

Original Article

Alternative reproductive tactics in female horseshoe crabs

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Consistent differences among females in mating with one (monandrous) or multiple males (polyandrous) may be a product of male behavior or may reveal the existence of female alternative reproductive tactics (ARTs). The distinction is an important one for understanding the evolution of sexually selected behavior. We evaluated whether ARTs exist in female horseshoe crabs (*Limulus polyphemus*), a species in which male alternative reproductive tactics are well known. In this species, attached pairs migrate to shore and spawn on high tides; the male fertilizes the female's eggs externally with free-swimming sperm as the eggs are being laid. Unattached males are attracted to pairs by visual and chemical cues and become satellites of some females while ignoring others. We used multiple lines of evidence, including mark/resighting; measurements of size, physical condition, and eggs laid; and field manipulations of female response to satellite males. We show that even at high nesting densities with intense male–male competition, some females mated only with their one attached male, and females were consistently monandrous or polyandrous across multiple nestings. Monandrous females did not attract satellites but when males were experimentally manipulated to join monandrous pairs, some females stopped nesting and left rather than nest with a satellite male. These females tended to be smaller than polyandrous females. Our results suggest condition-dependent differences between monandrous and polyandrous females that result in different context-dependent mating decisions by monandrous and polyandrous females to cope with sexual conflict. *Key words*: alternative reproductive tactics, horseshoe crabs, mate choice, sexual selection. [*Behav Ecol*]

INTRODUCTION

Females of many species attract and mate with multiple males. Such behavior is normally viewed either as a product of male–male competition and costly to females or as a way of improving the females' mating options. Benefits from multiple mating for females may arise by increasing their fertilization success or mate quality (trading up, good genes), by ensuring compatibility with their mates, or by increasing genetic diversity of their offspring (Zeh and Zeh 1997, 2001; Jennions and Petrie 2000; Yasui 2001; Evans and Marshall 2005; Simmons 2005). Variation in numbers of mates is normally thought to be continuous and normally distributed, but consistent discrete differences among females in mating with one (monandrous) or multiple (polyandrous) males may reveal the existence of alternative female mating tactics (Brennan et al. 2008; Oliveira et al. 2008). Alternative reproductive tactics (ARTs) that are associated with differences in numbers of mates have been found in females of a few species (Hatchwell and Davies 1990; Cordero et al. 1998; Byrne and Whiting 2011). Some evolve in response to mate conflict and male coercion (Magellan and Magurran 2006; Alonzo 2008), whereas others arise from differences in fecundity or investment in eggs (Alonzo and Sinervo 2001; Ruppell et al. 2001; Vercken et al. 2007) or from consistent differences among females in preferences for males (Hatchwell and Davies

1990; Van Gossum et al. 1999; Hunt and Simmons 2001; Sinervo et al. 2001).

Most clear examples of female ARTs are genetic polymorphisms with morph differences in color, morphology, and behavior (Cordero et al. 1998; Sinervo 1999; Sinervo et al. 2000; Morris et al. 2003; Svensson et al. 2005; Pryke et al. 2007; Dijkstra et al. 2009; Cox and Calsebeek 2011). In contrast, most male ARTs are known to be condition- or status-dependent (Gross 1996; Brockmann 2001), that is, the result of age, size, developmental, life-history, or physiological differences among males. Is this a real difference between the sexes or simply an artifact of more intense research having been conducted on male ARTs? Do consistent condition-dependent female ARTs exist (Alonzo 2008)? Most male ARTs involve direct male–male competition but females do not typically compete directly for resources or mates so one would expect female ARTs to be less common (Taborsky 2008). However, if female access to high quality or compatible mates is limited, then we might expect behavior to evolve to circumvent this limitation. Similarly, if reproductive costs and benefits change with the amount of resources available or accrued as juveniles, then we would expect condition-dependent ARTs to evolve for females of different ages or sizes (Lyon 1993; Henson and Warner 1997). Female ARTs may also arise from context-dependent female choice (e.g., Sih and Krupa 1992; Magurran et al. 1995; Alonzo and Sinervo 2001) or reproductive patterns (e.g., Goldschmidt et al. 1993; Ruppell and Heinze 1999; Westmeat et al. 2000). Whenever female ARTs arise, they will have important implications for understanding the evolutionary dynamics of reproduction, mate conflict, and sexual selection (Svensson et al. 2005). Differences in patterns of female choice, for example, can shape the evolution of male–male competition, sperm competition, and sexual dimorphism (Eberhard 1996; Hunt and Simmons 2000, 2001; Zeh and Zeh 2003). In this study, we examine whether female ARTs

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Figure 1

Photograph of nesting horseshoe crabs at Seahorse Key, FL. The pair on the left (the front portion of the female is buried) is nesting with 4 satellite males (polyandrous), whereas the nesting pair (female mostly buried in the sand) on the right is nesting without satellites (monandrous).

exist in horseshoe crabs (*Limulus polyphemus*), a species with condition-dependent male ARTs (Brockmann 2003a, 2003b).

Female horseshoe crabs, each with an accompanying male attached to her posterior spines, nest near the high tide line of Atlantic and Gulf coastal beaches (Shuster et al. 2003). The female deposits her eggs in the sand, which are fertilized externally by free-swimming sperm (Brockmann 2003b). Unattached males also come to the beach where they are attracted to spawning pairs by visual and chemical cues (Hassler and Brockmann 2001; Schwab and Brockmann 2007; Saunders et al. 2010) and crowd around them forming mating groups (Brockmann 2003b). Paternity analyses reveal that the attached male fertilizes all the female's eggs when spawning alone, but when unattached males are present, these satellites fertilize some to most of the female's eggs depending on satellite number, position, and other factors (Brockmann et al. 1994, 2000).

Horseshoe crabs often nest in dense aggregations, but even when the operational sex ratio is strongly male biased, some females nest only with their attached males so their offspring are fathered by one male, whereas at the same time and nearby, others nest in groups resulting in multiple male paternity for their offspring (Figure 1; Brockmann 1990). Pairs with few or no satellites are less likely to attract new satellites than are polyandrous females whose satellites have been removed (Brockmann 1996). Unattached males are attracted to polyandrous but not monandrous pairs (using visual and chemical cues; Hassler and Brockmann 2001; Saunders et al. 2010), before the eggs are laid and before other unattached males have arrived (Schwab and Brockmann 2007). These results suggest that the attractiveness of a pair to unattached males is due to the female or her attached male. However, Johnson and Brockmann (2010) showed through experimental manipulations in the field that multiple mating is costly for all females, but when allowed to nest naturally, monandrous and polyandrous females are equally successful. Taken together, the results of these earlier studies suggest 2 possible hypotheses: 1) from the male perspective, single versus group nesting results from differences in unattached male behavior toward females or their attached males (e.g., Brockmann et al. 2000; Hassler and Brockmann 2001) or 2) from the female perspective, single (monandrous) and multiple (with satellites, i.e., polyandrous) mating may be due to female mating decisions,

that is, female ARTs. Distinguishing between these possibilities is difficult in any system, particularly if the behavior is condition- or context-dependent, as seems likely here because there are no immediately obvious differences between monandrous and polyandrous females. To understand the evolution of reproductive behavior, we must know whether monandry (and single male paternity) and polyandry (multiple male paternity) result from the evolution of male or female decision rules.

Here, we present data to evaluate whether female ARTs exist in horseshoe crabs. 1) Spawning surveys measure the frequency of monandrous and polyandrous nesting at different nesting densities and operational sex ratios. If the differences in female behavior result from female ARTs, then monandry and polyandry will occur regardless of nesting density or numbers of males present. Alternatively, if monandry and polyandry result from differences in male behavior, then polyandry should be more common at higher operational sex ratios. 2) Marking and resighting studies evaluate the consistency of monandry and polyandry for individual females when mated with the same or different males. These data help to establish whether a female's mating tactic is affected by characteristics of the female or her attached male. If monandry and polyandry depend on female characteristics (i.e., condition-dependent tactics), then a female's tactic should remain the same even when paired with a different male. If female mating tactic depends on characteristics of the attached male (i.e., context-dependent tactics), however, then the mating tactic would be expected to change if the attached male changes. 3) A comparison of size and physical condition allows us to determine whether there are consistent differences between monandrous and polyandrous females or their attached males. If monandry and polyandry are condition-dependent female ARTs based on size or physical condition, then we should find that one tactic is consistently in poorer physical condition (as occurs with male ARTs in this species; Brockmann 2002) or smaller than the other. If monandry and polyandry are context-dependent, then polyandry should occur when females are attached to smaller males or males of lower quality (i.e., older males that are in poorer physical condition). Alternatively, unattached males may be attracted to larger, more fecund females or to pairs whose unattached males are smaller or in poorer condition. 4) Similarly, a study on the number, sizes, and developmental rates of eggs laid by monandrous and polyandrous females allows us to determine consistent differences between tactics. 5) One problem with a field study of this sort is that monandrous females might be just polyandrous females that have not yet attracted satellites. To evaluate this possibility, we conducted an experimental manipulation in which we attracted unattached males and encouraged them to join nesting monandrous pairs (using the method developed by Johnson and Brockmann 2010). This test identified some females as "intolerant" of nesting with satellite males (i.e., they left the beach when the satellite arrived) and some as "tolerant" (continued nesting when the satellite arrived). We then compared the size and physical condition of tolerant and intolerant females and their attached males to see if there were any consistent differences (see 3 above). In this study, we present evidence for consistent condition-dependent differences between monandrous and polyandrous females that may result in context-dependent mating decisions to cope with sexual harassment and to ensure successful mating with high quality or compatible males.

MATERIALS AND METHODS

The data for this study were compiled from a long-term study on a population of horseshoe crabs nesting along a 1-km

stretch of sandy beach at Seahorse Key (SHK), which is part of the Lower Suwannee National Wildlife Refuge (near Cedar Key, Levy County, FL) on the west (Gulf) coast of Florida (Brockmann and Johnson 2011). The research was conducted on high tides associated with the new and full moons (from 2 days before to 5 days after each new or full moon) when horseshoe crabs are known to spawn (Cohen and Brockmann 1983; Brockmann 1990, 1996). Previous research has shown that most females come to the breeding beaches during only 1 week within a year when they lay all their eggs during high tides in 1–5 nesting bouts (Brockmann 2003a; Brockmann and Johnson 2011). Data were collected in March–May 1993–1997, 2000, 2004–2005, and 2008–2009 (no data were collected in any of the intervening years). Although the details of data collection differed somewhat in the different years of the study as different questions were emphasized, the basic approach was the same from year to year, and we used the combined data in our analyses unless otherwise indicated.

What is the frequency of monandry and polyandry?

Spawning survey

During each high tide, just prior to the time of the maximum predicted high tide at SHK, we walked once along the 1-km beach and recorded the number of nesting pairs. We also counted the number of males that were not paired with a female (unattached males) and the number of males associated with each nesting pair in a spawning group (satellite males, i.e., those that are physically in contact with a pair or in contact with another individual that is in contact with the pair). The mating status of pairs with no satellites was classified as monandrous, and pairs with at least one satellite were polyandrous. From these data, we calculated the frequency of monandrous and polyandrous females under different nesting conditions.

Satellites added test

It is possible that monandrous females are simply polyandrous females that have not yet attracted a satellite male. To distinguish monandrous from polyandrous females, we used an experimental assay developed by Johnson and Brockmann (2010). We located a monandrous pair on the beach and observed them for 2 min to ensure that they were nesting and that they were not going to attract a satellite. A nearby, unattached male was then attracted to the pair (unattached males are attracted by dark moving objects and will readily follow your feet or can be gently guided toward a nesting pair) where he had to remain throughout the trial and interact with the attached male to be included in the data set. If the female left the beach spontaneously in less than 8 min after having been joined by the satellite male then she was considered intolerant and if she stayed past 8 min she was considered tolerant of satellite male presence. This 8-min cutoff was the median time that a female continued to nest after a satellite joined the pair in the Johnson and Brockmann (2010) experiment that used the same methodology. As the female left or after 8 min, the pair was pulled from the sand, marked, and placed in a wading pool for later measurement (see below).

Are individual females consistently monandrous or polyandrous? Tagging and resighting

Adult horseshoe crabs were picked up by hand along the shoreline during the high tide, males were detached from females, and the animals were placed in seawater-filled wading pools until after the tide when they were measured and released. Individuals were given a “temporary” tag (a thumb tack with a piece

of embossing tape bearing a unique number pressed into the lower lateral portion of the prosoma; Cohen and Brockmann 1983) as they were placed in a pool and their original mating status was recorded (monandrous or polyandrous and the number of satellites present). To evaluate whether females were consistently monandrous or polyandrous, we used data from 1996 to 1997 because in these years, our field methods emphasized locating and identifying marked animals as they returned to the beach to breed. On the tides that followed marking, we identified individuals by walking repeatedly up and down the beach during each high tide (from 2 h before to 2 h after the maximum high tide) recording the identities of all marked females and the number of associated satellite males. These data were then compared with the females’ original mating status at the time of marking (when attached males were removed).

In 2000, we conducted an experiment to determine whether females changed their mating status depending on whether or not they changed their original attached male. We either detached the attached male from a marked female (reaching underneath and pushing the male’s claws from the female’s posterior spines without otherwise disturbing her) or allowed the attached male to remain attached to his original mate (similarly jostling the female to control for disturbance). When females returned to nest on the following high tides, we recorded their mating status. If mating status depends on female size or physical condition, then we would expect no change in mating status whether the attached male had changed or not. However, if mating status is context-dependent, that is, determined by the quality or characteristics of the attached male, then we would expect females with experimentally detached males (new partners) to be more likely to change their original mating status, whereas females that did not change partners would retain their original mating status.

Do monandrous and polyandrous females or their attached males differ?

We measured each tagged horseshoe crab and evaluated his/her surface condition. Differences in female size or physical condition allowed us to determine whether they showed condition-dependent differences. Differences in attached male size or physical condition allowed us to evaluate context-dependent differences between females. We measured the interocular (IO) distance between the lateral eyes (measured dorsally with a tape measure), which is strongly correlated with carapace width (the width of the prosoma at its widest point, which has been used in other studies, e.g., Brockmann and Penn 1992). Horseshoe crabs have a terminal molt so as the adult ages its prosoma darkens (Penn and Brockmann 1995; Brockmann 2002), the amount of pitting from chitinoclastic bacteria and algae increases and the amount of mucus present on the surface declines (Harrington and Armstrong 1999, 2000; Harrington et al. 2008). We measured the physical condition of each individual using an index made up of 2 values: carapace color (light, medium, or dark scored as 5, 3, or 1) and the surface quality of the carapace (the amount of mucus and degree of pitting present on the prosoma scored as 1–5). An individual in perfect physical condition received an index value of 10.

Do monandrous and polyandrous females differ in egg number, egg development, or egg size?

We walked along the shore during high tide and identified one monandrous and one polyandrous female nesting at the same time. Nesting females are partially to completely buried in the sand (Schwab and Brockmann 2007), and each female

alternates periods of plowing forward in the sand, with her attached males and associated satellites in tow, with stationary periods of laying a cluster of eggs. We marked the locations and times of each of the female's egg-laying pauses by inserting wire flags on either side of her prosomal–opisthosomal hinge while keeping track of the presence or absence of satellites. After laying a number of egg clusters, the female bends, pulls herself out of the sand, and returns to the sea, leaving her eggs behind to develop deep in the sand. After the high tide receded (3–6 h after the eggs were laid), we returned to marked nests and dug up each flagged egg cluster. The eggs were counted by sieving them out of the egg–sand mixture (using 1-mm mesh plastic screening) and measuring the number of eggs by transferring them to a graduated cylinder (88 eggs/ml; Brockmann et al. 2000). In this way, we compared the number of eggs laid and the rate of egg laying (eggs/min) in the first 3 clusters we observed by monandrous and polyandrous females that were nesting at the same time. Sometimes during nesting the female paused as though she were laying eggs, and we placed flags in the sand to indicate this location but later when digging up the nest we found that no eggs were laid during this pause. Because monandrous and polyandrous females may differ in the frequency with which they fail to lay, we also counted the proportion of clusters that contained eggs (“proportion with eggs”).

The methods for measuring egg laying changed slightly from year to year (and no egg-laying data were taken in 1996, 1997, and 2000). In 1993–1995, we observed females while they laid 3–5 consecutive clusters of eggs at any point during the laying process (we just observed females as we came upon them). In 2004–2005, we observed a female from the moment she arrived on the beach through the first 5 egg clusters and in 2008, from the moment she arrived until she left spontaneously. If the number of eggs or the rate of egg laying declined (or increases) over a series of consecutive egg-laying bouts, then these differences in methods may account for year-to-year variation in our results.

In 2009, we reared eggs in the field to measure the success of monandrous and polyandrous females. We took a sample of sieved eggs (100–200) from randomly chosen clusters of monandrous and polyandrous nests (equal numbers matched for date and tide) and placed them in small bags made of plastic screening. The bags were then placed in the beach at the depth and location of the original cluster (bags from matched monandrous and polyandrous females were reared next to one another). We dug up the matched bags at the same time 13–29 days later, we checked to see if there was any development at 2 weeks and if not we reburied the bags and dug them up again 2 weeks later and recorded the proportion of eggs that had developed and the stage of development that was reached (young or old embryos or larvae).

We measured egg size (mean diameter) from 18 monandrous and 18 polyandrous females that were randomly collected from the shore. Unfertilized eggs were collected from the females by electro-ovulation according to the methods of Brown and Clapper (1981). Digital images (taken 20 h postrelease to ensure eggs had enough time to round out; unfertilized eggs go through the same changes as fertilized eggs in the first 20 h; Brown and Clapper 1981) of 10 unfertilized eggs/females were analyzed using SigmaScan Pro.

Analysis

Females and their attached males differed significantly from 1 year to the next in size (IO distance, female IO: $P \leq 0.0001$, degrees of freedom [df] = 8, 1568; attached male IO: $P \leq 0.0001$, df = 8, 1550) despite our always taking the size measurements in the same way. Therefore, we analyzed the size of

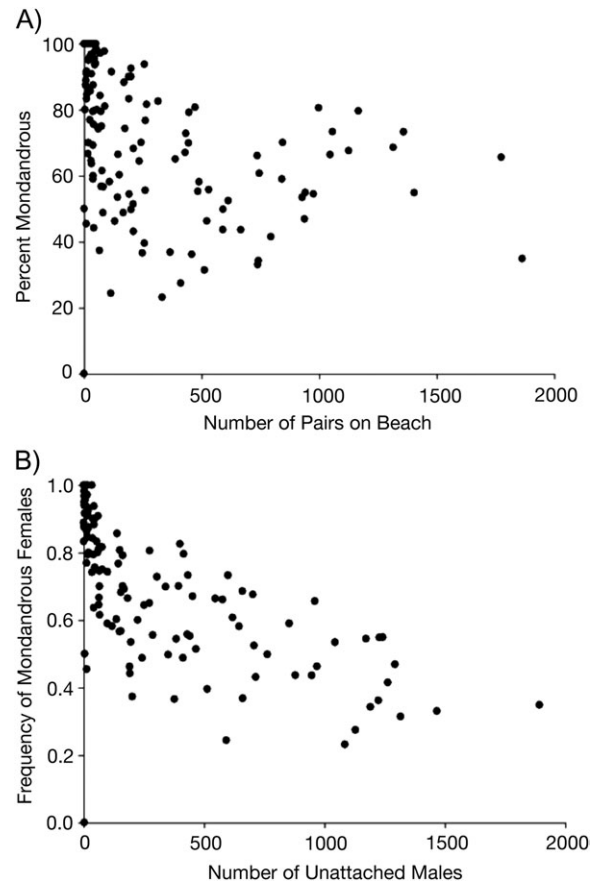


Figure 2

(A) The relationship between the proportion of female horseshoe crabs that are monandrous and the density of nesting pairs. (B) The relationship between the proportion of female horseshoe crabs that are monandrous, and the number of unattached males present on the nesting beach. The data are from 157 high tides on which we counted one or more pairs along a 1-km beach at Seahorse Key, FL during spring 2004, 2005, 2008, and 2009.

monandrous and polyandrous females and their attached males using a mixed model analysis of variance (R v. 2.10.1) with size as the dependent variable, female status (monandrous or polyandrous) as a fixed effect and year as a random effect. All other data were analyzed in JMP v. 8.0 or SigmaPlot v. 12.0. Data were inspected for normality. We use nonparametric analyses on nonnormal data and present medians for these data.

RESULTS

What is the frequency of monandry and polyandry?

Spawning survey

The proportion of females that were monandrous was 20% or more across a wide range of nesting densities (Figure 2A). This proportion declined significantly with an increased number of unattached males present on the nesting beach (Figure 2B; Spearman correlation coefficient -0.9 , $P < 0.001$) but remained above 20% even when unattached males were abundant.

Satellites added test

The satellites added test showed that 62% of monandrous females were intolerant and 38% ($N = 87$) were tolerant of nesting with satellite males.

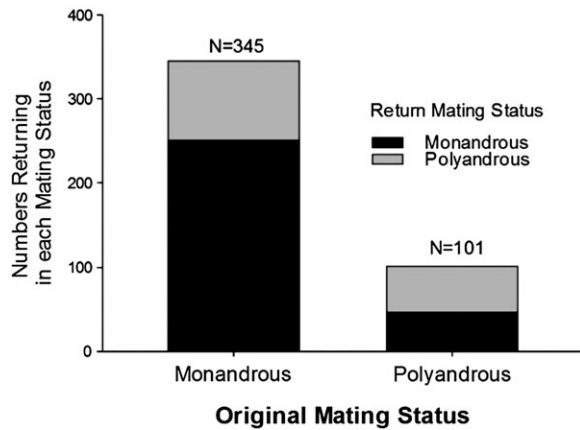


Figure 3

The number of marked monandrous and polyandrous females resighted in the same nesting status (monandrous or polyandrous) despite the fact that the original attached male had been removed during marking.

Are individual females consistently monandrous or polyandrous?

When attached males were removed from females, 72% ($N = 345$) of monandrous females nested again on subsequent tides without satellite males, whereas only 54% ($N = 101$) of females that were originally polyandrous nested again with satellites. Monandrous females were significantly more likely to retain their mating status than polyandrous females when attached males were removed (1996 and 1997 data; $\chi^2 = 21.3$, $df = 1$, $P < 0.001$; Figure 3). This result could arise if monandrous females were resighted more often than polyandrous females, but this was not the case (median 2, $P = 0.28$, Mann–Whitney U test). To compare monandrous and polyandrous females nesting at the same time with and without males removed, we conducted an experimental manipulation in 2000. Of those females that remained with their original attached males (controls), 83% ($N = 97$) maintained their original mating tactic, whereas only 68% ($N = 104$) of the experimentally detached females retained their original mating tactic ($\chi^2 = 4.7$, $df = 1$, $P = 0.03$). When only those females that were originally monandrous were considered, significantly more of the controls (90%) remained monandrous than the experimentals (74%; $\chi^2 = 6.4$, $df = 1$, $P = 0.01$).

Do monandrous and polyandrous females differ in size or condition?

Monandrous females were, on average, slightly smaller than polyandrous females (monandrous: mean = 13.75 cm \pm 0.033 standard error [SE], $N = 1059$; polyandrous: mean = 13.85 cm \pm 0.05 SE, $N = 507$; female status (fixed effect): t value = 2.23, $P = 0.026$). Monandrous females were also in somewhat better condition than polyandrous females (monandrous: median = 7, $N = 1060$; polyandrous: median = 6, $N = 509$; Mann–Whitney U statistic = 229379, $P < 0.001$). Intolerant monandrous females were smaller than tolerant monandrous females, though this difference was marginally nonsignificant (intolerant: median = 13.05 cm; tolerant: median = 13.65 cm; Wilcoxon test statistic = 120, $P = 0.058$, $N = 37$ pairs). There was no difference in the condition of tolerant and intolerant females (intolerant: median = 6, $N = 37$; tolerant: median = 6, $N = 37$; Wilcoxon test statistic = -11.5, $P = 0.82$).

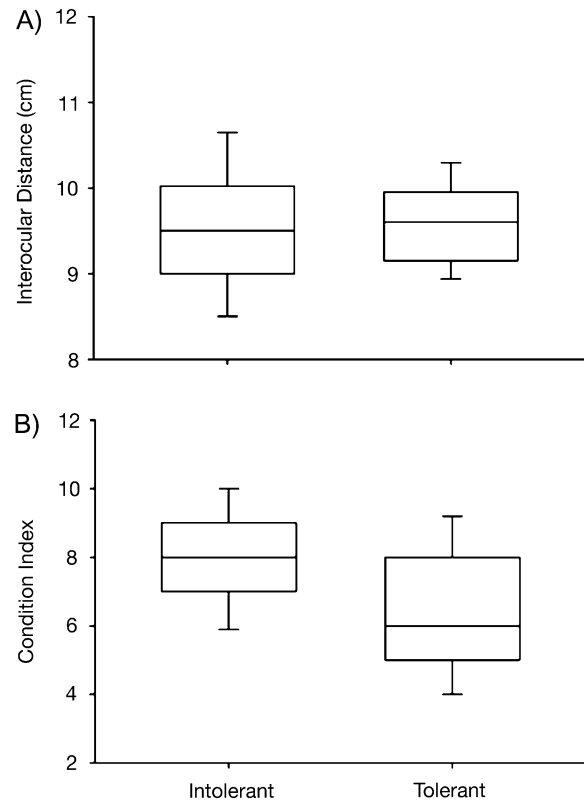


Figure 4

Median size (A) and condition (B) of the attached males of 2 types of monandrous female horseshoe crabs: intolerant females leave rather than nest with satellites and tolerant females will nest with satellites if one arrives. Box plots show 25th and 75th percentiles (box), median (line within box), and range (whiskers).

Do attached males of monandrous and polyandrous females differ in size or condition?

Attached males of monandrous and polyandrous females did not differ in size (monandrous: mean = 9.57 cm \pm 0.028 SE, $N = 1049$; polyandrous: mean = 9.61 cm \pm 0.039 SE, $N = 504$; female status (fixed effect): t value = 0.26, $P = 0.79$) or condition (monandrous: median = 8, $N = 1060$; polyandrous: median = 8, $N = 509$; Mann–Whitney U statistic = 261448, $P = 0.97$). Attached males of tolerant and intolerant monandrous females did not differ in size (intolerant: median = 9.5 cm; tolerant: median = 9.6 cm; Wilcoxon test statistic = 4.00, $P = 0.95$, $N = 37$ pair; Figure 4A), but attached males of intolerant females were in better condition than attached males of tolerant females (intolerant: median = 8; tolerant: median = 6; Wilcoxon test statistic = -187.00, $P = 0.0002$, $N = 37$ pair; Figure 4B).

Do monandrous and polyandrous females differ in egg number, egg size, or egg development?

Monandrous females laid fewer eggs than polyandrous females in all 6 years of the study and were significantly different in 2 of those years (Table 1). This result could occur if monandrous females laid more slowly than polyandrous females or if monandrous females failed to lay more often than polyandrous females, but Table 1 shows that monandrous and polyandrous females do not differ in the rate of egg laying and they lay with equal frequency. We found a positive relationship between female size (IO) and the number of eggs laid in 2004 ($r^2 = 0.12$, $P = 0.004$) but no relationship in any other year (1993:

Table 1

Median number of eggs laid in 3 clusters, the rate (number of eggs per minute) of laying, and the proportion of the clusters that contained eggs

Year	Median number of eggs laid			Rate of laying eggs per minute			Proportion of clusters with eggs (N)	
	Monandrous	Polyandrous	P^a	Monandrous	Polyandrous	P^a	Monandrous	Polyandrous
1993	3300	5280	0.36	316	499	0.63	0.88 (N = 36)	0.88 (N = 8)
1994	4004	4840	0.38	440	407	0.98	0.82 (N = 22)	1.0 (N = 6)
1995	3696	6820	0.03	337	487	0.11	0.86 (N = 69)	0.93 (N = 28)
2004	3476	5192	0.03	484	821	0.04	1.0 (N = 33)	1.0 (N = 35)
2005	2728	3080	0.76	660	598	1.00	0.84 (N = 50)	0.88 (N = 60)
2008	5500	5720	0.25	345	355	0.77	0.95 (N = 40)	0.96 (N = 55)

^a Mann–Whitney test.

$r^2 = 0.007$, $P = 0.57$; 1994: $r^2 = 0.015$, $P = 0.53$; 1995: $r^2 = 0.006$, $P = 0.44$; 2005: $r^2 = 0$, $P = 0.98$; 2008: $r^2 = 0.03$, $P = 0.10$).

It was not possible to determine whether tolerant and intolerant females laid different numbers of eggs because the intolerant females left quickly after being joined by a satellite male and therefore did not always lay.

Egg size (monandrous: median = 1.88 mm, $N = 18$; polyandrous: median = 1.87 mm, $N = 18$; chi square = 0.29; $df = 1$, $P = 0.60$) did not differ for monandrous and polyandrous females. The proportion of eggs that developed under natural conditions in the sand (monandrous: mean = 99.6%; polyandrous: mean = 99.2%; Wilcoxon test, $P = 0.39$, $N = 29$), the proportion that reached the last embryonic stage or more (monandrous: mean = 28%; polyandrous: mean = 35%, $P = 0.30$), and the proportion that reached the free-swimming larval stage (monandrous: mean = 18%; polyandrous: mean = 17%; $P = 0.81$) was the same for monandrous and polyandrous females.

DISCUSSION

Are monandry and polyandry alternative reproductive tactics (ARTs)?

We have presented evidence to suggest that mating with a single attached male (monandry and single male paternity) and mating with multiple males (polyandry and multi-male paternity) are female ARTs (Figure 1). Even when spawning densities are high and many unattached males are present (high operational sex ratio of males:female on the nesting beach), 20% of pairs remain monandrous (Figure 2). Similar results were obtained in a study of Delaware Bay horseshoe crabs (5–20% of pairs are monandrous; Brockmann 1996) where nesting densities and operational sex ratios are usually much higher than at our Florida study site. The satellites added test reveals that some monandrous females (62%) are exclusively monandrous, that is, they will leave the nesting beach rather than nest with a satellite male (intolerant monandrous females) and some will nest either as a monandrous or as a polyandrous female (38% tolerant monandrous females), but they seem not to attract satellites (or are not attractive to unattached males). We have also shown that the frequency of monandry declines as the number of unattached males increases (Figure 2B). Taken together, these results suggest that the presence of single and group nesting females is a result of both male behavior, as previously thought (Brockmann 1996; Brockmann et al. 2000; Hassler and Brockmann 2001), and female mating decisions (i.e., female ARTs). Similarly, in the dung beetle, male ARTs are driven by female provisioning behavior, so they are a result of both female behavior and male mating tactics (Hunt and Simmons 2000, 2001). Both male and

female behaviors also determine the variable mating patterns of dunnocks (Hatchwell and Davies 1990).

Our resighting of marked females shows that individual monandrous females are particularly likely to remain monandrous from one nesting to the next (Figure 3). This result is in agreement with a previous study conducted in Delaware Bay (Brockmann 1996) where monandrous pairs were more likely to stay monandrous than nearby polyandrous pairs that had all their satellites removed. In this previous study, females remained attached to the same males, but we show here that even when the attached male is replaced, monandrous females tend to remain monandrous. This suggests that the monandrous mating tactic is due to female behavior rather than being a property of her attached male or just the result of differences in satellite male behavior toward females. The satellites added test shows that many monandrous females leave the beach rather than nest with satellite males. Taken together then, it appears that monandry and polyandry are due to consistent and distinct differences among females in whether they spawn with satellite males or not.

Our results suggest that it is possible to misidentify monandrous and polyandrous females. Quick sightings during a spawning survey, for example, could identify a female as polyandrous when she is monandrous but has not yet had an opportunity to leave the beach or one could identify a female as monandrous when in fact she may be tolerant of nesting with satellites but has not yet attracted additional males. We have found no way to distinguish tolerant from intolerant monandrous females without conducting a satellites added test. In spite of these uncertainties, we were able to detect differences between females that mate with one versus multiple males.

Previous studies have shown that satellite males are attracted to nesting females by visual and olfactory cues and are able to detect small differences in female size (Schwab and Brockmann 2007), such as the difference in size between monandrous and polyandrous females. Unattached males are also more attracted by chemical cues from polyandrous pairs than from monandrous pairs (Hassler and Brockmann 2001; Saunders 2011), but it is not known whether the chemical cues are a female attractant (or for monandrous females a chemical repellent or lack of attractant) or a by-product of nesting (such as odors from eggs or ejaculates). Males are also attracted to odors from the eggs that females lay but careful observations of nesting pairs, and the timing of egg laying reveals that unattached males are often attracted to polyandrous pairs before the female has laid any eggs, which suggests that odor cues from the eggs are not the only source of chemical attraction (Schwab and Brockmann 2007; Saunders et al. 2010). None of these studies explicitly distinguishes between cues from the female or her attached male although it seems unlikely that satellite attraction cues would come directly from attached

males because their paternity is greatly reduced when satellites are present (Brockmann et al. 1994, 2000). Monandrous females continue to be monandrous when their mates have been replaced (this study), suggesting that cues emanate from the female, but an alternative hypothesis is that monandrous females always attract higher quality attached males and hence a cue that inhibits satellite approach may emanate from the attached males of monandrous females. The only way to distinguish between the 2 explanations is to conduct a manipulative experiment in which females are paired with pre-assigned males of known quality (such as exchanging the attached males of monandrous and polyandrous females to see whether females retain their original mating tactic with the new male).

Female ARTs are both condition- and context-dependent

Our marking and resighting observations and experiment suggest that female mating tactics have both condition-dependent and context-dependent effects. Females, particularly monandrous females, retain their mating tactic even when their original attached male has been removed and a new male has paired with her (Figure 3). This means that a female's mating tactic is a property of the individual female, that is, condition-dependent. However, in the experimental manipulation conducted in 2000, monandrous females that retained their attached male were significantly more likely to remain monandrous than monandrous females whose attached male had been removed. This result means that a female's mating tactic is affected by her attached male, that is, her mating tactic is context-dependent.

Condition-dependence of female ARTs in horseshoe crabs

In horseshoe crabs, monandrous females are smaller than polyandrous females and intolerant monandrous females are slightly smaller than tolerant ones. This result is consistent with previous studies (Brockmann 1996; Hassler 1999; Schwab and Brockmann 2007) that also found monandrous females to be smaller than polyandrous females, but they interpreted this result as caused by differences in male behavior. We and one previous study (Schwab and Brockmann 2007) found that monandrous females are in better condition than polyandrous females. Finally, this and previous studies found that monandrous females lay fewer eggs than polyandrous females (Brockmann 1996; Hassler 1999; Schwab and Brockmann 2007; Table 1), but there are no differences in egg size (this study) or energy content of the eggs (Hassler 1999) between monandrous and polyandrous females. Taken together these results support the view that female mating tactics (monandry and polyandry) are associated with condition-dependent differences (e.g., size, age, and physical condition) and possibly with differences in reproductive investment.

There are several possible explanations for the condition-dependent differences in female mating tactics of horseshoe crabs viewed from a male or female point of view. 1) Males may be more attracted to polyandrous females because they lay more eggs. Satellite males share paternity with the attached males and with other satellites (Brockmann et al. 1994, 2000), but polyandrous females lay more eggs, and it is possible that satellite males might benefit from joining a polyandrous female and sharing paternity rather than joining the less fecund monandrous females. Hassler (1999) calculated that as a first or second male on a polyandrous female, a male would on average gain more fertilizations than as the first male on a monandrous female. However, she showed that this explanation could not account for group sizes of more than 2 satellite males, which are common. This remains a viable hypothesis.

2) Alternatively, because polyandrous females lay more eggs than monandrous females (Table 1), they may attract additional males to ensure fertilization of all their eggs. However, this explanation seems unlikely because, as this study and 2 others have shown, in the field nearly all eggs develop in both monandrous and polyandrous females (Hassler 1999; Schwab 2006; Johnson and Brockmann 2010). Even when satellite males are removed from polyandrous females (Johnson and Brockmann 2010), nearly all eggs are fertilized. 3) Although it is widely believed that females have a terminal molt prior to sexual maturity (Smith et al. 2009), one possible explanation for our results is that the monandrous females are in a penultimate molt, whereas polyandrous females are in their final molt. This seems unlikely, however, because the differences in size (13.05 for intolerant monandrous females vs. 13.85 for polyandrous females) are not sufficient to be due to one less molt (one less molt would mean a size difference of $1.3\times$ or about 4 cm; Smith et al. 2009). Nonetheless, the difference in condition suggests that monandrous females have molted into their adult state more recently than the average polyandrous female (although age is probably not the only factor that influences condition). This suggests the hypothesis that multiple mating may be more costly for young adult females or perhaps they have less to gain from multiple mating than older adult females. 4) Differences in adult female size may be due to differences in resources accrued during juvenile development, which suggests that monandrous females and particularly intolerant monandrous females may have had fewer resources during development than polyandrous females. Although our data show that smaller females also tend to be in better condition, our condition measures indicate how recently a female molted into her adult state (i.e., age) rather than measuring a condition that would be affected by resource accumulation early in life. Taken together, our results suggest the hypothesis that multiple mating may be more costly for younger and/or smaller adult females or perhaps they have less to gain from multiple mating than the older and/or larger females.

Johnson and Brockmann (2010) showed that spawning with multiple males can be costly in terms of nesting success for all female horseshoe crabs: when satellite males are removed from polyandrous females, they lay more eggs than polyandrous females that retain their satellites and when satellites join monandrous females they lay fewer eggs than monandrous females nesting alone. However, when females are allowed to choose freely between nesting with or without satellites, they are equally successful in terms of nesting and developmental success. This means that polyandrous females have more to gain from their costly interactions with males than monandrous females have to gain if they nested with multiple males. Taken together, these results suggest that monandrous and polyandrous females differ in the costs and benefits of multiple mating and the different types of females choose different mating tactics appropriately for their condition and circumstances when they are free to do so. A similar conclusion comes from a species of damselfly with a female-limited color polymorphism: the 2 morphs differ in the costs of multiple mating (Siroty and Brockmann 2001), and each morph has her own tactics for avoiding costly male harassment (Siroty et al. 2003).

Context-dependence of female ARTs in horseshoe crabs

One possible context-dependent explanation for different mating tactics in female horseshoe crabs is that a female solicits satellite males when attached to a low quality or incompatible male, and she nests alone when her attached male is of high quality or compatibility. We did not detect any differences in

the condition of the attached males of monandrous and polyandrous females, but we did find that the attached males of intolerant monandrous females are in better condition than the attached males of tolerant monandrous females (Figure 4B). It is possible that there are differences in attached male quality (such as low sperm count) or compatibility that we could not detect in this field-based study. Furthermore, it is possible that smaller females or those in worse condition (a condition-dependent alternative) may attract poor quality or incompatible males and be forced to attract additional satellites to ensure high reproductive success (a context-dependent female choice).

Implications

This study illustrates the important point that male and female ARTs cannot be studied in isolation. By considering only the male perspective, previous studies missed important ways in which female behavior affects males and failed to consider how male traits affect females (Brockmann and Penn 1992; Brockmann 1996). In horseshoe crabs, the male ARTs, attached and unattached, are condition- and context-dependent (Brockmann 2002) with males switching from the attached to the unattached tactic as they age and their physical condition declines (Brockmann 2003b). These male tactics are also likely to be density- and frequency-dependent with the unattached tactic having higher success when rare (Brockmann and Taborsky 2008). Clearly, the success of the polyandrous female tactic depends on the frequency of unattached males in the population. In the present study, we have shown that female ARTs are affected by female size and physical condition and possibly by male quality. They may also be density- and frequency-dependent, with the intolerant monandrous tactic being more successful when rare. If so, then this means that the frequency of the monandrous female tactic will affect the payoff to the male tactics, and hence, the equilibrium frequency and the age at which males are predicted to switch from the attached to the unattached tactic. The same point was made by Alonzo and Warner (2000). Using a modeling approach, they show that female behavior can completely alter model predictions about the maintenance and expression of male ARTs. For example, condition-dependent female choice can cause male ARTs to be maintained when none were otherwise predicted and female behavior can suppress male ARTs when frequency- and condition-dependent mechanisms predict the maintenance of male ARTs (Jones 2002; Rios-Cardenas et al. 2007). Clearly, understanding the evolution of multiple mating and the maintenance of sexually selected traits requires that we study both female and male mating decisions, their outcomes and their complex interactions (Jennions and Petrie 1997; Alonzo and Warner 2000; Jones 2002; Luttbeg 2004; Mappes et al. 2008; Tobler et al. 2011).

In this study, we have shown that female tactics may be affected by male quality. If so, then this suggests that females are using previously unsuspected sources of information to assess males and acting on that information. Females paired with poor quality or incompatible males (Brockmann and Johnson 2011) may attract satellites, whereas those paired with high quality males remain monandrous. If so, then individual females from this externally fertilizing species must be able to detect quality based on the male's ejaculate or from his behavior during spawning. Use of such information is well known from the precopulatory and postcopulatory mate choice literature of internally fertilizing species (e.g., Eberhard 1996; Birkhead 1998) but is not well known from the literature on externally fertilizing species. Non-random mating is known from some externally fertilizing species (Pitcher and Neff 2007; Dziminski et al. 2008; Neff et al. 2008), potentially as a result of cryptic female choice (Rosengrave et al.

2008), so it is reasonable to suggest that this may also occur in female horseshoe crabs.

Our study also demonstrates that monandry and polyandry are affected by both condition- and context-dependent information. Such labile mating tactics are well known in other species. For example, in the blue-headed wrasse, females can choose between pair or group spawning modes based on immediate conditions, but nearly all females switch to pair spawning when they reach larger sizes (Warner 1985). In side-blotched lizards, females exhibit assortative mating prior to their first clutch, but preference of orange-throated females switches toward yellow-throated males prior to their second clutch (as female body condition deteriorates; Bleay and Sinervo 2007). Female ARTs often arise in response to conflict with males (e.g., avoiding male harassment). Females may differ in the degree to which they resist coercion due to differences in the cost or benefits of resisting, which could depend on the size or condition of the female and the environment in which the female is living (Sih and Krupa 1992; Alonzo 2008; Magellan and Magurran 2006). For example, in some insects, such as the water strider *Aquarius remegis*, female territorial behavior, where she feeds, and her prey-capture rates are affected by her size and the levels of male harassment she receives (Kaitala and Dingle 1993; Lauer et al. 1996). In guppies, where males may attempt to force copulations without courtship, females usually resist (Farr 1980), but they will switch between avoiding and accepting coercion based on predation risk and water velocity (Magurran and Seghers 1994; Magellan and Magurran 2006). Female guppies may also switch tactics based on age, using one tactic when younger and another when older (Jones 2002). In male horseshoe crabs, young males seek females offshore and spawn as attached males, whereas older males come ashore without females and spawn as satellites (Brockmann 2002). When the benefits of mating decisions vary among females of different sizes or condition or under different contexts (such as the quality of their mates), then female ARTs may be favored. When female ARTs evolve then this changes the payoffs and hence the maintenance of male tactics in the population and ultimately heritable variation in sexually selected male traits (Luttbeg 2004; Morris et al. 2010).

In summary, we hypothesize that differences between female horseshoe crabs in whether they mate with multiple males is due to an interplay between male and female behaviors. A male is attracted to a female when they stand to benefit from becoming a satellite of that female, which may be due to some attribute of the female (such as her large size and higher fecundity) or her attached male (perhaps because he is less able to compete in sperm competition). In horseshoe crabs, multiple mating is costly for all females (Johnson and Brockmann 2010), but only the intolerant monandrous females actively resist the male's satellite behavior, presumably because she has nothing to gain from mating multiply. Other females (tolerant monandrous) do not attract males but neither do they resist satellite males when they appear. Still other polyandrous females attract unattached males with chemical cues (Saunders et al. 2010), presumably because the gains from multiple paternity outweigh the costs. This means that females and males differ in the costs and benefits associated with multiple mating. For males, the differences are likely to arise from differences in paternity with different females. For females, the differences are likely to arise from differences in offspring fitness (no differences exist in fertilization success) when offspring are fathered by one versus many males. We predict that the developmental success of polyandrous females will be higher with satellite males than with the females' attached males, and monandrous females will have higher success with their attached males than with satellite males.

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