Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico

DAVID S. PORTNOY,* ANDREW N. PIERCY,† JOHN A. MUSICK,* GEORGE H. BURGESS† and JOHN E. GRAVES*

Virginia Institute of Marine Science, College of William and Mary, PO Box 1346, Gloucester Point, VA 23062-1346, USA, †Florida Program for Shark Research, Florida Museum of Natural History, University of Florida, PO Box 117800, Gainesville, FL 32611, USA

Abstract

To investigate patterns of polyandry in the sandbar shark (*Carcharhinus plumbeus*), 20 pregnant females were sampled from the western North Atlantic and Gulf of Mexico. Five species-specific microsatellite markers were used to genotype each shark and its litter. Of 20 litters, 17 (85%) were shown to have multiple sires. In multiply sired litters, the estimated minimum number of sires ranged from two to five with an average of 2.3 males per litter. Regression analysis did not demonstrate a significant relationship between female reproductive success and female body size or sire number and female body size. There was a high incidence of reproductive skew noted in litters, and two groups of males with significantly different mean reproductive success were observed. Analyses using Bateman's principles suggest that there is less direct benefit for females that acquire multiple mates than for males who bias paternity within litters. In light of past morphological and behavioural studies, these data suggest that patterns of polyandry in elasmobranchs may be determined by coercive mating, and that breeding behaviour has likely evolved in the context of sexual conflict.

Keywords: Bateman's principles, Carcharhinidae, genetic benefit, microsatellite, paternity analysis, sexually antagonistic co-evolution

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Introduction

Studies using high-resolution molecular markers have revealed that genetic polyandry is common across taxa (see Birkhead & Møller 1998 for a review). In aggregate spawning species with external fertilization, such as many bony fishes and amphibians, the presence of multiple sires per clutch is expected (DeWoody & Avise 2001; Myers & Zamudio 2004). However, polyandry has been demonstrated to be common in taxa with internal fertilization (e.g. mammals and birds) which were previously considered to be monogamous or polygynous (Gibbs *et al*. 1990; Carling *et al*. 2003; Goetz *et al*. 2003; Yamaguchi *et al*. 2004). These findings have lead many researchers to examine the potential benefit polyandry may provide to females that actively accept multiple copulations despite the associated costs.

Correspondence: David S. Portnoy, Fax: 804-684-7157; E-mail: dsport@vims.edu

Females may benefit directly or indirectly from multiple matings. Direct benefits, which increase reproductive success, may take the form of nutritive gifts that can be invested in the production of ova, as in insects (e.g. the decorated cricket, Sakaluk *et al*. 2006) or, increased sperm volume in species such as the American lobster (Gosselin *et al*. 2005). Species that are less sperm or energy limited, like the redwinged black bird or the freshwater sunfish, may benefit directly from polyandrous mating through shared parental care or territory usage (Gray 1997; Avise *et al*. 2002). Indirect genetic benefits do not affect reproductive success but may increase survivorship or reproductive success of offspring (Zeh & Zeh 2001). These benefits include increased additive genetic variance in progeny, bet-hedging in unstable environments, precopulatory or post-copulatory trading-up, and post copulatory defence against genetic incompatibility (Zeh & Zeh 1997; Newcomer *et al*. 1999; Jennions & Petrie 2000; Tregenza & Wedell 2000; Simmons 2003). However, many studies have been unable

to demonstrate female such benefit from polyandrous mating (Byrne & Roberts 2000; Garner & Schmidt 2003) raising doubt that genetic benefits alone can promote polyandry (Yasui 1998).

Mating partners that are genetically distinct have different ideal reproductive outcomes (Lessells 1999) which can lead to sexual conflict and greatly influence mating behaviour (Zeh & Zeh 2003; Parker 2006; Tregenza *et al.* 2006). A female's optimal mating frequency is determined by the balance between the costs associated with mating and the benefits of polyandry. Males, who generally produce greater amounts of energetically less costly gametes, can optimize their fitness by increasing the number of matings in which they participate (Bateman 1948; Arnqvist & Nilsson 2000) and/or by biasing sperm usage in multiply mated females. In situations where there is conflict over mating frequency, males may attempt to coerce resistant females into additional matings (Partridge & Hurst 1998). This dynamic may lead to antagonistic co-evolution, and in species where males have gained the advantage, the number of matings may be maintained above the female optima (Rowe & Arnqvist 2002). These superfluous matings increase the rate of genetic polyandry, often at a cost to female fitness (Warner *et al.* 1995; Byrne & Roberts 1999; Maklakov & Lubin 2004).

It is preferable to investigate changes in fitness associated with polyandry using controlled laboratory experiments, in which the number of matings can be carefully manipulated while benefits and costs to both sexes can be measured (Jones & Avise 2001). However, large vertebrates such as elasmobranchs (sharks, skates, and rays), do not lend themselves to such manipulation due to the difficulty of maintaining captive populations and aberrant behaviour resulting from the stress of captivity (Henningsen *et al.* 2004). Alternatively, high-resolution microsatellite markers allow for kinship analyses (Blouin *et al.* 1996; Fiumera *et al.* 2001; Jones & Ardren 2003). In situations where entire litters can be genotyped, detailed information about male and female reproductive output can be collected. Comparative approaches utilizing phylogenetic information can then be used to investigate the adaptive significance of reproductive behaviour (Harvey & Pagel 1991).

Elasmobranchs are a basal vertebrate lineage with internal fertilization. Some elasmobranchs feature prolonged maternal care in the form of long gestation periods and reproductive cycles greater than 1 year (Carrier *et al.* 2004). Mating is physically costly to females, as copulation requires males to grasp and hold on to females with their jaws (Pratt & Carrier 2001). Despite the substantial cost, varying levels of polyandry have been observed in most species examined. In the nurse shark, *Ginglymostoma cirratum* (Ohta *et al.* 2000; Saville *et al.* 2002), and the lemon shark, *Negaprion brevirostris* (Feldheim *et al.* 2002; Feldheim *et al.* 2004), the majority of litters examined had multiple sires. In contrast,

the majority of litters in the bonnethead, *Sphyrna tiburo*, had one sire (Chapman *et al.* 2004) as did the single litter examined in the banded houndshark, *Triakis scyllium* (Ohta *et al.* 2000). The balance of costs and benefits that have lead to differences in the rate of polyandry across these species is not entirely clear. A comparison of rates of polyandry, demography and life history across related shark species will help elucidate the evolutionary implications of polyandry in elasmobranchs and may be instructive for further inquiries into the fitness consequences of polyandry in species where mating incurs significant cost.

The sandbar shark, *Carcharhinus plumbeus*, is part of a monophyletic unit (Carcharhinidae), with *N. brevirostris* and *S. tiburo* (Naylor 1992). Therefore, patterns of polyandry in sandbar sharks are of interest from a comparative evolutionary perspective. This species is also of interest from a conservation perspective because it is cosmopolitan and exploited throughout much of its range (Compagno 1984). The western North Atlantic population, which extends into the Gulf of Mexico (Bigelow & Schroeder 1948; Springer 1960; Heist *et al.* 1995), is a primary target of the commercial shark fishery (Burgess & Morgan 2002). Like other carcharhinids, it is long lived, slow to mature, and has a low fecundity, making its lifetime reproductive output more similar to that of a cetacean than a bony fish (Smith *et al.* 1998). Understanding factors that affect levels of polyandry may be important in maintaining viable populations in the face of exploitation (Martinez *et al.* 2000; Rowe & Hutchings 2003).

We characterized the prevalence of multiple paternity in sandbar sharks in the western North Atlantic using highly polymorphic microsatellite markers. We investigated whether there is direct female benefit to genetic polyandry by examining the relationship between mating success (the number of sires) and female reproductive success (number of offspring) (Bateman 1948; Jones *et al.* 2000; Jones *et al.* 2002). As an alternative, we examined whether female reproductive success simply varied with size. Since sandbar shark mating is violent in nature (Springer 1960), we hypothesized that small and large females might exhibit different mating rates, which would be reflected in sire number. If large females can better absorb the costs of mating and benefit indirectly from genetic polyandry, then the number of sires would be positively correlated with female size. Alternatively, if indirect benefits are small, and large females can resist coercive mating better than smaller females, the number of sires would be negatively correlated with female size. Since polyandry creates a forum for sperm competition, even when male success comes at the expense of female fitness (Chapman *et al.* 1995), we investigated male fitness by examining the relationship between male reproductive success and the number of competing sires.

Materials and methods

Collection and genotyping

Twenty pregnant sandbar sharks were collected in the western North Atlantic Ocean and Gulf of Mexico through two fishery independent longline surveys and the Florida Museum of Natural History's Commercial Shark Fishery Observer Program. These animals were considered to be sampled from a single population based on the results of prior molecular analyses (Heist *et al.* 1995), tagging studies (Musick, unpublished data) and an analysis of the current data using STRUCTURE 2.1 (Pritchard *et al.* 2000). Fork length (FL), measured from the tip of the snout to the fork of the tail, was determined for each shark. The paired uteri were dissected from each female, placed on ice, and frozen upon return to the laboratory for later analysis. All pups were removed from the uteri and measurements of pup FL were taken.

Tissue samples, in the form of fin clips, were taken from all pups. Either fin clips or uterine tissue were taken from adult female sharks for genetic analysis. Tissue was stored in dimethyl sulfoxide (DMSO) buffer (Seutin *et al.* 1991) or 95% ethanol at 4 °C. DNA was subsequently extracted using the Chelex protocol described by Estoup *et al.* (1996). After 2 min of centrifugation at 16 000 g, 0.3 μL of the supernatant was used directly as a template for 5-μL polymerase chain reactions (PCRs). Five highly polymorphic microsatellite markers (Cpl-90, Cpl-128, Cpl-132, Cpl-166, Cpl-169) isolated from an enriched genomic library were amplified using IR-700 and IRD-800 labelled forward primers for each mother and her litter (Table 1). Descriptions of the primers and PCR conditions are reported elsewhere for four of the markers (Portnoy *et al.* 2006). The fifth marker Cpl-132 (F: CTCCCTTCCCTACCATATTTCC, R: AATACAGGAGGCTTTGCACGC, GenBank Accession no.: DQ191808) was optimized for this study. Cpl-132 reactions contained 20 mM Tris-HCl (pH 8.4), 1.2 mM MgCl$_2$, 0.001 mg/μL BSA, 0.2 mM dNTP mix, 20 pmol of primer. This marker required a step-up PCR protocol. The reaction conditions consisted of a denaturation at 95 °C for 4 min followed by 5 cycles at 94 °C for 1 min, 58 °C for 0.5 min and 72 °C for 1 min, followed by 25 cycles at 94 °C for 1 min, 65 °C for 1 min and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. All amplicons were electrophoresed through 25 cm 6.5% polyacrylamide gels using a LiCor 4200 Global IR^2 system. A 50–350 bp size standard was run in the first, middle, and last lanes of each gel and locus-specific standards were run in every 8th lane. Alleles were scored manually with the aid of GENE IMAGIR 4.05 (Scanalytics). Twenty percent of samples were randomly selected and rescored to ensure accurate scoring.

Genetic data analyses

Allele frequencies were calculated for each locus with FSTAT (Goudet 2001) using 67–73 adult individuals, including the 20 adults collected for this study, from throughout the species range in the western North Atlantic and Gulf of Mexico. Conformance to the expectations of Hardy–Weinberg equilibrium was calculated for each locus in GENEPOP (Raymound & Rousset 1995) using exact tests with 10 000 iterations. These same individuals were used to calculate the probability of excluding incorrect sires, given a known maternal genotype, for each individual locus and across all loci in GERUD 2.0 (Jones 2005) using the methodology of Dodds *et al.* (1996). The probability of detecting multiple paternity (PrDM) was calculated using PRDM software (Neff & Pitcher 2002), which only allows the user to input frequency data for 30 alleles per locus. Since Cpl-166 and Cpl-169 both have more than 30 alleles, low frequency alleles that did not appear in the maternal genotype were binned two at a time until only 30 states were left. For each litter, scenarios specific to the maternal genotype with different levels of paternal skew were considered. For example, for a monogamous litter with 10 offspring we evaluated the PrDM under several scenarios in which the litter actually had two sires. In each scenario, we used a different ratio of paternal contribution.

Genotypic arrays were visually inspected to ensure that all progeny shared at least one allele at each locus with their mother. The number of paternal alleles for each locus across a litter was then summed. A litter was considered polyandrous if two or more loci across a litter had three or more paternal alleles. Allele counts for each locus allowed for an initial estimate of the number of sires. For litters in which all loci had only two paternal alleles, Fisher's exact tests were used to determine whether loci conformed to the expectations of Mendelian segregation in a monogamous mating. GERUD 2.0 (Jones 2005) was then used to estimate the minimum number of fathers that sired a litter and the number of progeny per sire from the array of genotypes expressed by the female and her progeny. For cases in which no unique solution was found, up to 50 solutions

Table 1 Summary statistics for five microsatellite markers: allele number (*A*); gene diversity (*h*) calculated in FSTAT; number of individuals screened (*N*); conformance to HW equilibrium [*p*(*hw*)] calculated in GENEPOP; exclusion probabilities [*P*(*e*)] calculated in GERUD

Locus	Motif	*A*	*h*	*N*	*p*(*hw*)	*P*(*e*)
Cpl-90	(AC)$_{24}$	27	0.930	70	0.45	0.856
Cpl-128	(CA)$_{13}$TA(CA)$_{13}$	16	0.870	70	0.66	0.746
Cpl-132	(TG)$_{16}$	12	0.836	71	0.50	0.670
Cpl-166	(GT)$_{17}$	45	0.972	67	0.90	0.930
Cpl 169	(TG)$_{42}$	36	0.942	73	0.12	0.870

with the highest priority scores were ranked. COLONY 12 (Wang 2004), a program that clusters full sibling families within half sibling families using multilocus gene arrays, was also used to estimate the number of fathers that sired a litter and their relative contribution. Paternal genotypes reconstructed by both programs were examined to determine whether any sires had contributed to multiple litters. Reconstructed fathers were screened for the presence of multiple alleles across loci that were in high frequency in the population, as this may indicate multiple males being treated as one (Myers & Zamudio 2004). Estimates of sire number and patterns of paternal contribution obtained by the different algorithms were subsequently compared to ensure more robust results.

Possible relationships between female reproductive success (litter size) and body size (FL) as well as the number of sires and female size were determined through linear regression analysis. Chi-square analysis was used to test the null hypothesis that male reproductive success was random and would therefore conform to a Poisson distribution (Zar 1999). For each litter, the male with the greatest reproductive success in terms of number of offspring sired was designated as the most successful male. Bateman (1948) stated that variance in reproductive success was indicative of intrasexual selection and that the correlation between reproductive success and mate number was the cause of this selection. Therefore, by using Bateman's principles, the direct benefit for females who mate multiply can be compared to the benefit for males that limit additional male contribution to litters. It is important to note that although the latter relationship was not expressly discussed by Bateman, his principles can be applied because the correlation between reproductive success and number of additional sires still measures the fitness component of selection, and the variance in reproductive success still measures its strength. To make these comparisons, reproductive success was regressed against mate/sire number for females and the most successful males. Point estimates and confidence intervals of the slopes (B) were then used to examine relative benefit (Arnold & Duvall 1994; Arnold 1994; Jones *et al.* 2002). The standardized variances in reproductive success (I) were calculated by dividing the variance in reproductive success by the squared mean of reproductive success for each sex, allowing for the comparison of the strength of selection on each sex (Wade 1979; Wade & Arnold 1980; Jones *et al.* 2002).

Results

The distribution of genotypes at all loci conformed to the expectations of Hardy–Weinberg equilibrium (Table 1). The number of alleles present at each locus ranged between 12 and 45 (Table 1). Exclusion probabilities were high for each locus and the cumulative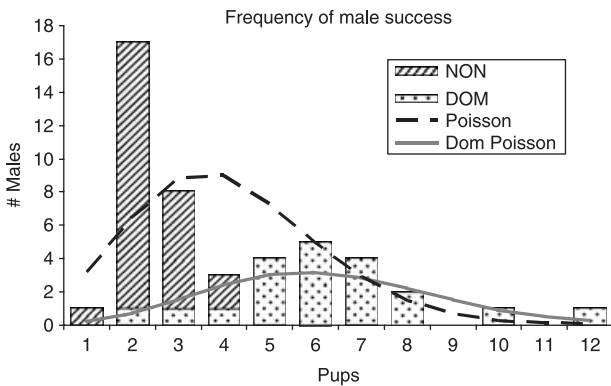 exclusion probability was greater than 0.99 (Table 1). A low frequency null allele (0.014) was discovered in two mothers and their litters at locus Cpl-169 (litters A and C). All pups in both of these litters amplified at least one allele at this locus. In addition, within litter allele counts were consistent between Cpl 169 and the other four loci. Since Cpl-169 also conformed to the expectations of Hardy–Weinberg equilibrium, the use of this locus did not bias our estimation of paternal contribution.

Genetic polyandry was detected in 17 of 20 litters (85%) by allele counts. Litters A, D and R had four or fewer parental alleles for each locus examined, consistent with genetic monogamy. Fisher's exact tests were nonsignificant in these litters indicating that all loci conformed to expectations of Mendelian segregation, supporting the conclusion that they were genetically monogamous. PrDm was lowest in the genetically monogamous litters (65%) when reproductive skew was assumed to be high (12–1), but increased rapidly as skew was decreased. Of the polyandrous litters, the number of sires per litter estimated by GERUD 2.0 varied between two and four, while COLONY estimated between two and five sires per litter. The average numbers of sires per litter as estimated by GERUD 2.0 and COLONY were 2.30 and 2.65, respectively. GERUD 2.0 produced a unique paternity solution in seven litters. For the remaining 10 litters, priority scores produced by GERUD 2.0 were used to rank scenarios. Only litter J and K had more than 50 solutions prior to ranking. For seven of these litters, all solutions predicted the same number of progeny per sire but differed in paternal genotypes. Litters J and Q had two solutions with different progeny per sire ratios. However, the same progeny per sire ratios appeared in the majority of solutions, most of which had higher-ranking priority scores. Only litter O resulted in more than two solutions with differing progeny per sire ratios. Even so, four of six solutions for this litter predicted the most successful male sired six of the pups (Table 2). COLONY results were the same as the highest-ranking GERUD results for eight of the polyandrous litters. In eight of the remaining nine litters, COLONY predicted the same number of progeny for the most successful sire but more total sires or different paternal contribution ratios. For litter J, COLONY predicted fewer offspring for the most successful sire than GERUD (Table 2). No reconstructed male genotypes appeared more than once across litters and none had an overabundance of high frequency alleles.

GERUD 2.0 and COLONY results showed similar trends and significance in most subsequent analyses therefore only the results using GERUD 2.0 data are presented below. The regression of reproductive success as a function of maternal fork length, had a slope that was not distinguishable from zero with fairly tight 95% confidence intervals (B = –0.015, P = 0.80, CI 95% = –0.14 < B < 0.11). The slope of relationship between the number of sires and female length was also not significantly different from zero (B = –0.027,

Table 2 Summary of GERUD and COLONY estimates of paternal contribution for *Carcharhinus plumbeus* litters; Atlantic (SA), Gulf of Mexico (GOM) minimum number of sires suggested by GERUD (Sires), most likely ratio of paternal contribution obtained from GERUD (Skew), number of GERUD solutions which returned the same paternal contribution ratio (#), total number of GERUD solution (Total), number of additional GERUD solutions with different paternal contribution (Alternative), number of sires suggested by COLONY (Sires 2), ratio of paternal contribution obtained from COLONY (COLONY)

Litter	Location	Size	Sires	Skew	#	Total	Alternative	Sires 2	COLONY
A	SA	10	1	NA	1	1	NA	1	NA
B	GOM	6	2	3:3	1	1	NA	2	3:3
C	GOM	10	3	6:2:2	20	20	NA	4	6:2:1:1
D	GOM	12	1	NA	1	1	NA	1	NA
E	SA	9	2	7:2	2	2	NA	2	7:2
F	GOM	9	2	7:2	1	1	NA	3	7:1:1
G	SA	8	2	6:2	1	1	NA	3	6:1:1
H	GOM	10	3	6:2:2	12	12	NA	4	6:2:1:1
I	GOM	4	2	2:2	6	6	NA	3	2:1:1
J	GOM	12	4	5:3:2:2	41	50	1	5	4:3:2:2:1
K	GOM	13	4	5:3:3:2	50	50	NA	4	5:3:3:2
L	GOM	9	2	5:4	2	2	NA	2	5:4
M	SA	10	2	8:2	1	1	NA	2	8:2
N	SA	10	2	7:3	1	1	NA	2	7:3
O	SA	11	3	6:4:1	1	6	3	3	6:4:1
P	SA	7	2	4:3	1	1	NA	2	4:3
Q	GOM	10	3	6:2:2	4	6	1	3	6:3:1
R	GOM	8	1	NA	1	1	NA	1	NA
S	SA	9	3	5:2:2	4	4	NA	4	5:2:1:1
T	SA	10	2	7:3	1	1	NA	2	7:3

Fig. 1 Frequency distribution of male reproductive success for males who sired greatest number of progeny in a given litter (DOM) and males who sired remainder of progeny in a given litter (NON). Dashed line is the expected distribution of mating success for all males, if it was determined by random processes (mean reproductive success calculated from data = 4.1). Solid line is the expected distribution of mating success for the most successful males only, if it was determined by random processes (mean reproductive success calculated from data = 6.2).

$P = 0.26$, CI 95% = $-0.076 < B < 0.022$). The distribution of reproductive success across all 46 males did not conform to the expectations of a Poisson distribution (d.f. = 7, χ^2-value = 25.38, $P < 0.01$, Fig. 1). When the data were partitioned into the reproductive success of the most successful males in each litter vs. other sires, the success of the most successful males conformed to the expectations of a Pois-

son distribution (d.f. = 7, χ^2-value = 4.42, $P > 0.75$, Fig. 1). The mean reproductive success of the most successful males was 6.3 pups per litter while the mean success for all other males was 2.4 pups per litter (*t*-test, d.f. = 22, $P < 0.001$).

Slope estimates for the regression of female reproductive success as a function of sire number differed depending on whether GERUD or COLONY results were used (GERUD: B = 0.98, 95% CI = $-0.11 < B < 2.1$; COLONY: B = 0.43, 95% CI = $-0.46 < B < 1.31$, Fig. 2), however, neither slope was significantly different from zero (GERUD: $P = 0.076$, COLONY: $P = 0.32$). The regression of the most successful males reproductive output against the number of sires per litter showed an inverse relationship, with consistent estimations of slope between GERUD and COLONY (GERUD: B = -1.30, 95% CI = $-2.42 < B < -0.19$; COLONY: B = -1.12, 95% CI = $-1.95 < B < -0.30$, Fig. 2). In both cases, the slopes were significantly different from zero (GERUD $P = 0.024$; COLONY $P = 0.01$). The standardized variance in reproductive success was higher for the most successful males (GERUD: I = 0.13; COLONY: I = 0.14) than females (I = 0.05).

Discussion

Genetic polyandry occurs with high frequency in *Carcharhinus plumbeus*. Of the 20 litters examined, 17 (85%) had multiple sires. This level of polyandry is consistent with some previous studies which reported 86% polyandry in *Negaprion brevirostris* and 100% polyandry in *Ginglymostoma cirratum* (Ohta *et al.* 2000; Saville *et al.* 2002; Feldheim *et al.* 2004). In *Sphyrna tiburo* however, genetic polyandry was
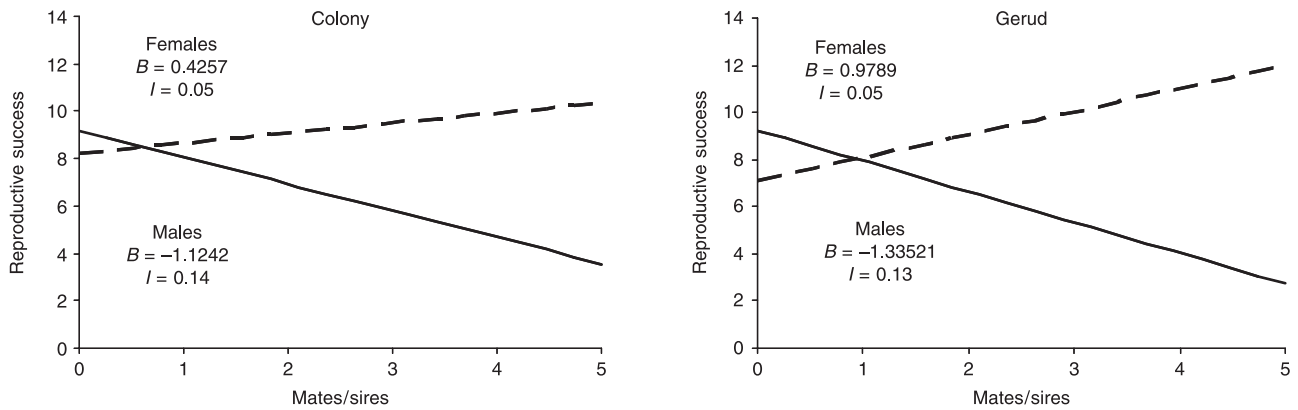
Fig. 2 Relationships between reproductive success and number of mates per litter for females (dash line) and reproductive success and number of additional sires for 'successful males' (solid line) using GERUD and COLONY data. Estimates of the intensity of selection (*I*) support point estimates of the slope (*B*) calculated by least squares regression. In this case, the larger *B* and *I* values for the male data suggest that there may be direct benefit for males that limit the number of additional sires in a litter, while there is no direct benefit for females who are multiply inseminated.

found in less than 19% of the litters examined (Chapman *et al*. 2004). Average litter sizes of polyandrous females were approximately 15 (*N* = 2) in *N. brevirostris* (Feldheim *et al*. 2002) and 29 (*N* = 3) in *G. cirratum* (Ohta *et al*. 2000; Saville *et al*. 2002). For *S. tiburo,* multiply sired females had an average litter size of 14 (*N* = 4) with significantly larger litters than monogamous mating females (Chapman *et al*. 2004). Despite smaller average litter size in *C. plumbeus* [just over 9, (*N* = 20)], polyandry was the dominant reproductive mode. Even the smallest litter (4) had multiple sires. In addition, male reproductive success was highly skewed within litters. Of the 17 polyandrous litters examined, nine had one male siring at least 60% of the total progeny. A similar pattern was observed in *S. tiburo* (Chapman *et al*. 2004) where high skew in male success in polyandrous litters was also present.

While the present study was unable to distinguish whether the mating system in *C. plumbeus* is truly polyandrous or is in fact polygynandrous, previous observational and experimental approaches in other shark species have revealed polygyny (Pratt & Carrier 2001; Feldheim *et al*. 2004). Theoretically, polygyny increases the fitness of any male able to sire multiple litters (Bateman 1948); therefore we feel it is likely that polygynous mating occurs in the sandbar shark.

We were unable to detect a relationship between female size and reproductive success. This may be due, in part, to sample size and/or the small range in litter sizes (between 4 and 13) observed in this study. Our point estimate of the slope, however, was very small and negative (–0.015 pups/cm). Taken literally this slope would mean that a female shark that grew 70 cm would have a decrease in reproductive success of one pup. Given that the species matures at 150-cm fork length and the largest females are

around 215 cm in fork length (Casey & Natanson 1992; Sminkey & Musick 1995) this point estimate lacks biological meaning. Similarly, we were unable to find a relationship between female size and sire number. Once again, the slope was quite small and negative (–0.027 sires/cm) lacking biological meaning throughout most of the 95% confidence interval. These data suggest that size is unrelated to the number of sires either because female sandbar sharks show no preference for number of matings or are unable to control mating frequency.

The development and use of highly variable microsatellite markers is critical to this type of study because the increased genetic resolution offsets the decreased probability of detecting sires when the number of offspring sampled are small (Neff & Pitcher 2002). In addition, the molecular markers provided fairly consistent results when estimating paternal contribution with programs that use different algorithms to estimate paternal contribution. The major difference in the output between the two programs is due to how each algorithm treats two unassigned progeny. GERUD 2.0 produces a more conservative estimate of the number of sires, as it will attribute these two offspring to one father. COLONY 1.2 will assign them to one or two fathers depending on the likelihood of each outcome (determined by population allele frequencies and the number of shared alleles between progeny). However, the simulations were consistent, allowing us to explore fitness benefits to both sexes in relation to patterns of genetic polyandry and reproductive skew. The direct benefit to multiple mating can be examined by estimating the slope of the least squares regression between reproductive success and number of mates (Bateman 1948; Arnold 1994; Arnold & Duvall 1994; Jones *et al*. 2000). In *C. plumbeus*, these slopes were flat (B = 0.43, B = 0.98) and not significantly different

from zero, suggesting that there may be little direct benefit for multiply inseminated females (Andersson & Iwasa 1996). The slope produced through linear regression results in the best approximation of selection gradients but may not be the best fit for the data (Lande & Arnold 1983). The absolute values of point estimates of slopes were larger for males than females (B = 1.12, B = 1.30) and significantly different from zero, suggesting males receive direct benefit by limiting the number of additional males gaining access to a female's ova. Since the estimated B for females varied depending on whether GERUD or COLONY results were used and confidence intervals were large, a second measure was used to validate our conclusions. Calculating the standardized variance of reproductive success (I) for males and females allows for an estimate of the amount of selective force the sexes are experiencing (Wade 1979; Wade & Arnold 1980). These measures corroborated the above conclusion as males had larger I-values than females, suggesting there is greater opportunity for selection on males to limit the number of additional sires contributing to a litter than there is for females to acquire additional sires. Together, these measures suggest that while there may be little direct benefit to females who mate multiply, the ability to bias paternity should be selected for in male sandbar sharks. While this study was unable to distinguish whether male *C. plumbeus* bias paternity through precopulatory (behavioural) or postcopulatory (physiological) mechanisms and direct observational data on this species reproduction are lacking, it seems likely that intrasexual competition is important in the evolution of male reproductive behaviour in this species.

The widespread genetic polyandry seen in *C. plumbeus*, in the absence of strong direct selection for females to mate multiply, may indicate that genetic benefits promote the maintenance of polyandry. Since these benefits affect an organism's inclusive fitness (reproductive success of offspring) they are difficult to demonstrate, but have been shown in a number of taxa (reptiles, Olsson *et al*. 1996; eutherian mammals, Keil & Sachser 1998; bony fishes, Evans & Magurran 2000; metatherian mammals, Kraaijeveld-Smit *et al*. 2002). In internally gestating animals such as the sandbar shark, the avoidance of genetic incompatibility, often caused by inbreeding (Zeh & Zeh 1997), may be an important genetic benefit for females who mate multiply. Mating in sharks is particularly costly to females due to blood loss caused by male biting (Springer 1960) and from vaginal lesions (Pratt 1979) resulting from the anchor-like morphology of the distal end of the male's splayed intromittent organs. One might expect polyandry to be common in sharks with small population sizes and low dispersal capabilities such as *G. cirratum*, or in sharks that show philopatry to isolated breeding grounds such as *N. brevirostris*. In these sharks, the genetic benefits of inbreeding avoidance may be great enough to outweigh the costs of mating.

Conversely, highly dispersive species with larger population sizes may be more likely to breed monogamously because the chances of inbreeding are lower while the costs of mating are still high (Chapman *et al*. 2004). In the western North Atlantic the sandbar shark has a wide range, large population size, and centralized mating location (Springer 1960); characteristics that would lead to the expectation of monogamy. However, genetic monogamy does not appear to be common.

Increased within-litter genetic variance caused by polyandrous mating may be a more important form of genetic benefit for female *C. plumbeus.* For females with reproductive cycles greater than 1 year, mating opportunity is limited and polyandrous mating may ensure increased genetic variation in progeny over a lifetime. In serially monogamous species that mate annually, this benefit may not be great enough to outweigh the cost of mating. Female sandbar sharks are believed to require a quiescent period between reproductive efforts, and likely do not mate annually (Springer 1960; Joung & Chen 1995). The same is true of both *G. cirratum* and *N. brevirostris* (Pratt & Carrier 2001; Feldheim *et al*. 2002). Female *S. tiburo*, in which monogamy is common, reproduce annually (Chapman *et al*. 2004). This pattern lends support to the idea that reproductive periodicity may be important in determining the rate of polyandry. The benefit of increased genetic variation across litters, however, affects a female's inclusive fitness. Such indirect benefits are thought to be smaller than direct benefits and therefore may not outweigh mating costs (Cameron *et al*. 2003).

Alternatively, while there may be some form of indirect female benefit, the lack of relationship between female size and number of sires may reflect the inability of female *C. plumbeus* of any size to control mating frequency. When mating is physically costly, genetic benefits may not improve fitness enough to encourage multiple matings beyond the minimum required to ensure the fertilization of all ova (Brown *et al*. 2004; Maklakov & Lubin 2004). While sandbar sharks in the western North Atlantic have a one-to-one sex ratio overall, segregation of the sexes results in sex ratios that vary in space and time (Springer 1960; Musick *et al*. 1993; Burgess, unpublished data). Females migrate long distances to give birth in nursery grounds such as Chesapeake Bay and Delaware Bay, where adult males are seldom seen. Mating, on the other hand, takes place at centralized mating grounds off the coast of Florida. However, because females are thought to reproduce once every 2 years and males annually, there is likely a male-biased operational sex ratio (OSR) on the mating grounds. The number of attempts by males to force or steal copulations has been shown to increase across taxa as OSR becomes more male biased (Shine *et al*. 2003; Byrne & Roberts 2004; Fitze *et al*. 2005; Head & Brooks 2006). Population densities may also change intersexual contact rates and consequently

reproductive behaviour (Westneat & Sherman 1997). As the density and persistence of males increases, female resistance may become difficult. In shark species, multiple males have been observed attempting to breed simultaneously or blocking female access to refugia (Carrier *et al.* 1994; Pratt & Carrier 2001).

When the costs associated with resistance outweigh the costs of matting, females may engage in convenience polyandry (Thornhill & Alcock 1983) and the level of genetic polyandry may be maintained above the female optima. This dynamic has been previously documented in other taxa (Rowe 1994; Lee & Hays 2004). Since females must cooperate to allow successful copulation, in species like the sandbar shark, female mating rates should be seen as evolving reaction norms, by which females seek to situationally maximize their fitness, rather than fixed optima (Arnqvist & Nilsson 2000). Experimental work with damselflies and guppies demonstrated that females were more likely to engage in superfluous copulations when the costs associated with resistance were great (Kelly *et al.* 1999; Cordero & Andres 2002). In these situations, more aggressive or persistent males may gain additional copulations, while more resistant females are able to avoid superfluous harmful matings. The increase in fitness for both sexes at the phenotypic extremes of aggression and resistance can lead to sexually antagonistic co-evolution (Holland & Rice 1998; Chapman *et al.* 2003). The results of such contests are the evolution of secondary characteristics used to ameliorate the costs of mating or involved directly in male aggression or female resistance (Lessells 2006). In elasmobranchs the thick skins of female sharks (Pratt & Carrier 2001), sexual segregation (Klimley 1985), and the seasonal development of mating teeth by males of many Batoids (Kajiura & Tricas 1996) may be examples of such characters. Parallel characters that are seen in insects where sexually antagonistic co-evolution is thought to operate include male and female grasping/antigrasping structures in water striders (Rowe & Arnqvist 2002) and the use of accessory gland products (Chapman *et al.* 1995).

In this study, we found high levels of genetic polyandry in western North Atlantic sandbar sharks. Our findings, however, suggest that neither direct female benefits nor avoidance of genetic incompatibility adequately explain the pattern of male fertilizations in our data. Additionally, our data suggest there may be more selective pressure for males to bias paternity than for females to mate multiply, indicative of intense intrasexual competition. While other cryptic genetic benefits for females cannot be discounted, we feel that coercive male mating tactics are likely important in dictating the number of matings in which a female engages. When examining patterns of polyandry in wild populations it is therefore important to account for intramasculine competition, as well as differing male and female motivations for reproductive behaviour.

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DS Portnoy is a student at the Virginia Institute of Marine Science (VIMS) and conducted this study as part of his PhD research on the population dynamics, reproductive behavior, and phylogeography of *C. plumbeus*. J. Musick is the Acuff professor of Marine Science at VIMS and his research interests include a variety of topics involving aspects of the ecology, conservation, systematics, and morphology of marine vertebrates. He serves as Co-chair of the International Union for the Conservation of Nature (IUCN) Shark Specialist group. A Piercy is a research biologist at the Florida Museum of Natural History and a PhD student at the University of Florida. His research is focused is on shark reproduction and age and growth. GH Burgess is the director of the Florida Program for Shark Research at the Florida Museum of Natural History. Mr. Burgess' research interests include fish ecology, systematics, and conservation. J. Graves, also a professor at VIMS, studies the processes of molecular evolution in marine organisms, with a focus on the population structure and movements of large pelagic fishes. He is also chair of the U.S. Advisory Committee for the International Commission for the Conservation of Atlantic Tunas.