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Are indirect genetic benefits associated with polyandry? Testing predictions in a natural population of lemon sharks

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Abstract

Multiple mating has clear fitness benefits for males, but uncertain benefits and costs for females. We tested for indirect genetic benefits of polyandry in a natural population, by using data from a long-term genetic and demographic study of lemon sharks (*Negaprion brevirostris*) at Bimini, Bahamas. To do so, we followed the fates of individuals from six cohorts (450 age-0 and 254 age-1 fish) in relation to their individual level of genetic variation, and whether they were from polyandrous or monoandrous litters. We find that offspring from polyandrous litters did not have a greater genetic diversity or greater survival than did the offspring of monoandrous litters. We also find no evidence of positive associations between individual offspring genetic diversity metrics and our surrogate measure of fitness (i.e. survival). In fact, age-1 individuals with fewer heterozygous microsatellite loci and more genetically similar parents were *more* likely to survive to age-2. Thus, polyandry in female lemon sharks does not appear to be adaptive from the perspective of indirect genetic benefits to offspring. It may instead be the result of convenience polyandry, whereby females mate multiply to avoid harassment by males. Our inability to find indirect genetic benefits of polyandry despite detailed pedigree and survival information suggests the need for similar assessments in other natural populations.

Keywords: fitness, heterozygosity, indirect genetic benefits, internal relatedness, microsatellites, polyandry

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Introduction

Multiple mating has long been known to provide fitness benefits to males (Bateman 1948), but the potential benefits to females are more controversial (i.e. polyandry; Birkhead 2000; Tregenza & Wedell 2000; Fedorka & Mousseau 2002). Females produce a limited number of ova, and so a single mating event often assures fertilization, whereas the physical act of mating may impose additional costs. Increased susceptibility to predation, physical injury, disease, and energetic loss are all potential female mating costs (Daly 1978; Blanckenhorn *et al*. 2002). In some species, these direct costs to mothers may be offset by direct benefits to offspring, such as increased parental care, nuptial gifts, protection, or access to territories (Arnqvist & Nilsson 2000; Wiklund *et al*. 2001). In other species, however, males contribute no material resources other than sperm (e.g. Pearse & Avise 2001), and here, multiple mating can be more difficult to explain. Furthermore, although multiple mating by females often leads to several males contributing paternity to a single litter (polyandry), this is not always the case (monoandry). Thus, we here define female multiple mating as the act of mating more than once in a single breeding season (either with the same male or different males), and polyandry as multiple males contributing to a female's litter.

One potential explanation for female multiple mating in these resource-free mating systems is indirect genetic benefits, such as greater genetic compatibility between parents, 'good genes', or increased genetic variability of offspring (Zeh & Zeh 2001; Neff & Pitcher 2005). Such

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benefits may produce offspring of higher genetic quality, thus increasing their survivorship or subsequent reproductive success (Zeh & Zeh 2001). Indirect genetic benefits have now been demonstrated in a broad range of taxa (Pearse & Avise 2001; Fedorka & Mousseau 2002; Foerster *et al*. 2003; Blouin-Demers *et al*. 2005), although their form and prevalence in nature remains controversial (Jennions & Petrie 2000; Brown *et al*. 2004; Lee & Hays 2004; Fitze *et al*. 2005; Kleven & Lifjeld 2005; Akçay & Roughgarden 2007). This controversy arises, at least in part, from most previous studies failing to effectively eliminate potential direct benefits (Jennions & Petrie 2000). Another problem is that viable alternatives are rarely considered. For example, multiple mating by females may simply be the result of coercion by males (Lee & Hays 2004; Fitze *et al*. 2005). In such cases, multiple mating may provide no benefit to offspring but instead function as a means by which females avoid excessive harassment. We attempt to address these issues by testing for indirect genetic benefits of polyandry in lemon sharks (*Negaprion brevirostris*), a species where direct benefits are absent and harassment is likely.

Indirect genetic benefits of polyandry might be tested by assessing whether offspring from litters sired by a single male differ in fitness from those sired by multiple males. Such experiments have shown that polyandry can enhance offspring survival in artificial settings (Fedorka & Mousseau 2002; Dunn *et al*. 2005; Ivy & Sakaluk 2005), but tests in natural populations are rare (but see Garant *et al*. 2005). Another possibility is to test whether the genetic diversity or genetic similarity of parents influences offspring viability (Birkhead 2000; Tregenza & Wedell 2000). Indeed, previous studies in birds have shown that females increase offspring heterozygosity and fitness through extra-pair matings (for example see Foerster *et al*. 2003). Finally, one can directly test whether individual offspring with low genetic diversity differ in fitness from those with high genetic diversity (Brown & Brown 1998; Kruuk *et al*. 2002). Here, many studies of wild vertebrate populations have reported associations between genetic diversity and key fitness components (Lieutenant-Gosselin & Bernatchez 2006), such as reproductive success (Slate *et al*. 2000; Charpentier *et al*. 2005) and juvenile survival (Coltman *et al*. 1998; Coulson *et al*. 1999; Garant *et al*. 2005).

Tests for relationships between genetic diversity and fitness in natural populations have recently been improved through the development of new multilocus genetic diversity metrics for individuals (Coltman & Slate 2003). One metric is the proportion of heterozygous microsatellite loci within an individual, and referred to here as 'standardized multilocus heterozygosity' (sMLH; Coltman *et al*. 1999). Another metric is the relatedness of an individual's parents (Duarte *et al*. 2003), but this method can only be used with genetic information on both parents. If such information is lacking, one can use 'internal relatedness' (IR), a direct derivative of Queller & Goodnight's (1989) relatedness coefficient, but with shared rare alleles weighted more heavily than common alleles (Amos *et al*. 2001). Recent work, however, has brought into question the accuracy of these two widely used multilocus estimators of genetic diversity. Aparicio *et al*. (2006) suggest that although sMLH does partly correct for bias among loci, it also ignores allele frequency. Similarly, IR is thought to underestimate heterozygosity of individuals carrying rare alleles. Thus, a new metric, homozygosity by loci (HL), has been developed, which avoids these problems by weighing the contribution of each locus to the homozygosity index depending on their allelic variability (Aparicio *et al*. 2006). We employ all of these genetic diversity metrics in the present study in order to compare their relative efficiency.

Polyandry in sharks

Sharks provide an interesting natural system for exploring the relationship between genetic variation and fitness because many species exhibit a combination of highly polygamous mating (Feldheim *et al*. 2001a; Saville *et al*. 2002; Daly Engel *et al*. 2006) and breeding site fidelity (i.e. philopatry; Hueter *et al*. 2005), with the latter increasing the chances of inbreeding. The pervasiveness of polyandry in most sharks studied to date has led some authors to suggest that this behaviour provides fitness benefits to females (Feldheim *et al*. 2004; Daly Engel *et al*. 2006). Any such benefits would have to be indirect because female sharks and their offspring do not receive direct material benefits from males, although females may mate multiply simply to guard against male infertility or sperm depletion. Moreover, any indirect benefits would likely have to be strong because mating costs seem likely: a male will grip a female's fin and trunk with his teeth, sometimes resulting in large areas of torn or missing flesh (Pratt Jr & Carrier 2001). Female sharks are also subject to cloacal injuries inflicted by intromittent organs (Pratt Jr & Carrier 2001), and these deep cuts have the potential to cause extensive blood loss and infection. If indirect benefits are found to be lacking, then a likely alternative is that females engage in multiple mating simply to reduce the costs of coercion by males (Portnoy *et al*. 2007).

The lemon shark is a large, placentally viviparous coastal species. It is found in the western Atlantic from New Jersey to Brazil, in coastal Atlantic waters off of West Africa, and in the eastern Pacific from Baja California to Colombia (Compagno 1984). Adult females of this species use shallow-water nursery areas for mating and parturition, and give birth to between 4 and 18 juvenile pups every 2 years (Feldheim *et al*. 2002). We have intensively studied one such nursery site (Bimini, Bahamas) every year since 1995. The enclosed nature of the Bimini nursery lagoon allows for a remarkable sampling efficiency of offspring in each year, with a very high proportion of these individuals then recaptured in subsequent years (Gruber *et al*. 2001; DiBattista *et al*. 2007). The nursery lagoon serves as both a mating and birthing ground, and recent genetic studies have found that pregnant females exhibit philopatry to this site (Feldheim *et al*. 2002; Feldheim *et al*. 2004). Past work has also shown that females mate with multiple males, but that males rarely mate with multiple females (Feldheim *et al*. 2004).

Predictions

If polyandry in the Bimini lemon shark population confers indirect genetic benefits to offspring, then several predictions might hold. First, offspring from polyandrous litters might have higher survival than offspring from monoandrous litters. Second, offspring from polyandrous litters might have higher genetic diversity than offspring from monoandrous litters. Third, more genetically diverse offspring might survive at a higher rate. Genetic pedigree reconstruction in this population allows us to categorize each litter as monoandrous or polyandrous, and to estimate the genetic diversity of individual offspring. Detailed mark–recapture data for juveniles then allows us to examine whether these genetic properties are related to offspring survival.

Materials and methods

Field data

Bimini, Bahamas (25°44′N, 79°16′W), is a mangrove-fringed chain of islands located 85 km east of Miami, Florida. The Bimini islands enclose a 21-km^2 shallow lagoon (0–120 cm deep at low tide) that serves as a nursery site for approximately 300 juvenile and sub-adult lemon sharks (Morrissey & Gruber 1993). Each year since 1995, approximately 99% of the juveniles have been captured in two areas of the Bimini lagoon: North Sound and Sharkland (Gruber *et al*. 2001). This exhaustive sampling allows for the accurate and unbiased estimation of mortality, which is 44% between age-0 and age-1, and 45% between age-1 and age-2 (DiBattista *et al*. 2007). These mortality rates should provide an excellent opportunity to test whether juvenile survivorship is related to polyandry and genetic diversity.

Sampling takes place just after pupping by adult females. Newborn and juvenile sharks are captured in 180-m long × 2-m deep gill nets (Manire & Gruber 1991). Sub-adult (males: 70–175 cm; females: 70–185 cm) and adults (males > 175 cm, females > 185 cm; Compagno 1984) are captured using rod and reel or longline fishing gear. When feasible, each shark is weighed (kilograms), measured for precaudal length (PCL, tip of snout to precaudal pit in millimetres; Compagno 1984), and tagged intramuscularly with an individually coded passive integrated transponder (PIT) tag. Each subsequent time a shark is captured, we record its tag number and again measure its length and mass. A small piece (2 mm^2) of fin tissue is taken from every shark for subsequent DNA extraction. The present study focuses on pedigree data of newborn and juvenile sharks sampled from 1995 to 2000 (Table 1).

The following methods allowed us to assign ages to 704 of 774 sampled juvenile sharks (450 age-0 and 254 age-1), with the remainder excluded from analyses. Umbilical status was used to identify newborn (age-0) sharks in 1997 through 2000. The open umbilical scar slowly closes during the first few months of life, enabling age-0 sharks to be distinguished from other year classes. Age-0 sharks in these samples were always less than 52 cm PCL, and so we used this as a conservative cut-off point to identify age-0 sharks when umbilical status was not recorded (1995 and 1996). For the few sharks caught for the first time at age-1 or age-2, we determined the year of birth (and therefore age) based on length measurements and an average annual growth rate at Bimini of 5.2–7.1 cm (Barker *et al*. 2005). For individuals where the above method yielded ambiguous results (e.g. near an age cut-off), microsatellite analyses were used to match individuals of unknown age to their siblings of known age (for details see below and Feldheim *et al*. 2004).

Survival in the first (age-0 to age-1) and second (age-1 to age-2) year of life was estimated by recaptures in the nursery area between 1996 and 2005. If a shark survived from age-0 to age-1, and did not emigrate during that interval, we had a 0.99 probability of recapturing it at age-1 (DiBattista *et al*. 2007). If a shark survived from age-1 to age-2, and did not emigrate during that interval, we had a 0.92 probability of recapturing it at age-2 (DiBattista *et al*. 2007). Moreover, numerous lines of evidence indicate that very few sharks emigrate from the study site before age-2: (i) telemetry studies reveal high site fidelity and limited movement (Morrissey & Gruber 1993); (ii) juvenile sharks displaced 4–16 km from their observed home ranges returned 97% of the time (Edrén & Gruber 2005); and (iii) age-0 and age-1 sharks are rarely captured outside of the nursery area (Gruber *et al*. 2001; DiBattista *et al*. 2007). For all of these reasons, we are confident that tagged sharks not recaptured after a given interval had died during that interval.

Genetic data

Genomic DNA was extracted from fin samples with a salting-out protocol (Sunnucks & Hales 1996). Samples were then genotyped with nine dinucleotide microsatellite primer pairs. Microsatellite screening and polymerase chain reaction (PCR) amplification conditions are described elsewhere (Feldheim *et al*. 2001b; Feldheim *et al*. 2002). Multilocus genotypes were obtained for a minimum of

Table 1 Summary of newborn (age-0) and age-1 juvenile lemon sharks caught, tagged, and genetically analysed, including annual survival rates to age-1 or age-2

Cohort	Captured sharks (*N*)*	Survival†	Genetically assigned or reconstructed mothers‡	Genetically assigned or reconstructed fathers‡	Both parents genotyped
Age-0 sharks§					
1995	83	0.59	0.95	0.82	0.82
1996	41	0.63	1.00	0.71	0.71
1997	96	0.55	0.97	0.71	0.71
1998	66	0.56	0.99	0.88	0.88
1999	95	0.61	0.95	0.71	0.71
2000	68	0.49	0.97	0.62	0.62
Total	450	0.57	0.97	0.74	0.74
Age-1 sharks§					
1996	50	0.64	0.96	0.76	0.76
1997	35	0.54	0.97	0.66	0.66
1998	58	0.53	0.99	0.78	0.78
1999	41	0.42	0.98	0.83	0.83
2000	70	0.41	0.98	0.73	0.73
Total	254	0.50	0.98	0.75	0.75

*All sharks caught were genotyped for at least seven of nine microsatellite loci; †survival represents the proportion of individuals surviving to age-1 (for age-0 sharks) or age-2 (for age-1 sharks); ‡proportion of individuals with genetically assigned or genetically reconstructed mothers and fathers; §fish ages are the first year of life (age-0) and the second year of life (age-1).

seven loci for all juvenile sharks (Table 1). Deviations from Hardy–Weinberg and linkage equilibrium were tested in GENEPOP version 3.4d (Raymond & Rousset 1995; see Appendix I). The presence of null alleles was examined by using MICRO-CHECKER version 2.2.3 (van Oosterhout *et al*. 2004).

Parentage analysis was based on two steps. We first used the maximum-likelihood program CERVUS version 2.0 (Marshall *et al*. 1998) to assign individual offspring to the few candidate parents that we were able to catch (i.e. eight adult females and five adult males, see Feldheim *et al*. 2002). We considered both a relaxed (80%) and strict (95%) confidence level in CERVUS, and included any parents assigned under these criteria. However, for the remaining, unassigned offspring (87% and 85% of age-0 and age-1 sharks, respectively), we constructed progeny arrays between all pairs of newborn sharks within a cohort, thus revealing full- and half-sibling groups (i.e. KINSHIP 1.3; Goodnight & Queller 1999). These groups were then used to manually reconstruct the genotypes of parents that we had not been able to catch (for more details see Feldheim *et al*. 2002, 2004). In this way, we were able to genetically assign or reconstruct 97% of the mothers of age-0 sharks and 98% of the mothers of age-1 sharks (Table 1). Paternal reconstruction was less successful (74% and 75% for age-0 and age-1 sharks, respectively), but we were usually able to ascertain when multiple fathers sired a litter (Feldheim *et al*. 2004). The mean number of fathers per litter was 2.086 ± 0.083, with a range of one to four. Furthermore, there was little evidence of within-litter reproductive skew. On average, in litters with two fathers, one male sired the majority of the offspring (66%), but in litters sired by three or more sires, no one male sired the majority of the offspring (see Fig. 1).

Based on a pedigree reconstructed using the above methods, we classified each female's litter as having been fertilized by a single male (monoandry) or by several males (polyandry). Litters in which we sampled two or fewer juveniles (5 out of 86 litters) were excluded because of the difficulty of inferring multiple paternity. We also ignored multiple mating by males within years — because this was the case for less than 6% of the parental males. Finally, because some mating events may not produce offspring, our results directly inform the genetic consequences of female polyandry, which may or may not closely reflect the fitness consequences of female multiple mating.

Hypothesis testing was based on several different measures of offspring genetic diversity that have proven useful in other natural populations of vertebrates (e.g. Garant *et al*. 2005; Aparicio *et al*. 2006; Da Silva *et al*. 2006). First, we calculated sMLH for each individual, which measures the proportion of loci that are heterozygous, weighted at each locus by the expected heterozygosity (Coltman *et al*. 1999). Not all individuals were typed at the same number of loci — and so sMLH values were standardized through division by the maximum value observed at that locus, which reduces the influence of highly polymorphic

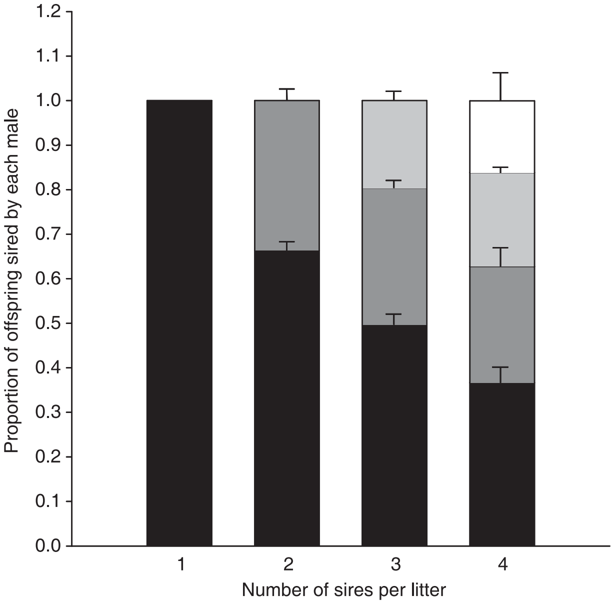

Fig. 1 Mean paternal contribution within litters of newborn lemon sharks, expressed as the proportion of offspring sired ± 1 SEM. Litters are sired by either one ($N = 17$), two ($N = 42$), three ($N = 20$), or four males ($N = 2$); the first father (black bars) represents males siring the largest proportion of offspring within each litter, the second father (dark grey bars) represents males siring the second largest proportion, third fathers (light grey bars) sire the third largest proportion, and fourth fathers (open bars) sire the fourth largest proportion.

loci (see Amos *et al*. 2001). Second, we calculated IR, which estimates the relatedness of each individual's parents and thus the relative level of inbreeding. This metric is adapted from Queller & Goodnight's (1989) relatedness coefficient, and it measures the genetic correlation between alleles at a locus within an individual, using population allele frequencies as a weighting factor (Amos *et al*. 2001). When calculated across multiple loci, IR values are approximately normally distributed around a mean of zero, with negative (positive) values suggesting relatively 'outbred' ('inbred') individuals (Amos *et al*. 2001). Third, we calculated HL, which weights the contribution of each locus to the homozygosity index depending on their allelic variability (Aparicio *et al*. 2006); similar to IR, higher values are indicative of increased homozygosity, and thus lower genetic diversity.

To ensure that IR provides an accurate reflection of parental relatedness, we estimated the relationship coefficient between all genetically assigned or reconstructed parents in our data set by using Goodnight & Queller's (1999) software program KINSHIP 1.3. The reference population used to calculate allele frequencies in these analyses was all the individuals caught over the course of the study. We then calculated the correlation between relatedness coefficients obtained in KINSHIP, and IR estimated for those offspring of each parental pair. In both age-0 and age-1 sharks, positive correlations were evident (age-0, $r = 0.46$, $P < 0.0001$; age-1, $r = 0.42$, $P < 0.0001$), and subsequent analyses based on both metrics were similar (despite a smaller sample size for relatedness coefficients: $N = 306$ or 178 for age-0 and age-1 juveniles, respectively), thus only IR values are reported here. This also suggests that IR provides a reasonable surrogate for parental relatedness in matings for which relatedness cannot be calculated directly.

Statistical analyses

Means are presented as ± 1 SEM and $\alpha = 0.05$. Most data (molecular metrics and survival values) did not conform to a normal distribution, even after transformations. When testing for the benefits of polyandry, we therefore used randomization tests with 10 000 permutations, as implemented in RESAMPLING version 1.3 (Hovell 2002). This program compares the proportion of times data randomly reshuffled between all treatment groups gives an effect (i.e. t or F-value) greater than that of the original dataset. For our first prediction, we used this approach to test whether survival rates differ between the offspring of monoandrous vs. polyandrous litters. For our second prediction, we used it to test whether average genetic diversity differs between monoandrous vs. polyandrous litters. No significant survival or genetic diversity differences were detected among cohorts, and so data from 1995 to 2000 were pooled for all analyses. However, age-0 and age-1 sharks were analysed separately because some individuals were in both data sets, which were therefore not independent.

Our third prediction was that more genetically diverse offspring would survive at a higher rate. Here, we analysed relationships between each genetic diversity metric (sMLH, IR, HL) and *individual* survival in separate logistic regression models with binomial error structures (e.g. Da Silva *et al*. 2006). Potential effects of relevant variables on survival were first considered: sex, cohort, population density (i.e. number of unique individuals caught each year), and relative condition factor (calculated as 10 000 mass PCL^{-b}, where b is the slope of the regression line of $\log_{10}$ mass on $\log_{10}$ PCL for the entire data set; see Schulte-Hostedde *et al*. 2005). Logistic regressions exclude individuals that are missing data for one or more variables, and so we adopted a two-step procedure. First, all of the non-genetic terms, which were known for most individuals, were fit to the model for age-0 to age-1 survival and (separately) to the model for age-1 to age-2 survival. The non-genetic full models were then reduced by removing each nonsignificant term. Genetic terms were then added individually to the reduced models. All interactions were nonsignificant and thus not reported here.

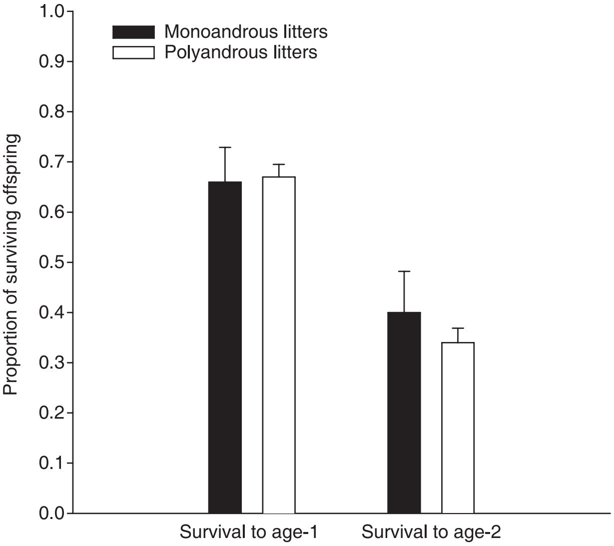

Fig. 2 Mean survival of monoandrous (1 sire; $N = 17$) and polyandrous (2, 3, or 4 sires; $N = 64$) litters. Survival represents the mean proportion of individuals within each litter surviving to age-1 (for age-0 sharks) or age-2 (for age-1 sharks). All values are means ± 1 SEM.

Results

The microsatellite loci exhibited high heterozygosity (H_O: 0.537–0.950) and allelic diversity, with 5–40 alleles per locus (see Appendix I). Three loci showed Hardy–Weinberg deficits (LS11, $P < 0.001$; LS15, $P = 0.011$; LS22, $P = 0.036$) and all pairs of loci were out of linkage equilibria (all $P < 0.05$). Feldheim *et al.* (2002) found similar results, and attributed these departures from neutrality to sampling a large proportion of siblings. The fact that we found no evidence of null alleles ($P > 0.05$ in all cases), and that we sampled a single localized juvenile population (i.e. pronounced sibling structure, but no Wahlund effect), supports this assertion.

We found no genetic or survival differences between monoandrous and polyandrous litters. Most obviously, the proportion of offspring within a litter that survived to age-1 or age-2 was not significantly different between monoandrous and polyandrous litters (survival to age-1, $t = 0.044$, d.f. = 80, $P = 0.97$; survival to age-2, $t = -0.87$, d.f. = 80, $P = 0.39$; Fig. 2). Similarly, within-litter sMLH ($t = 0.402$, d.f. = 80, $P = 0.69$), IR ($t = -0.90$, d.f. = 80, $P = 0.37$), and HL ($t = -0.28$, d.f. = 80, $P = 0.79$) did not differ between monoandrous and polyandrous litters (Fig. 3). We also failed to find any relationship between the number of males siring a litter (i.e. one to four males) and the proportion of offspring that survived (survival to age-1: $F_{3,80} = 0.58$, $P = 0.77$; survival to age-2: $F_{3,80} = 0.82$, $P = 0.39$), within-litter genetic diversity (sMLH: $F_{3,80} = 0.45$, $P = 0.74$), parental relatedness (IR: $F_{3,80} = 0.71$, $P = 0.56$), or HL ($F_{3,80} = 0.31$, $P = 0.89$). The moderate sample sizes in these analyses may have limited statistical significance in these comparisons, but biological significance also seems lacking given that effect sizes were very small ($d < 0.2$ in all cases). We did, however, find that polyandrous litters were significantly larger than monoandrous ones ($t = 3.16$, d.f. = 80, $P = 0.002$), and that litter size increased as a function of the number of sires ($F_{3,80} = 5.91$, $P = 0.001$).

HL was highly correlated with both sMLH (age-0 data set: $r = -0.97$, $P < 0.001$; age-1 data set: $r = -0.97$, $P < 0.001$) and IR (age-0 data set: $r = 0.94$, $P < 0.001$; age-1 data set:

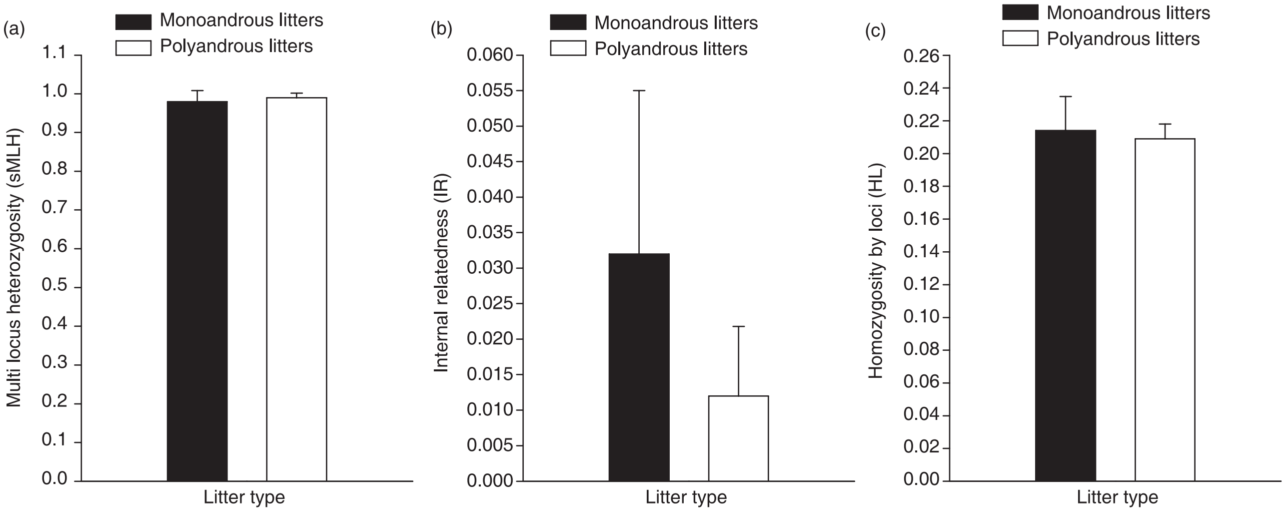

Fig. 3 Mean standardized multilocus heterozygosity (sMLH; a), internal relatedness (IR; b), or homozygosity by loci (HL; c) of monoandrous (1 sire; $N = 17$) and polyandrous (2, 3, or 4 sires; $N = 64$) litters. All genetic diversity metrics were calculated among offspring for each litter, and then averaged over all litters for each treatment group (i.e. monoandry vs. polyandry). All values are means ± 1 SEM.

Table 2 Logistic regression models of juvenile survival to age-1 ($N = 450$) or age-2 ($N = 254$)

Model terms*	d.f.	Wald statistic (χ^2)	Coefficient (SE)	*P* value
Age-0 sharks				
Non-genetic terms				
Cohort	5	3.63	3.63 (8.21)	0.60
Sex	1	0.35	–0.11 (0.19)	0.56
CF†	1	0.43	–0.52 (0.79)	0.51
Pop. density	5	0.22	–3.83 (8.21)	0.64
Genetic terms				
sMLH	1	1.52	–0.68 (0.55)	0.22
IR	1	1.10	0.66 (0.64)	0.30
HL	1	1.26	0.85 (0.75)	0.26
Age-1 sharks				
Non-genetic terms				
Cohort	4	7.93	–0.34 (0.46)	0.094
Sex	1	5.86	–0.63 (0.26)	**0.016‡**
CF†	1	0.12	–0.43 (1.26)	0.73
Pop. density	4	7.69	–0.97 (0.44)	0.10
Genetic terms				
sMLH	1	4.92	–1.56 (0.70)	**0.027‡**
IR	1	5.49	1.92 (0.82)	**0.019‡**
HL	1	3.96	1.89 (0.95)	**0.046‡**

*Abbreviations: mean standardized multilocus heterozygosity (sMLH); internal relatedness (IR); homozygosity by loci (HL); relative condition factor (CF). †Relative condition factor was calculated as 10 000 mass PCL^{-b}, where b is the slope of the regression line of log$_{10}$ mass on log$_{10}$ PCL for the entire data set (age-0: $b = 2.83$, $r = 0.72$; age-1: $b = 2.77$, $r = 0.88$). ‡Significant effects are shown in bold.

$r = 0.93$, $P < 0.001$), and did not provide qualitatively different results. Given that HL is supposed to provide comparatively more statistical power for a given sample size compared to existing metrics, agreement in our results reinforces the robustness of our genetic diversity findings.

The probability of survival for individuals was related to few potential predictor variables. For the age-0 model, survival to age-1 was not influenced by non-genetic terms (Table 2), nor by sMLH ($P = 0.20$), IR ($P = 0.28$), or HL ($P = 0.26$). For the age-1 model, the only non-genetic effect was that males had higher survival than females ($P = 0.032$). Interestingly, survival decreased for individuals with higher sMLH ($P = 0.027$), but lower IR ($P = 0.019$) and HL ($P = 0.046$). This suggests that age-1 offspring are more likely to survive to age-2 if they are less genetically diverse. When each locus was considered separately, however, the two least variable loci (LS54 and LS75, each with five alleles) appeared to drive this result (Table 3), with the remaining loci not significant and variable in the direction of their respective correlations.

Finally, we tested whether the genetic diversity of individuals was correlated with their size at age-1 (i.e. PCL) or with their first year growth (expressed as centimetres grown in a year). Spearman's rank order correlations revealed that more genetically diverse and less inbred individuals were larger at age-1 (sMLH vs. PCL: $r = 0.15$, $P = 0.015$; IR vs. PCL: $r = -0.14$, $P = 0.027$; HL vs. PCL: $r = -0.12$, $P = 0.043$) and grew faster (sMLH vs. growth: $r = 0.16$, $P = 0.023$; IR vs. growth: $r = -0.17$, $P = 0.017$; HL vs. growth: $r = -0.13$, $P = 0.037$). These correlations potentially help explain the lower survival of more genetically diverse individuals, because larger and faster growing individuals have a lower probability of juvenile survival at Bimini (DiBattista *et al*. 2007).

Discussion

We examined mating patterns (i.e. polyandry), genetic diversity, and fitness (i.e. survival) in a natural population of lemon sharks. In contrast to recent studies in other taxa (Garant *et al*. 2005; Ivy & Sakaluk 2005; Fisher *et al*. 2006), we find no evidence of indirect genetic benefits for 1- and 2-year-old offspring from polyandrous females.

Polyandry and indirect genetic benefits

Polyandry is a common phenomenon in elasmobranchs (Feldheim *et al*. 2001a; Saville *et al*. 2002; Daly Engel *et al*. 2006; but see Chapman *et al*. 2004). The lemon shark, in particular, is highly polyandrous (Feldheim *et al*. 2004), and we identified litters with up to four fathers. With few exceptions, females produced offspring with a particular

Table 3 Logistic regression model for the effects of single-locus heterozygosity (HET), internal relatedness (IR), or homozygosity by loci (HL) on juvenile survival to age-1 ($N = 450$) or age-2 ($N = 254$). To isolate individual effects, significance was assessed by including all loci in the model, and then sequentially removing terms. All *P* values were Bonferroni adjusted to account for multiple comparisons. Interactions with all non-genetic terms considered in this study (cohort, sex, relative condition factor, and population density) were not significant and thus not presented in this table

Locus		*P* value (survival to age-1)	Coefficient (SE)	*P* value (survival to age-2)	Coefficient (SE)
LS11	HET	0.29	0.15 (0.14)	0.93	0.016 (0.19)
	IR	0.31	–0.15 (0.15)	0.94	–0.016 (0.20)
	HL	0.29	–0.21 (0.20)	0.93	–0.023 (0.27)
LS15	HET	0.64	–0.091 (0.19)	0.16	–0.38 (0.26)
	IR	0.65	0.089 (0.19)	0.074	0.48 (0.27)
	HL	0.64	0.11 (0.24)	0.16	0.47 (0.33)
LS22	HET	0.51	0.17 (0.27)	0.23	–0.45 (0.38)
	IR	0.52	–0.17 (0.27)	0.23	0.46 (0.38)
	HL	0.51	–0.19 (0.29)	0.23	0.50 (0.42)
LS30	HET	0.37	–0.13 (0.14)	0.62	0.093 (0.19)
	IR	0.35	0.14 (0.15)	0.52	–0.13 (0.20)
	HL	0.37	0.18 (0.20)	0.62	–0.13 (0.27)
LS48	HET	0.33	–0.41 (0.42)	0.80	–0.13 (0.50)
	IR	0.34	0.40 (0.42)	0.76	0.15 (0.50)
	HL	0.33	0.43 (0.44)	0.80	0.13 (0.53)
LS54	HET	0.26	–0.12 (0.10)	**0.032***	–0.30 (0.14)
	IR	0.39	0.10 (0.12)	**0.014***	0.39 (0.16)
	HL	0.26	0.22 (0.19)	**0.032***	0.55 (0.25)
LS75	HET	0.48	–0.10 (0.14)	0.057	–0.36 (0.19)
	IR	0.53	0.091 (0.14)	**0.041***	0.39 (0.19)
	HL	0.48	0.14 (0.20)	0.057	0.52 (0.28)
LS52	HET	0.72	0.14 (0.38)	0.60	–0.27 (0.53)
	IR	0.71	–0.15 (0.38)	0.61	0.27 (0.52)
	HL	0.72	–0.15 (0.41)	0.60	0.29 (0.56)
LS82	HET	0.19	–0.23 (0.17)	0.86	0.040 (0.22)
	IR	0.19	0.23 (0.18)	0.88	–0.035 (0.23)
	HL	0.19	0.30 (0.23)	0.86	–0.053 (0.29)

*Significant effects are shown in bold.

male only once, and males rarely contributed offspring to more than one litter in a given year. Females may therefore mate with multiple males within a season, or perhaps different males in different seasons. The latter is possible owing to sperm storage in paired oviducal glands (Pratt Jr 1993), where sperm may remain viable for several years (Pratt Jr & Tanaka 1994). The specific patterns of polyandry may then be the result of pre- or postcopulatory processes, with the latter including differential sperm utilization or investment in particular offspring before parturition (Eberhard 1996; Neff & Pitcher 2005).

What benefits, if any, might female sharks gain by mating with more than one male? Direct benefits seem unlikely given that sharks do not form stable pair bonds after copulation, do not provide postnatal parental care, and do not derive material resources from mating (Pratt Jr & Carrier 2001). Alternatively, females may mate multiply to guard against infertility or sperm depletion of their mating partners. This possibility cannot be ruled out given the strong relationship between litter size and the number of genetic mating partners detected in this study, as well as a lack of male reproductive skew. An additional alternative, and the one that we test here, is that females may gain an indirect genetic benefit for their offspring. These benefits might relate to genetic compatibility, 'good genes', or genetic variation per se (Zeh & Zeh 2001; Neff & Pitcher 2005). We now consider each of these possibilities in turn, while also recognizing that they are not mutually exclusive.

First, polyandrous females increase the chances of obtaining a genetically compatible (i.e. dissimilar) male (Tregenza & Wedell 2000; Zeh & Zeh 2001; Neff & Pitcher 2005). Females might then bias paternity towards that male, or preferentially provision that male's offspring (Tregenza & Wedell 2000), which should then increase offspring survival. Under this scenario, offspring from polyandrous litters should have less related parents and higher survival, which was not the case in our study (Figs 2 and 3). In fact, survival from age-1 to age-2 was *higher* for juveniles with more genetically similar parents (Table 2), although this result was mostly based on only two loci (see below). We must also caution that we cannot assess incompatibility effects based on other genes, such as the major histocompatibility complex (Jennions & Petrie 2000), or effects that manifest themselves during gestation.

A second hypothesis is that polyandrous females bias paternity or investment towards the best male or the best offspring. The difference from the previous hypothesis is that females here benefit from specific good genes (additive genetic effects), rather than genetic compatibility (nonadditive genetic effects; Birkhead & Moller 1992). Under this hypothesis, we would predict that (i) there should be reproductive skew in polyandrous litters with 'genetically superior' males siring the majority of young, and (ii) those males should produce offspring with the highest fitness. As with the genetic compatibility hypothesis, we find little support for good genes benefits. Although one male sired the majority of offspring (66%) in litters with only two fathers, males did not contribute a disproportionate amount of offspring to increasingly polyandrous litters (i.e. three or four sires, see Fig. 1). Furthermore, within all polyandrous litters, offspring survival was no different between males that sired the largest proportion of the litter, and all other contributing males ($P > 0.385$ in all cases, data not shown). Although our results fail to support the good genes hypothesis, we cannot completely rule it out. For example, females may preferentially mate with males that are homozygous for particular genes, which may also explain the observed positive association between offspring homozygosity and survival. Alternatively, females may obtain good genes that only manifest themselves as reproductive benefits for their sons (sexy-sons hypothesis; see Neff & Pitcher 2005).

A third hypothesis is that polyandrous females increase genetic diversity among offspring within a given litter, which may hedge against uncertain future environmental conditions (Ridley 1993). That is, by increasing the genetic diversity of her offspring in a single breeding season, a female may increase the probability that at least one is adapted to the current environmental conditions, or is able to adapt to changing conditions. Under this hypothesis, we would expect polyandrous litters to have greater genetic diversity among offspring and to have higher average survival, neither of which proved to be the case (Figs 2 and 3). We caution, however, that the benefits of polyandry might only be evident when following a female over her entire reproductive life, which is often prohibitive for long lived organisms such as lemon sharks (i.e. mature at 10–12 years of age, live up to 40 years; Brown & Gruber 1988).

Genetic diversity and survival

Various studies have found positive relationships between neutral genetic diversity and survival in the wild (Coltman *et al*. 1998; Coulson *et al*. 1999; Foerster *et al*. 2003; Garant *et al*. 2005) — but this was not the case in our study. Many possible explanations exist for our counter-intuitive finding, with one being physical linkage between neutral marker genes and genes experiencing balancing selection (see Acevedo-Whitehouse *et al*. 2006). Our data do not refute this possibility because two marker loci are clearly having a stronger effect than the others. However, these loci were also the least variable, and so high variability at the other loci may mask a true negative effect (i.e. a greater proportion of individuals are heterozygous at these loci).

It also remains possible that genetic diversity is correlated with other (unmeasured) fitness components, such as embryo survival *in utero*, survival to adulthood, or lifetime reproductive success. The first of these possibilities seems remote given that parental relatedness, and thus the relative level of inbreeding, is not related to the number of offspring produced in each lemon shark litter (Feldheim *et al*. 2004). The other two possibilities cannot be assessed owing to the difficulty of tracking these mobile and long-lived animals. Yet another possibility is that genetic diversity may show opposite correlations with different agents of juvenile mortality, such as predation, starvation, or disease. These offsetting effects may mask overall fitness effects (Acevedo-Whitehouse *et al*. 2003), although little is known about the relative frequency of causes of juvenile mortality in the lemon shark.

We here suggest that high heterozygosity can also reduce survival. This might occur in two ways. First, high heterozygosity per se might influence specific traits in ways that are detrimental to survival. Indeed, juveniles with higher heterozygosity generally grow faster and are larger at age, a relationship often seen in natural populations (Coltman *et al*. 1998; Coulson *et al*. 1998; Pujolar *et al*. 2005). In these other taxa, increased growth is thought to improve fitness but that is not the case here. At Bimini, fast growth and large size are unequivocally associated with *reduced* juvenile survival (DiBattista *et al*. 2007) — perhaps because fast growth requires the adoption of more risky foraging strategies (see Mangel & Stamps 2001). A second possibility is that higher heterozygosity might reflect outbreeding depression. This does seem possible for Bimini lemon sharks because (i) they differ significantly in growth and size from other lemon shark populations (Barker *et al*. 2005; Freitas *et al*. 2006), and (ii) males apparently move extensively between nursery sites (Feldheim *et al*. 2001b). It is therefore possible that mating with a genetically different male at Bimini will mean mating with a male from a different population that carries genes best suited for a different environment, which should then reduce offspring survival.

Mate choice, sexual conflict and convenience polyandry

Nothing in our data suggests genetic benefits of polyandry in female lemon sharks. As noted above, we may simply have missed benefits evident at other life-history stages or at other genes. If not, however, we are left with the question of why female sharks mate multiply. So little is known about the mating behaviour of lemon sharks, or related species, that we can only speculate as to how much control females have over mating. Some choice may be possible given that female sharks in other species can actively avoid copulation with some males and permit it with others (Pratt Jr & Carrier 2001). This ability may be limited, however, because multiple males often breed simultaneously with a single female, or forcefully exclude her from refugia (Pratt Jr & Carrier 2001). If female sharks are unable to easily control how often and with whom they mate, sexual conflict and convenience polyandry may provide a plausible explanation for polyandry. That is, male sharks may try to maximize their fitness through coercion, with females accepting superfluous matings to avoid the costs associated with resistance to aggressive males (Arnqvist & Nilsson 2000; Brown *et al*. 2004; Lee & Hays 2004; Fitze *et al*. 2005; Portnoy *et al*. 2007).

Convenience polyandry has been demonstrated for some insects (Rowe 1992; Weigensberg & Fairbairn 1994), has been inferred in a reptile (Lee & Hays 2004), and is a reasonable explanation when females mate multiply with no obvious benefits and high costs. Thus, female mating rates may reflect the 'best-of-a-bad-job' (see Lee & Hays 2004), with male antagonistic traits or behaviour driving female mating rates away from those that would be optimum in the absence of harassment. We feel that a key question in resolving this uncertainty is when the greatest costs are incurred. If the costs of avoiding harassment are greater than those during actual mating, then convenience polyandry would be favoured. If, instead, the costs of avoiding harassment are less than those during mating, then convenience polyandry would be disfavoured. Direct observations of lemon shark mating are needed to infer potential costs incurred by females.

Conclusion

We found no evidence that polyandry in lemon sharks provides indirect genetic benefits in the form of increased juvenile survival. Although we cannot conclusively rule out the possibility that we missed the most important period, traits, or genetic markers (see above), it is reassuring that our results are clearly weaker than those observed in at least some other studies (Coltman *et al*. 1998; Coulson *et al*. 1999; Foerster *et al*. 2003; Garant *et al*. 2005). These findings leave us with the impression that polyandry may not provide any benefits for the offspring of female lemon sharks, suggesting that alternative hypotheses for the evolution of polyandry warrant further investigation. Increased monitoring of mating behaviour in our study population would help in this regard. Thus, our findings are informative in the context of the mating system of this and other closely related coastal shark species, but should be applied with caution to studies examining indirect genetic benefits in other taxa. Indeed, future work examining the occurrence of convenience polyandry in nature and its relationship to mating systems in general, are needed.

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References

Acevedo-Whitehouse K, Guilland F, Greig D, Amos W (2003) Disease susceptibility in California sea lions: inbreeding influences the response of these animals to different pathogens in the wild. *Nature*, **422**, 35.

Acevedo-Whitehouse K, Spraker TR, Lyons E *et al*. (2006) Contrasting effects of heterozygosity on survival and hookworm resistance in California sea lion pups. *Molecular Ecology*, **15**, 1973–1982.

Akçay E, Roughgarden J (2007) Extra-pair paternity in birds: review of the genetic benefits. *Evolutionary Ecology Research*, **9**, 855–868.

Amos W, Worthington Wilmer JM, Fullard K *et al*. (2001) The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 2021–2027.

Aparicio JM, Ortego J, Cordero PJ (2006) What should we weigh to estimate heterozygosity alleles or loci? *Molecular Ecology*, **15**, 4659–4665.

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, **60**, 145–164.

Barker MJ, Gruber SH, Newman SP, Schluessel V (2005) Spatial and ontogenetic variation in growth of nursery-bound juvenile lemon sharks, *Negaprion brevirostris*: a comparison of two age-assigning techniques. *Environmental Biology of Fishes*, **72**, 343–355.

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349–368.

Birkhead TR (2000) Defining and demonstrating postcopulatory female choice. *Evolution*, **54**, 1057–1060.

Birkhead TR, Moller AP (1992) *Sperm Competition in Birds*. Academic Press, London, UK.

Blanckenhorn WU, Hosken DJ, Martin OY *et al*. (2002) The costs of copulating in the dung fly *Sepsis cynipsea*. *Behavioral Ecology*, **13**, 353–358.

Blouin-Demers G, Gibbs HL, Weatherhead PJ (2005) Genetic evidence for sexual selection in black ratsnakes, *Elaphe obsoleta*. *Animal Behaviour*, **69**, 225–234.

Brown J, Brown E (1998) Are inbred offspring less fit? Survival in a natural population of Mexican jays. *Behavioral Ecology*, **9**, 60–63.

Brown C, Gruber SH (1988) Age assessment of the lemon shark, *Negaprion brevirostris* using tetracycline validated vertebral centra. *Copeia*, **3**, 747–753.

Brown WD, Björk A, Schneider K, Pitnick S (2004) No evidence that polyandry benefits females in *Drosophila melanogaster*. *Evolution*, **58**, 1242–1250.

Chapman DD, Prodhöl PA, Gelsleichter J, Manire C, Shivji MS (2004) Predominance of genetic monogamy by females in a hammerhead shark, *Sphyrna tiburo*: implications for shark conservation. *Molecular Ecology*, **13**, 1965–1974.

Charpentier M, Setchell JM, Prugnolle F *et al*. (2005) Genetic diversity and reproductive success in mandrills (*Mandrillus sphinx*). *Proceedings of the National Academy of Sciences, USA*, **102**, 16723–16728.

Coltman DW, Slate J (2003) Microsatellite measures of inbreeding: a meta-analysis. *Evolution*, **57**, 971–983.

Coltman DW, Bowen WD, Wright JM (1998) Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proceedings of the Royal B: Biological Sciences*, **265**, 803–809.

Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, **53**, 1259–1267.

Compagno LJV (1984) *FAO Species Catalogue: Sharks of the World*. Food and Agriculture Organization of the United Nations, Rome.

Coulson T, Pemberton JM, Albon SD *et al*. (1998) Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 189–195.

Coulson T, Albon SD, Slate J, Pemberton JM (1999) Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution*, **53**, 1951–1960.

Da Silva A, Luikart G, Yoccoz NG, Cohas A, Allainé D (2006) Genetic diversity-fitness correlation revealed by microsatellite analyses in European alpine marmots (*Marmota marmota*). *Conservation Genetics*, **7**, 371–382.

Daly M (1978) The cost of mating. *American Naturalist*, **112**, 771–774.

Daly-Engel TS, Grubbs RD, Holland KN, Toonen RJ, Bowen BW (2006) Assessment of multiple paternity in single litters from three species of carcharhinid sharks in Hawaii. *Environmental Biology of Fishes*, **76**, 419–424.

DiBattista JD, Feldheim KA, Gruber SH, Hendry AP (2007) When bigger is not better: selection against large size, high condition, and fast growth in juvenile lemon sharks. *Journal of Evolutionary Biology*, **20**, 201–212.

Duarte LC, Bouteiller C, Fontanillas P, Petit E, Perrin N (2003) Inbreeding in the greater white-toothed shrew, *Crocidura russula*. *Evolution*, **57**, 638–645.

Dunn DW, Sumner JP, Goulson D (2005) The benefits of multiple mating to female seaweed flies, *Coelopa frigida* (Diptera: Coelpidae). *Behavioral Ecology and Sociobiology*, **58**, 128–135.

Eberhard WG (1996) *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, New Jersey.

Edrén SMC, Gruber SH (2005) Homing ability of young lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes*, **72**, 267–281.

Fedorka KM, Mousseau TA (2002) Material and genetic benefits of female multiple mating and polyandry. *Animal Behaviour*, **64**, 361–367.

Feldheim KA, Gruber SH, Ashley MV (2001a) Multiple paternity of a lemon shark litter (Chondrichthyes: Carcharhinidae). *Copeia*, **3**, 781–786.

Feldheim KA, Gruber SH, Ashley MV (2001b) Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Molecular Ecology*, **10**, 295–303.

Feldheim KA, Gruber SH, Ashley MV (2002) The breeding biology of lemon sharks at a tropical nursery lagoon. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 1655–1661.

Feldheim KA, Gruber SH, Ashley MV (2004) Reconstruction of parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark, *Negaprion brevirostris*. *Evolution*, **10**, 2332–2342.

Fisher DO, Double MC, Blomberg SP, Jennions MD, Cockburn A (2006) Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature*, **444**, 89–92.

Fitze PS, Le Galliard JF, Federici P, Richard M, Clobert J (2005) Conflict over multiple-partner mating between males and females of the polygynandrous common lizards. *Evolution*, **59**, 2451–2459.

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, **425**, 714–717.

Freitas RHA, Rosa RS, Gruber SH, Wetherbee BM (2006) Early growth and juvenile population structure of lemon sharks *Negaprion brevirostris* in the Atol das Rocas biological reserve, off north-east Brazil. *Journal of Fish Biology*, **68**, 1319–1332.

Garant D, Dodson JJ, Bernatchez L (2005) Offspring genetic diversity increases fitness of female Atlantic salmon (*Salmo salar*). *Behavioral Ecology and Sociobiology*, **57**, 240–244.

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, **8**, 1231–1234.

Gruber SH, De Marignac JRC, Hoenig JM (2001) Survival of juvenile lemon sharks at Bimini, Bahamas, estimated by mark-depletion experiments. *Transactions of the American Fisheries Society*, **130**, 376–384.

Hovell DC (2002) *RESAMPLING v 1.3*: Available from URL: http://www.uvm.edu/~dhowell/StatPages/Resampling.

Hueter R, Heupel MR, Heist EJ, Keeney DB (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science*, **35**, 239–247.

Ivy TM, Sakaluk SK (2005) Polyandry promotes enhanced offspring survival in decorated crickets. *Evolution*, **59**, 152–159.

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 21–64.

Kleven O, Lifjeld JT (2005) No evidence for increased offspring heterozygosity from extrapair mating in the reed bunting (*Emberiza schoeniclus*). *Behavioral Ecology*, **16**, 561–565.

Kruuk LEB, Sheldon BC, Merilä J (2002) Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society B: Biological Sciences*, **269**, 1581–1589.

Lee PLM, Hays GC (2004) Polyandry in a marine turtle: females make the best of a bad job. *Proceedings of the National Academy of Sciences, USA*, **101**, 6530–6535.

Lieutenant-Gosselin M, Bernatchez L (2006) Local heterozygosity-fitness correlations with global positive effects on fitness in threespine stickleback. *Evolution*, **60**, 1658–1668.

Mangel M, Stamps J (2001) Trade-offs between growth and mortality and the maintenance of individual variation in growth. *Evolutionary Ecology Research*, **3**, 583–593.

Manire CH, Gruber SH (1991) Effect of M-type dart tags on field growth of juvenile lemon sharks. *Transactions of the American Fisheries Society*, **120**, 776–780.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Morrissey JF, Gruber SH (1993) Habitat selection by juvenile lemon sharks, *Negaprion brevirostris*. *Environmental Biology of Fishes*, **38**, 311–319.

Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: An integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19–38.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Pearse DE, Avise JC (2001) Turtle mating systems: behavior, sperm storage, and genetic paternity. *Journal of Heredity*, **92**, 206–211.

Portnoy DS, Piercy AN, Musick JA, Burgess GH, Graves JE (2007) Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico. *Molecular Ecology*, **16**, 187–197.

Pratt Jr HL (1993) The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environmental Biology of Fishes*, **38**, 139–149.

Pratt Jr HL, Carrier JC (2001) A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes*, **60**, 157–188.

Pratt Jr HL, Tanaka S (1994) Sperm storage in male elasmobranchs: a description and survey. *Journal of Morphology*, **219**, 297–308.

Pujolar JM, Maes GE, Vancoillie C, Volckaert FAM (2005) Growth rate correlates to individual heterozygosity in the European eel, *Anguilla anguilla* L. *Evolution*, **59**, 189–199.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Raymond M, Rousset F (1995) GENEPOP v. 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **83**, 248–249.

Ridley M (1993) Clutch size and mating frequency in parasitic *Hymenoptera*. *American Naturalist*, **142**, 892–910.

Rowe L (1992) Convenience polyandry in a water strider: foreign conflicts and female control of copulation frequency and guarding duration. *Animal Behaviour*, **44**, 189–202.

Saville KJ, Lindley AM, Maries EG, Carrier JC, Pratt HL Jr (2002) Multiple paternity in the nurse shark, *Ginglymostoma cirratum*. *Environmental Biology of Fishes*, **63**, 347–351.

Schulte-Hostedde AI, Zinner B, Millar JS, Hickling GJ (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology*, **86**, 155–163.

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH (2000) Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1657–1662.

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, **13**, 510–524.

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, **9**, 1013–1027.

Weigensberg I, Fairbairn DJ (1994) Conflict of interest between the sexes: a study of mating interactions in a semiaquatic bug. *Animal Behaviour*, **48**, 893–901.

Wiklund C, Karlsson B, Leimar O (2001) Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness. *Proceedings of the Royal Society B: Biological Sciences*, **268**, 1661–1667.

Zeh JA, Zeh DW (2001) Reproduction mode and the genetic benefits of polyandry. *Animal Behaviour*, **61**, 1051–1063.

This study forms part of Joseph DiBattista's PhD thesis on quantitative genetics and the evolution of mating systems, using a natural population of lemon sharks as a model system. Kevin Feldheim is interested in shark population genetics. He is also involved in a number of other projects employing microsatellite markers to infer relatedness and parentage. Andrew Hendry is currently investigating factors that influence the evolution of biological diversity, including natural selection, gene flow, adaptation, and reproductive isolation. He conducts research in a number of study systems, in such exotic places as the Galapagos islands (Darwin's Finches), Trinidad and Tobago (Guppies), British Columbia (Sticklebacks), and Alaska (Sockeye Salmon). Samuel Gruber has been doing elasmobranch research for the past 40 years, and his current focus is on the ecology and conservation biology of sharks. He founded both the American Elasmobranch Society (AES) and the Bimini Biological Field Station (BBFS), and looks forward to his forthcoming retirement.

Appendix I

Summary characteristics for nine dinucleotide microsatellite loci isolated in the lemon shark (*Negaprion brevirostris*), based on the analysis of 574 unique individuals (age-0 and age-1 juveniles) sampled from Bimini, Bahamas

Locus	N	Size range (bp)	N_A	H_O	H_E
LS11	572	231–341	40	0.651	0.681
LS15	572	154–249	25	0.805	0.814
LS22	574	119–167	18	0.891	0.902
LS30	574	188–232	14	0.679	0.710
LS48	572	176–226	25	0.950	0.942
LS54	571	156–166	5	0.537	0.548
LS75	571	212–222	5	0.679	0.703
LS52	572	160–242	37	0.941	0.947
LS82	569	165–239	21	0.765	0.762

N, number of individuals successfully typed at a particular locus; N_A, number of alleles scored; H_O and H_E, observed and expected heterozygosities, respectively.