment, and mating strategy on reproductive success in natural populations and the challenges associated with unraveling them.

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