Assessing the potential for post-copulatory sexual selection in elasmobranchs

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Abstract

This review highlights the potential role that post-copulatory sexual selection plays in elasmobranch reproductive systems and the utility of this group to further understanding of evolutionary responses to the post-copulatory processes of sperm competition and cryptic female choice. The growing genetic evidence for female multiple mating (polyandry) in elasmobranchs is summarized. While polyandry appears to be common in this group, rates of multiple paternity are highly variable between species suggesting that there is large variance in the strength of post-copulatory sexual selection among elasmobranchs. Possible adaptations of traits important for post-copulatory sexual selection are then considered. Particular emphasis is devoted to explore the potential for sperm competition and cryptic female choice to influence the evolution of testes size, sperm morphology, genital morphology and sperm storage organs. Finally, it is argued that future work should take advantage of the wealth of information on these reproductive traits already available in elasmobranchs to gain a better understanding of how post-copulatory sexual selection operates in this group.

Keywords

cryptic female choice; genitals; multiple paternity; oviducal gland; sperm competition; testis

INTRODUCTION

Darwin (1871) realized that survival alone is not enough to secure the transmission of genes to future generations and suggested that another form of selection, which he called sexual selection, influences the evolution of traits that determine mating success. Sexual selection can promote the evolution of costly pre-copulatory (before mating) traits, such as weapons and extravagant ornaments, that enhance mating success during male-male competition and mate choice (Andersson, 1994). In most animals, however, females mate with multiple males within a single reproductive episode and sperm from different males can overlap both temporally and spatially within the female's reproductive tract. Consequently, sexual

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selection can continue after mating through the post-copulatory processes of sperm competition, the competition among ejaculates from rival males for fertilization of the female's ova (Parker, 1970), and cryptic female choice, when females influencing the outcome of sperm competition through variation in their behaviour, physiology or morphology (Thornhill, 1983; Eberhard, 1985, 1996). Evidence from numerous taxa reveals that these post-copulatory processes influence the evolution of sexual behaviours, ejaculate traits (*e.g.* ejaculate volume and sperm morphology, swimming speed and viability), genital and reproductive tract morphology, and reproductive physiology (Birkhead, 1998; Hosken & Stockley, 2004; Snook, 2005; Birkhead *et al.*, 2009; Evans & Meisner, 2009; Montgomerie & Fitzpatrick, 2009). Indeed, due to the prevalence and importance of post-copulatory sexual selection in influencing fitness, sperm competition and cryptic female choice are now recognized as a potent selective force influencing the evolution of myriad sexual traits (Birkhead & Møller, 1998; Birkhead *et al.*, 2009).

Over the past four decades, studies of post-copulatory sexual selection have grown almost exponentially to include most major groups of animals (Birkhead & Møller, 1998; Birkhead *et al.*, 2009). Studies of post-copulatory sexual selection in fishes have notably lagged behind that of other taxa (Montgomerie & Fitzpatrick, 2009), however, and this is particularly obvious for elasmobranchs. In this review, the available evidence for post-copulatory sexual selection in elasmobranchs is reviewed and hypotheses are presented on the role that it plays in shaping male reproductive traits in this group. As will be clear from this review, although post-copulatory sexual selection is an inevitable consequence of their reproductive biology, studies of sperm competition and cryptic female choice are a very underdeveloped field in elasmobranchs. Therefore, the many features that make this group ideal for gaining novel insights into post-copulatory sexual selection are highlighted in order to identify fruitful avenues for future research. The evidence that females mate with multiple males in elasmobranchs is reviewed first, as this is a prerequisite for any putative model system of post-copulatory sexual selection. Then, a range of possible adaptations for post-copulatory sexual selection displayed by elasmobranchs are considered, focusing on traits that are known to play important roles in sperm competition, cryptic female choice and sexual conflict in other taxa.

MULTIPLE PATERNITY IN ELASMOBRANCHS

Direct evidence that females mate with multiple males over the course of a single breeding season (polyandry) comes either from infrequent behavioural observations of multiple matings in the field (Chapman *et al.*, 2003) or, more commonly, from genetic studies where advances in molecular techniques have led to an increase in the number of studies assessing multiple paternity (the siring of a single brood of offspring by multiple fathers) in elasmobranchs (Feldheim *et al.*, 2001; Chapman *et al.*, 2004; Daly-Engel *et al.*, 2006). Genetic studies have supported the conclusion that polyandry occurs in elasmobranchs, thereby enabling researchers to confidently infer patterns of reproductive behaviour without direct behavioural observations. While the application of molecular methods to assign paternity is relatively recent in elasmobranchs, the available evidence demonstrates that polyandry is both prevalent and highly variable in this group. Furthermore, among sharks, it is likely that multiple paternity evolved relatively early, prior to the division of the two major shark superorders (Squalomorphii and Galeomorphii) *c.* 350 million years ago (Heinecke *et al.*, 2009). Of the eight extant orders of shark, five have been shown to exhibit multiple paternity: Carcharhiniformes, Orectolobiformes, Hexanchiformes, Lamniformes and Squaliformes.

Multiple paternity has been documented in 12 of the 15 species (80%) of elasmobranchs summarized in this review (including 14 species of sharks and one skate; Table I). The

frequency of multiple paternity, however, varies widely among elasmobranch species. For example, Daly-Engel *et al.* (2010) and Chapman *et al.* (2004) reported a predominance of genetic monogamy in the shortspine spurdog *Squalus* cf. *mitsukurii* Jordan & Snyder 1903 and the bonnethead shark *Sphyrna tiburo* (L. 1758), respectively, while a predominance of genetic polyandry has been reported in the lemon shark *Negaprion brevirostris* (Poey 1868) and the small spotted catshark *Scyliorhinus canicula* (L. 1758) (Feldheim *et al.*, 2001; DiBattista *et al.*, 2008; Griffiths *et al.*, in press; see Table I). Rates of multiple paternity can also vary among populations of a single species. In the sandbar shark *Carcharhinus plumbeus* (Nardo 1827), rates of multiple paternity range from 40% in a tropical population in the central Pacific Ocean to 85% in a temperate population in the north-west Atlantic Ocean (Daly-Engel *et al.*, 2007; Portnoy *et al.*, 2007). Similarly, in the spiny dogfish *Squalus acanthias* L. 1758 populations sampled along the east coast of North America, rates of multiple paternity vary almost two-fold, ranging from 17 to 30% (Lage *et al.*, 2008; Veríssimo *et al.*, 2010).

The occurrence of polyandrous mating among elamobranchs may actually be underestimated in the studies reviewed in Table I for a number of reasons. First, the molecular techniques used to assess rates of multiple paternity rely on adequate sample size to give authentic frequency estimates. Many studies of multiple paternity in elasmobranchs, however, particularly for rare or endangered species, utilize opportunistic analyses on a single brood, thereby precluding an accurate frequency estimate. Indeed, of the 15 species summarized in Table I, multiple paternity has been found in every case where more than one litter was examined. Further, species whose life history includes litter sizes smaller than three pups, a common trait in a number of elasmobranchs, cannot be easily examined for multiple paternity. Finally, factors such as fertilization bias due to sperm competition and cryptic female choice will lead to the underestimation of polyandry in elasmobranchs, as these processes inevitably exclude genetic representation by some mated males (García-González, 2008). Regardless of these limitations, genetic techniques are still useful for detecting the occurrence of multiple paternity in elasmobranchs, and the number of studies documenting this phenomenon continues to grow.

EVOLUTIONARY RESPONSES TO POST-COPULATORY SEXUAL SELECTION IN ELASMOBRANCHS

The preceding evidence that multiple mating occurs in numerous species of elasmobranchs (Table I) illustrates the potential role for post-copulatory sexual selection to shape reproductive trait evolution in this group. Little attention, however, has been paid to understanding such evolutionary processes in elasmobranchs, much less the underlying mechanisms responsible. This section explores a range of putative reproductive traits that may be influenced by post-copulatory sexual selection in elasmobranchs, including testes size, sperm morphology, genital morphology and sperm storage organs in females. These traits are known to be important determinants of competitive fertilization success and influence the outcome of post-copulatory sexual selection in other taxa and, as this review demonstrates, there is increasing evidence that these traits are shaped by similar evolutionary forces in elasmobranchs.

TESTES SIZE

Investment in testicular tissue is highly variable among species (Harcourt *et al.*, 1981; Møller, 1991; Todd, 2008; Montgomerie & Fitzpatrick, 2009) and is influenced by a variety of factors including body size, seasonal effects and clutch size (Møller, 1991; Stockley *et al.*, 1996; Montgomerie & Fitzpatrick, 2009). After controlling for these factors, however, relative (*i.e.* body size corrected) investment in testicular tissue often reflects the extent of

multiple mating by females in a population, and thus the strength of post-copulatory sexual selection (Parker *et al.*, 1997; Birkhead & Møller, 1998; Montgomerie & Fitzpatrick, 2009). Larger testes produce greater numbers of sperm, as revealed by the positive relationship between testes size (*i.e.* combined testes mass) and the number of sperm ejaculated (Parker, 1982; Møller, 1989; Marconato & Shapiro, 1996; Schärer *et al.*, 2004). Thus, males with larger testes are able to ejaculate a greater number of sperm, and consequently enjoy a competitive advantage over rival males in contests to fertilize a female's ova (Parker, 1982). Highly polyandrous species are, therefore, expected to have larger testes relative to closely related monogamous species, a prediction that has been validated across a wide range of taxa (Parker *et al.*, 1997).

Given the clear link between mating behaviours and relative testes size, the use of size corrected testes mass is commonly used as a proxy measure for the risk of sperm competition (Parker *et al.*, 1997). Therefore, to provide possible insights into how post-copulatory sexual selection influences testes size in elasmobranchs, the relationship between testes mass (M_T) and body size (total length, L_T) is explored in an effort to identify species whose relatively large or small testes may provide insight into sexual selection. Using information from the literature and unpublished work, data were compiled on M_T (whenever possible using peak values to account for seasonal effects) and adult male L_T for 43 species of sharks (data available in Table SI, Supporting Information). As with other fishes (Montgomerie & Fitzpatrick, 2009), there was a strong positive correlation between M_T and L_T in sharks [$r = 0.87$, $P < 0.001$, Fig. 1(a)]. It is clear, however, that there are several species that invest either relatively more or less in testes size than would be expected when only considering L_T as an explanatory variable [Fig. 1(a)]. Interspecific variation in investment in M_T is known to be a hallmark of the effect of post-copulatory sexual selection in shaping testes size in other taxa (Harcourt *et al.*, 1981). Of the species of elasmobranchs examined [Fig. 1(a)], several may offer valuable insights into how selection acts on testes size and some of these are discussed in greater detail.

Data on L_T and M_T were available for five species where rates of multiple paternity have been established (Table I): *Squalus* cf. *mitsukurii*, *S. tiburo*, *S. acanthias*, *C. plumbeus* and *S. canicula*. To explore the possible link between rates of multiple paternity and investment in M_T, residual M_T was calculated from a regression of $\log_{10} L_T$ and $\log_{10} M_T$. These residual M_T values were then compared to observed rates of multiple paternity. Residual values were used rather than the more traditional gonado-somatic index [$I_G = 100 M_T (M - M_T)^{-1}$, where M body mass] as the latter has been criticized on statistical grounds (Tomkins & Simmons, 2002). Because of the small sample size, residual M_T values were used here for illustrative purposes only. This preliminary examination of the data reveals that species with higher rates of multiple paternity also invest relatively more in testicular tissue [Fig. 1(b)], suggesting that elasmobranch species experiencing a greater risk of sperm competition have relatively larger testes than those with lower risk of sperm competition.

Evidence of population-specific investment in M_T was found in two elasmobranch species, which may indicate population-specific responses to the risk of sperm competition. For *C. plumbeus*, male investment in testes differs between populations, with males from Atlantic Ocean populations investing relatively more in testes compared with males from Australian populations [Fig. 1(a)]. This difference in testes size may result from geographically variable rates of multiple paternity observed in *C. plumbeus* populations. Although the degree of multiple paternity is currently unknown in Australian populations, in the north-west Atlantic Ocean population 85% of litters have multiple sires (Portnoy *et al.*, 2007), suggesting that sperm competition may be prevalent in this population. The rate of multiple paternity in north-west Atlantic Ocean *C. plumbeus* is among the highest observed in elasmobranchs (Table I) and more than double that of the Hawaiian population of *C.*

plumbeus (Daly-Engel *et al.*, 2007). If rates of multiple paternity in the Australian population are similar to those in the Hawaiian population, the observed difference in testicular investment could represent a local response to differences in mating behaviours. Similarly, M_T varies among three populations of *S. acanthias* [Fig. 1(a)], which may be another species where rates of multiple paternity vary among populations (Table I). Such intraspecific variance in testes size in response to differential rates of multiple paternity is not without precedent (Firman & Simmons, 2008) and probably represents a population-specific response to the level of sperm competition.

SPERM MORPHOLOGY AND QUALITY

The considerable variation in sperm morphology and components of sperm 'quality' (*e.g.* sperm swimming speed, viability or the amount of variation in sperm morphology) observed in numerous taxa is thought to reflect variance in the strength of post-copulatory sexual selection (Hunter & Birkhead, 2002; Montgomerie & Fitzpatrick, 2009; Pitnick *et al.*, 2009; Fitzpatrick & Baer, 2011). Thus, sperm traits that make a male's ejaculate more competitive than sperm from rival males are expected to provide a selective advantage in post-copulatory sexual selection. Many studies have upheld this prediction by showing that males with faster swimming sperm (Gage *et al.*, 2004; Casselman *et al.*, 2006; Liljedal *et al.*, 2008; Gasparini *et al.*, 2010) or sperm that are better suited to the characteristics of a females' reproductive tract (García-González & Simmons, 2007) fertilize more eggs than sperm from rival males. Furthermore, several recent studies have demonstrated a link between the length of various sperm components (*e.g.* head, mid-piece and flagellum) and sperm swimming speed (Gomendio & Roldan, 1991, 2008; Fitzpatrick *et al.*, 2009, 2010; Lüpold *et al.*, 2009; Mossman *et al.*, 2009; Firman & Simmons, 2010; Helfenstein *et al.*, 2010), leading researchers to speculate on the role of sperm competition in favouring relatively long, and therefore fast-swimming, sperm (Gomendio & Roldan, 1991, 2008; Fitzpatrick *et al.*, 2009). Characteristics of the female's reproductive tract can also influence the evolution of sperm morphology, as some females can exert cryptic choice for relatively long sperm (Pitnick *et al.*, 2009). In many taxa, variance in sperm morphology is explained not only by the level of sperm competition but also by the length of the female's reproductive tract (Pitnick *et al.*, 2009). These latter studies reveal the importance of female traits in influencing selection on sperm length and suggest that the relationships observed between sperm length and the level of sperm competition may reflect more complex evolutionary patterns of selection on ejaculate traits.

Among elasmobranch species, researchers have been aware of the extensive inter-specific variation in sperm morphological traits for almost a century (Fig. 2; Leigh-Sharpe, 1920). Recently, Jamieson (2005) offered a more in-depth examination of the level of variation in sperm morphology, summarizing the extensive interspecific variation in sperm morphology across elasmobranchs. Of the 30 elasmobranch species considered in Jamieson's (2005) analysis, all sperm components varied approximately three-fold in length, with sperm head length ranging from 33 μm in *C. plumbeus* to 93 μm in the roughskin dogfish *Centroscymnus owstoni* Garman 1906, sperm mid-piece length ranging from 7 μm in the dusky smooth hound *Mustelus canis* (Mitchill 1815) to 21 μm in the Japanese swellshark *Cephaloscyllium umbratile* Jordan & Fowler 1903 and sperm flagellum length ranging from 49 μm in the tiger shark *Galeocerdo cuvier* (Péron & LeSueur 1822) to 143 μm in the Japanese angelshark *Squatina japonica* Bleeker 1858 (Jamieson, 2005). This pattern of inter-specific variation in sperm length components could be characteristic of an evolutionary response in sperm size to the selective pressures associated with post-copulatory sexual selection.

Unfortunately, the functional significance and selective forces responsible for generating the observed variation in elasmobranch sperm morphology have yet to be investigated. Consequently, there remains extensive scope for examinations of how selection is acting on sperm morphology. At the intraspecific level, competitive fertilization studies which take advantage of recent artificial insemination techniques in elasmobranchs (Luer *et al.*, 2007) would help to determine if variation in sperm morphology influences competitive fertilization success. At the interspecific level, phylogenetically controlled studies assessing whether sperm size increases in response to post-copulatory sexual selection in elasmobranchs, as is often the case in other taxa (Gomendio & Roldan, 1991, 2008; Fitzpatrick *et al.*, 2009), would offer further insights into the evolution of sperm traits in this group.

GENITAL MORPHOLOGY

The primary function of male genitalia is to transfer sperm to females during copulation, while the role of the female genitalia is to receive the male intromittent organ. In many species, however, genitalia also serve a sexually selected role. In competitive matings, males with certain (species-specific) genital morphologies are more successful at inseminating females and fertilizing ova than rival males (Arnqvist & Danielsson, 1999; Danielsson & Askenmo, 1999; House & Simmons, 2003; Simmons *et al.*, 2009; Evans *et al.*, 2011). The strength of selection on male genitalia is therefore expected to co-vary with mating systems, and previous studies in insects have demonstrated that male genitalia exhibit dramatic divergence in morphology in polyandrous species compared to monandrous species (Arnqvist, 1998). Comparative studies in mammals also provided some evidence of a positive correlation between genital length and sperm competition risk at the species level (Ramm, 2007). Post-copulatory sexual selection may also influence the morphology of male genitalia to act as a mechanism to remove sperm from rival males from the female's reproductive tract, thereby reducing the likelihood of sperm competition (Waage, 1979).

Female genital morphology and reproductive tracts, which can also be highly variable across closely related species, may co-evolve with male genitals as a result of post-copulatory sexual selection and sexual conflict (Arnqvist & Rowe, 2002; Hosken & Stockley, 2004; Evans *et al.*, 2011). For example, female genital and reproductive tract morphologies may serve as an avenue for extending cryptic female choice for males with particular reproductive traits (Eberhard, 1985). In many species, however, the underlying evolutionary interest of males and females differs, leading to sexual conflict over control of inseminations and fertilizations (Eberhard, 1985; Hosken & Stockley, 2004; Arnqvist & Rowe, 2005). Sexual conflict can have a particularly important influence on genital evolution if genital traits provide advantages to one sex at the expense of the other during matings (Arnqvist & Rowe, 2005). Clear evidence for sexual conflict influencing genital evolution comes from studies of insects, where in many species male genitalia are armed with spines that can physically injury females during copulation and reduce a female's likelihood of re-mating (Hosken & Stockley, 2004). Thus, the harmful effects of male genital armaments can benefit males at the expense of females, as males benefit by reducing the intensity of sperm competition experienced by their ejaculate, while induced female chastity prevents females from securing genetic benefits associated with multiple mating (Jennions & Petrie, 2000). In cases such as these, females often exhibit counter-adaptations (*e.g.* protective 'pads' or behaviours) that minimize the harmful effects of male appendages (Rönn *et al.*, 2007).

In elasmobranchs, males have external, paired intromittent appendages, claspers, which extend from the posterior base of the pectoral fins (Fig. 3). Male elasmobranchs use either one or both of their claspers during copulation to transfer sperm to the female. Claspers have been used extensively as an indicator of male maturity because, as a male matures the

claspers become calcified and rigid and the base of the clasper is able to rotate so that it can be directed anteriorly (Clark & von Schmidt, 1965). Given the importance of assessing reproductive maturity in fisheries science (Walker, 2005) and the relative ease with which male maturity may be estimated, there are extensive historical and contemporary data available on clasper length for a wide variety of elasmobranch species. In a pioneering series of papers, the external clasper morphology of 87 species of elasmobranchs has been described (Leigh-Sharpe, 1920, 1921, 1922*a*, b, c, 1924*a*, b, 1926*a*, b, c, d), and a review of the literature over the last 30 years uncovered clasper length data for an additional 70 species. Despite the abundance of data available and the known role of sexual selection in shaping genital morphology in other taxa, no study to date has attempted to assess whether clasper morphology is influenced by sexual selection and mating strategy. Several possible directions for future research on this topic in elasmobranchs are therefore presented here.

The first of these takes advantage of the fact that male genital (clasper) size and morphology can vary dramatically (Fig. 3), even among closely related elasmobranchs. For example, after comparing the reproductive biology of three species of catsharks in the family Scyliorhinidae, Flammang *et al.* (2008) reported that clasper lengths for mature brown catsharks *Apristurus brunneus* (Gilbert 1892) and long-nose catsharks *Apristurus kampae* Taylor 1972 were 8·3 and 8 6% of the male L_T, respectively. In contrast, claspers of the filetail catshark *Parmaturus· xaniurus* (Gilbert 1892) were 13·4% of the adult L_T (Flammang *et al.*, 2008). It remains unclear if this variation in clasper length is functionally important or influenced by the selective force of post-copulatory sexual selection. Tentative evidence suggests, however, that sperm competition may influence the relative investment in clasper length in cat-sharks as I_G values, a proxy measure for sperm competition risk, were greater in *P. xaniurus* than *A. brunneus* (Flammang *et al.*, 2008). Due to the relative ease in maintaining catshark populations in captivity (Griffiths *et al.*, in press), this family therefore represents an intriguing avenue for research.

In terms of intraspecific variation in male genital size and shape and concomitant adaptations in female genital traits, there are striking similarities between elasmobranchs and other taxa including teleosts (Evans & Meisner, 2009; Evans *et al.*, 2011), insects (Arnqvist & Rowe, 2005; Röonn *et al.*, 2007) and mammals (Dixson, 1987; Baryshnikov *et al.*, 2003; Ramm, 2007). In some shark species, the terminal cartilages of claspers are armed with sharp ridges, spurs or hooks [Fig. 3(c)] that act as holdfasts during copulations but can subsequently cause damage to the females' reproductive tract (Pratt & Carrier, 2005). Vaginal scars caused by claspers are commonly observed in sharks and can be used as an indicator of recent mating activity (Pratt, 1979). Together with male behaviours of aggressively biting the fins and bodies of females during matings (Carrier *et al.*, 1994; Kajiura *et al.*, 2000), these vaginal scars suggest that mating can be costly for female elasmobranchs. In at least one species of shark, the blue shark *Prionace glauca* (L. 1758), females have thick-walled vaginas that may represent a co-evolutionary adaptation to the evolution of clasper spurs and hooks (Pratt, 1979; Pratt & Carrier, 2005). Thus, the presence of both male and female genital modifications suggests that post-copulatory sexual selection may be an important selective force driving genital evolution in elasmobranchs.

CRYPTIC FEMALE CHOICE AND SPERM STORAGE ORGANS

The most direct method for biasing fertilization success available to females is to exert pre-copulatory mate choice for preferred males (Andersson, 1994). Even in the absence of pre-copulatory processes, however, females can exert cryptic choice for preferred males, and thereby control male reproductive success, through a variety of post-copulatory processes. During copulation, or immediately thereafter, females can bias paternity by either accepting fewer sperm from undesirable males during matings (Pilastro *et al.*, 2004) or by ejecting

sperm from undesirable males directly after mating has occurred (Pizzari & Birkhead, 2000). After mating, biochemical interactions between ejaculates and the ovarian fluid in the female's reproductive tract can influence male fertilization success by differentially influencing sperm swimming speed among competing males to disadvantage sperm from undesirable males (Gasparini & Pilastro, 2011). In addition, in species where females mate multiply and store sperm in specialized sperm storage organs, females may be able to differentially store and utilize sperm to fertilize their eggs with sperm from preferred males (Eberhard, 1996). In some insect species, such differential use of sperm from competing males is facilitated by the presence of multiple sperm storage organs that allows sperm from different males to be partitioned and increases female control of patterns of paternity (Eberhard, 1996; Hellriegel & Bernasconi, 2000; Snow & Andrade, 2005).

Evaluating the evolutionary significance of cryptic female choice in elasmobranchs can be challenging, particularly due to the inherent difficulties in documenting reproductive behaviours in this group (Pratt & Carrier, 2005). Studies of the oviducal gland a paired organ in the female reproductive tract where sperm are stored that is also the site of ooctye fertilization and egg case manufacture (Pratt, 1993; Hamlett *et al.*, 1998; Carrier *et al.*, 2004), represent an interesting avenue for future investigation. Sperm storage in the oviducal gland, which has been documented in numerous elasmobranch species (Parsons *et al.*, 2008), can last for days to years and allows females to decouple mating with fertilizations (Pratt, 1993; Parsons *et al.*, 2008). Following insemination, sperm are stored in the main cavity of the oviducal gland's terminal zone or packaged into discrete seminiferous tubules embedded in the epithelium (Hamlett *et al.*, 1998). Intriguingly, the prevalence of female multiple mating (see Table I) coupled with the evidence that sperm storage is common in elasmobranchs, suggests that sperm from multiple males may be stored separately and that this may facilitate cryptic female choice. Additionally, the complex anatomy of the oviducal gland in elasmobranchs and the various ways that sperm may be stored and partitioned suggest that the oviducal gland has the potential to enhance post-copulatory sexual selection by alternately facilitating or inhibiting sperm mixing prior to fertilization (Hamlett *et al.*, 1998; Carrier *et al.*, 2004). While the function of the oviducal gland in facilitating cryptic female choice remains speculative at this point, future investigations of the role that this organ plays in influencing patterns of paternity, and comparative investigations of whether oviducal gland morphology co-varies with the strength of post-copulatory sexual selection would greatly enhance understanding of how cryptic female choice might proceed in elasmobranchs.

CONCLUSIONS

This review has argued that post-copulatory sexual selection is a powerful selective force operating in elasmobranchs, and highlighted several reproductive traits that are likely to be shaped by sperm competition, cryptic female choice and sexual conflict. From the available evidence, it is clear that there is extensive interspecific variation in patterns of polyandry, testes size, sperm morphology and genital morphology in elasmobranchs. In some cases, species exhibit intraspecific variation in these reproductive traits, suggesting that this variation may also be shaped by population-specific responses to the level of post-copulatory sexual selection. The major challenge now is to explain the adaptive significance of this variation and the evolutionary processes that have shaped it in elasmobranchs and to greatly enhance understanding of the importance of female traits during post-copulatory episodes of sexual selection. The latter would necessitate a thorough investigation of whether the oviducal gland facilitates cryptic female choice and would hopefully lead to a better understanding of the physiological processes that might allow females to exert such cryptic choice.

Although an understanding of how post-copulatory sexual selection operates in elasmobranchs is limited, there is a wealth of information on reproductive traits in elasmobranchs that are available to address these questions. With recent advances in understanding of the phylogenetic relationships among elasmobranchs (Vélez-Zuazo & Agnarsson, 2011), future comparative studies promise to offer robust phylogenetically controlled analyses of how post-copulatory sexual selection influences reproductive traits. Furthermore, recent successes in the development of artificial insemination protocols for elasmobranchs (Luer *et al.*, 2007) will be instrumental in future studies that aim to determine how various sperm traits influence competitive mating success. Additionally, examining how genital morphology influences reproductive success in elasmobranch species where mating can be observed in the field and in captive environments promises to greatly enhance understanding of how selection acts on both genital morphology and reproductive strategies. While often challenging, incorporating post-copulatory sexual selection into studies of elasmobranch reproductive biology is key to understanding how male and female reproductive traits evolved in this ancient group of fishes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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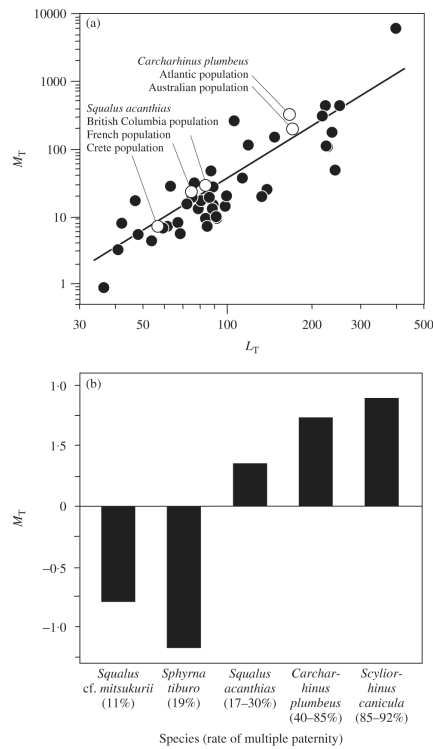
Fig. 1.
Testes mass (M_T) and post-copulatory sexual selection in elasmobranchs. (a) Relationship between mean M_T and adult male total length (L_T) in 43 species of sharks. Data are available in Table SI, Supporting Information. (b) Relationship between rates of multiple paternity for five shark species and relative investment in testes as measured using residual M_T values. Rates of multiple paternity are presented in Table I. Residual M_T values were calculated from a $\log_{10}$–$\log_{10}$ plot of M_T and L_T for *Squalus acanthias* (F. Hazin, unpubl. data), *Squalus* cf. *mitsukurii* (M. Broadhurst, unpubl. data), *Sphyrna tiburo* (G. Parsons, unpubl. data), *Carcharhinus plumbeus* (I. Baremore & L. Hale, unpubl. data) and *Scyliorhinus canicula* (Kousteni *et al.*, 2010). For all species, the geographic location where testes and multiple paternity data were collected was matched as closely as possible.
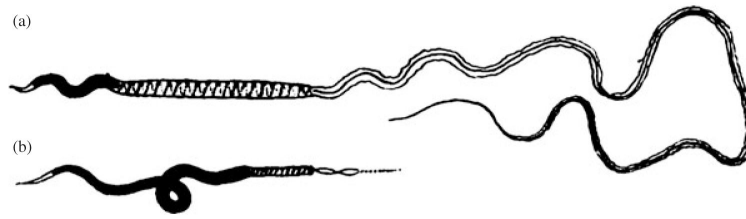
Fig. 2.
Variation in sperm morphology in elasmobranchs. A drawing of the sperm morphology of (a) *Scyllium canicula* and (b) *Raia circularis* (Modified from Leigh-Sharpe (1920) and reprinted with permission from the *Journal of Morphology*).

Fig. 3.
Variation in clasper morphology in elasmobranchs: (a) *Mustelus stevensi* and (b) *Neotrygon kuhlii*. For clarity, the tail has been removed in the pictures. (c) The clasper of *Etmopterus baxteri* showing the clasper spur.

Table I

A compilation of all known studies on elasmobranch multiple paternity (MP) to date (N, number of broods sampled). For both N and % MP, a range is presented if multiple studies are cited under the same species or if the author gave a range as a finding. As 10 is considered the minimal number of broods able to generate a frequency estimate, studies with $N < 10$ are listed as multiple paternity 'Found' or 'Not found'

Common name	Latin name	N	% MP	Methodology	Reference
Lemon shark	Negaprion brevirostris	45–46	81–86	Kinship reconstruction with microsatellites	Feldheim et al. (2001), DiBattista et al. (2008)
Spiny dogfish	Squalus acanthias	10–29	17–30	Brood sampling, microsatellites	Lage et al. (2008), Veríssimo et al. (2010)
Shortspine spurdog	Squalus cf. mitsukurii	27	11	Brood sampling, microsatellites	Daly-Engel et al. (2010)
Bonnethead shark	Sphyrna tiburo	22	19	Brood sampling, microsatellites	Chapman et al. (2004)
Sandbar shark	Carcharhinus plumbeus	20	40–85	Brood sampling, microsatellites	Daly-Engel et al. (2007), Portnoy et al. (2007)
Small-spotted catshark	Scyliorhinus canicula	13	85–92	Brood sampling, microsatellites	Griffiths et al. (in press)
Thornback ray	Raja clavata	4	Found in all	Brood sampling, microsatellites	Chevolot et al. (2007)
Shortfin mako shark	Isurus oxyrinchus	4	Found in all	Brood sampling, microsatellites	Gubili et al. (2012)
Nurse shark	Ginglymostoma cirratum	1–2	Found in all	Brood sampling with MHC genes and RFLP	Ohta et al. (2000), Saville et al. (2002)
Bignose shark	Carcharhinus altimus	1	Found	Brood sampling, microsatellites	Daly-Engel et al. (2006)
Bluntnose sixgill shark	Hexanchus griseus	1	Found	Brood sampling, microsatellites	Larson et al. (2011)
White shark	Carcharadon carcharias	1	Found	Brood sampling, microsatellites	Gubili et al. (2012)
Banded houndshark	Triakis scyllium	1	Not found	Brood sampling with MHC genes and RFLP	Ohta et al. (2000)
Galapagos shark	Carcharhinus galapagensis	1	Not found	Brood sampling, microsatellites	Daly-Engel et al. (2006)
Whale shark	Rhincodon typus	1	Not found	Brood sampling, microsatellites	Schmidt et al. (2010)