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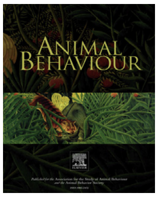

The evolution and maintenance of sexual size dimorphism in horseshoe crabs: an evaluation of six functional hypotheses

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Ultimate hypotheses to explain sexual size dimorphism (SSD) generally focus on differences in reproductive roles between males and females. For example, intrasexual competition for mates can favour larger males in some taxa, and fecundity selection can favour larger females in other taxa. However, SSD is not simply due to one selection pressure on males or females, but is the outcome of numerous conflicting pressures on both sexes. Despite this, few studies have tested multiple hypotheses to explain the factors underlying SSD. Horseshoe crabs, *Limulus polyphemus*, have female-biased SSD in all populations, and are an interesting species for the study of SSD because of their unusual evolutionary history and life-history strategy. We used the hypothetico-deductive method to evaluate multiple predictions from six hypotheses to understand the evolution and maintenance of SSD in horseshoe crabs. We examined these hypotheses using field studies and data from the literature. We found little support for four hypotheses. However, predictions for the fecundity advantage hypothesis were supported (e.g. larger females lay more eggs than smaller females). Our results predict that, for every centimetre increase in size, females will lay 1672 ± 285 more eggs per spawning bout. Thus, selection is likely to favour the future compounded gain in fecundity from one extra year of growth over the gain from one additional mating opportunity if females mature earlier. Predictions for the protandry hypothesis were also supported. The costs for males maturing at smaller sizes (i.e. a large-size advantage for competing males, and increased ejaculate volume) appear to be minimal. Therefore, males that mature and reproduce earlier will likely be selected for, given the demographic advantage of their offspring hatching and reproducing earlier. Our study illustrates the importance of investigating multiple hypotheses for both males and females in the study of SSD.

Sexual size dimorphism results from sexually antagonistic selection favouring different optimal body sizes for males and females. The evolution of independent size optima is constrained by genetic correlations between the sexes, as well as physiological, developmental and phylogenetic constraints (Blanckenhorn, 2007; Fairbairn, 2007). Given these constraints, it is surprising that sexual size dimorphism (SSD) is so widespread across the animal kingdom. Many adaptive hypotheses have been proposed to explain the evolution and maintenance of SSD, and most focus on the differences in reproductive roles between the sexes. For example, male competition for mates is the likely cause of male-biased SSD in fallow deer, *Dama dama* (McElligott et al., 2001); whereas fecundity selection likely explains the female-biased SSD in spiders (Head, 1995). In reality, SSD is not simply the result of one selection pressure on males or females, but rather the product of a suite of conflicting pressures on both sexes (Badyaev, Hill, Stoehr, Nolan, & McGraw, 2000; Badyaev, Whittingham, & Hill, 2001; Blanckenhorn, 2000; Moore, 1990). Despite this, few studies have examined multiple hypotheses to explain the ultimate factors underlying SSD (Székely et al., 2007; but see Corl, Davis, Kuchta, Comendant, & Sinervo, 2010; Fairbairn, 1990; Ficetola, Bonardi, Colleoni, Padoa-Schioppa, & Scali, 2013; Serrano-Meneses & Székely, 2006). Here we use the hypothetico-deductive method to evaluate six functional hypotheses to understand why female-biased SSD may be adaptive in horseshoe crabs. We examine these hypotheses with field studies of reproducing individuals, and with a comparative analysis of multiple horseshoe crab populations along the Atlantic and Gulf coasts of North America using data from the literature.

Study Species

Horseshoe crabs are an interesting species for the study of SSD because of their unusual evolutionary history and life-history strategy. They come from an ancient lineage and are widely

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regarded as 'living fossils' that have not changed since the Mesozoic era (Fisher, 1984). Moreover, just four species of horseshoe crabs share an entire class (Meristomata) of the subphylum Chelicerata. They are long-lived marine arthropods (15–18 years) that take many years to reach sexual maturity (9–10 years), and they have determinant growth (at sexual maturation they undergo a terminal moult into adulthood; Koons, 1883; Riska, 1981; Shuster, 1955; Shuster & Sekiguchi, 2003; Sokoloff, 1978). Female horseshoe crabs are consistently larger than males in all populations, and the female-to-male size ratio is approximately 1.3 (Brockmann & Smith, 2009). At Seahorse Key, Florida, U.S.A., only 5% of the population overlaps in size ($N = 5856$; Brockmann & Smith, n.d.). Furthermore, the proximate cause of SSD in this species is known (Smith, Mandt, & MacDonald, 2009): females are larger because they have one additional moult and one additional year of growth compared to males (i.e. they show sexual bimaturation).

Horseshoe crabs are divided into genetically distinct subpopulations (King, Eackles, Spidle, & Brockmann, 2005), which vary in a number of traits such as population size, population density, operational sex ratio and morphology (Brockmann & Smith, 2009; Riska, 1981; Shuster, 1982). They are external fertilizers, and usually nest on sandy, low-energy beaches of bays and barrier islands. Within a breeding season, spawning occurs during daily and nightly high tides around the new and full moons. The mating system of horseshoe crabs is best characterized as 'explosive mating assemblage polygyny', where receptive females are abundant for a brief time during which mating is frequent (Brockmann, 1990; Thornhill & Alcock, 1983). Spawning is highly synchronized both temporally (Barlow, Powers, Howard, & Kass, 1986; Rudloe, 1980) and spatially (Penn & Brockmann, 1994), and this often results in high nesting densities. Individual females generally nest for only 1 week in a breeding season, whereas males continue to nest for many weeks (Brockmann & Johnson, 2011; Brockmann & Penn, 1992; Smith, Brousseau, Mandt, & Millard, 2010). For many populations this results in a highly male-biased operational sex ratio (OSR) on spawning beaches (Botton & Loveland, 1992). High nesting densities and a male-biased OSR are characteristics of a population with strong intrasexual competition (Shuster & Wade, 2003). Indeed, there is often intense competition among horseshoe crab males as they push against and crawl over each other for access to females.

A well-established negative correlation between age and condition has been demonstrated. For example, because horseshoe crabs do not moult as adults, the physical condition of the carapace deteriorates over time (Brockmann, 1996; Brockmann & Penn, 1992). Moreover, the condition of the carapace correlates with the physical performance of horseshoe crabs (Brockmann & Penn, 1992; Penn & Brockmann, 1995). While there is no method to measure absolute horseshoe crab age, relative age can be determined based on the slipper shell, *Crepidula fornicate*. This mollusc attaches to horseshoe crabs as larvae where they will live and grow for many years (Botton & Ropes, 1988). Brockmann (1996) found that *Crepidula* are larger on horseshoe crabs with more deteriorated carapaces (i.e. they had been living there longer). Hence, older horseshoe crabs tend to be in poorer condition, and these factors can have important influences on their mating behaviour. Indeed, male horseshoe crabs exhibit two condition-dependent, alternative mating tactics (Brockmann, 2002; Brockmann & Penn, 1992). Males that are younger and in better condition attach to a female out at sea and arrive on spawning beaches paired with her in amplexus. These males usually remain with the female until she has completed egg laying for the year, then they detach and seek another female. This attached tactic imposes a cost of nutritional stress during the breeding season (Smith, Schrank, & Brockmann, 2013). Older males that are in poorer condition do not attach to females, but rather roam the shoreline in search of mating pairs. These unpaired males join spawning pairs as satellites and engage in sperm competition with the attached male and any other satellite males that may be present (Brockmann, 1990, 2002; Penn & Brockmann, 1995). Satellite males that achieve the most desirable positions are quite successful in fertilizing a significant proportion of eggs (Brockmann, Colson, & Potts, 1994; Brockmann, Nguyen, & Potts, 2000; Brockmann & Penn, 1992). On average, a satellite male gains 40% of fertilizations, although this decreases as the number of satellites present increases. Attached and satellite males clearly differ in age, condition and behaviour; but they do not differ in size (Brockmann, 2002; Penn & Brockmann, 1995), and there is no large-size advantage for attached males. However, whether there is a size advantage for satellite males in competition with other satellites is not known. The frequent highly male-biased OSR and high female nesting densities, along with the presence of alternative mating tactics may contribute to the evolution and maintenance of SSD in horseshoe crabs.

Adaptive Hypotheses that Explain SSD

We evaluate multiple predictions from six alternative hypotheses to better understand the evolution and maintenance of SSD in horseshoe crabs. We recognize that these hypotheses are not mutually exclusive, and likely, multiple selection pressures affect the female-biased SSD in this species. We organize these hypotheses into three groups, those that refer to (1) natural selection favouring a divergence in size between the sexes, (2) natural and sexual selection favouring small male size and (3) natural and sexual selection favouring large female size.

NATURAL SELECTION FOR DIVERGENCE IN SIZE

Competitive Displacement Hypothesis

The resources used by individuals are determined in part by their body size, which means that intersexual competition for a limiting resource (e.g. food) may favour sexual size dimorphism (Hedrick & Temeles, 1989; Schoener, 1967; Shine, 1989; Slatkin, 1984). For example, larger individuals could capture or consume larger food items than smaller individuals, or larger individuals could access food items that require more strength to open. Thus, differences in size between males and females could reduce intersexual competition for food. Studies have demonstrated competitive displacement resulting in sexual dimorphism of overall body size (Kruger, 2005; MacDonald, 1985; Madsen, 1983) and of trophic structures such as bill size (Jones, 1997; Selander, 1966; Temeles, Pan, Brennan, & Horwitt, 2000). This hypothesis differs from the others in that SSD is not directly related to reproductive roles, and thus does not predict the direction of SSD (only that males and females should diverge).

One important assumption of the competitive displacement hypothesis is that males and females eat different types of food or forage in different locations (Table 1). Few studies have examined the natural feeding patterns of horseshoe crabs in the wild, and so differences between males and females might explain SSD in this species. We tested this assumption by using stable isotope analyses of $\delta^{15}N$ and $\delta^{13}C$. Values of $\delta^{15}N$ indicate the trophic level at which animals are feeding: as animals feed at higher trophic levels, the value of $\delta^{15}N$ in their tissues increases (Michener & Schell, 1994). In marine organisms, $\delta^{13}C$ values can indicate where animals are feeding relative to the shore: inshore food webs are typically more enriched in $\delta^{13}C$ values than offshore food webs, and so animals feeding closer to the shore would have