

RECONSTRUCTION OF PARENTAL MICROSATELLITE GENOTYPES REVEALS FEMALE POLYANDRY AND PHILOPATRY IN THE LEMON SHARK, *NEGAPRION BREVIROSTRIS*

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Abstract.—Because sharks possess an unusual suite of reproductive characteristics, including internal fertilization, sperm storage, relatively low fecundity, and reproductive modes that range from oviparity to viviparity, they can provide important insight into the evolution of mating systems and sexual selection. Yet, to date, few studies have characterized behavioral and genetic mating systems in natural populations of sharks or other elasmobranchs. In this study, highly polymorphic microsatellite loci were used to examine breeding biology of a large coastal shark, the lemon shark, *Negaprion brevirostris*, at a tropical lagoon nursery. Over six years, 910 lemon sharks were sampled and genotyped. Young were assigned into sibling groups that were then used to reconstruct genotypes of unsampled adults. We assigned 707 of 735 young sharks to one of 45 female genotypes (96.2%), and 485 (66.0%) were assigned to a male genotype. Adult female sharks consistently returned to Bimini on a biennial cycle to give birth. Over 86% of litters had multiple sires. Such high levels of polyandry raise the possibility that polyandry evolved in viviparous sharks to reduce genetic incompatibilities between mother and embryos. We did not find a relationship between relatedness of mates and the number of offspring produced, indicating that inbreeding avoidance was probably not driving pre- or postcopulatory mate choice. Adult male sharks rarely sired more than one litter at Bimini and may mate over a broader geographic area.

Key words.—Genotype reconstruction, lemon shark, microsatellites, parentage assignment, polyandry, relatedness.

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Although not traditionally included in behavioral ecology studies, sharks possess a suite of reproductive characteristics that make them particularly suitable for exploring mating system hypotheses. Mode of fertilization has long been an important component of mating system theory (Williams 1975; Dawkins and Carlisle 1976; Gross and Shine 1981); sharks have specialized copulatory organs and represent the oldest vertebrate group possessing internal fertilization. Compared to teleost fish, sharks have low fecundity and high maternal investment in individual offspring, thus it should be expected that females would exercise some form of pre- or postcopulatory mate choice. Sperm competition may play an important role in shark reproduction because females store sperm in a specialized oviducal gland, and sperm may be viable for more than a year (Castro et al. 1988; Pratt 1993). Among the major vertebrate groups, sharks are uniquely diverse in reproductive mode, spanning oviparity to ovoviviparity to viviparity. Unfortunately, both behavioral and genetic information on mating and reproduction in sharks and other elasmobranchs has been limited. As with any large, mobile marine species, the temporal and spatial scopes of shark reproductive behaviors make direct observations challenging. Many aspects of shark mating systems will have to be inferred using genetic techniques, as has been the case for other marine organisms (Kichler et al. 1999; Worthingham Wilmer et al. 1999). Genetic studies have also been difficult, hampered by the slow development of highly variable mark-

ers for elasmobranchs, such as microsatellites, needed for parentage studies. To date, parentage assessment of litters has been performed in just two species of sharks, nurse sharks, *Ginglymostoma cirratum* (Ohta et al. 2000; Saville et al. 2002) and lemon sharks, *Negaprion brevirostris* (Feldheim et al. 2001a, 2002). Both studies found litters sired by multiple males, but sample sizes were very small.

These preliminary findings raise the possibility that at least some sharks belong to the growing list of organisms that exhibit polyandry, as revealed by the increasing use of genetic methods for determining the parentage of offspring. The pervasiveness of polyandry has shifted the focus of sexual selection studies from male mating strategies to the female perspective. Recently, Zeh and Zeh (2003) went so far as to suggest that sexual selection research is in the throes of a paradigm shift. The traditional Darwinian paradigm of promiscuous males and choosy, effectively monogamous females needs to be modified to accommodate polyandry. Advantages of polyandry in sharks would need to be substantial because breeding entails a high cost to females. Copulating male sharks bite female's fins and trunk, sometimes resulting in large areas of torn or missing flesh from females (Pratt and Carrier 2001).

Several hypotheses have been proposed to explain multiple mating in species such as sharks where females receive only sperm from males (see reviews by Yasui 1998; Jennions and Petrie 2000; Tregenza and Wedell 2000; Zeh and Zeh 2001). One leading theory involves sperm competition, whereby females mate polyandrously to obtain increased fertilization success for their sons (sexy sperm hypothesis; Harvey and May 1989; Keller and Reeve 1995; Simmons 2003). Hy-

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potheses involving genetic benefits include the trading-up hypothesis (Petrie and Kempenaers 1998; Jennions and Petrie 2000) that suggests fertile, mated females obtain additional matings from superior males, based on some criterion to determine male quality (Jennions and Petrie 2000). The good genes hypothesis (Watson 1991; Yasui 1998) predicts that females simply mate with multiple males to help ensure fertilization by the highest quality male available. The genetic diversity hypothesis (Yasui 1998; Landry et al. 2001) suggests multiple mating leads to higher genetic variability among offspring and higher fitness, especially in a fluctuating environment (Yasui 1998). Alternatively, females may benefit from a genetically diverse clutch by reducing competition for limited resources among full-sibs (sib competition avoidance; Ridley 1993; Yasui 1998).

Other recent sexual selection theories have brought attention to the sexual conflict between male and female genomes (Chapman et al. 2003; Zeh and Zeh 2003). The genetic incompatibility hypothesis proposes females mate polyandrously to prevent fertilization by males who are genetically incompatible with the female (Zeh and Zeh 1996, 1997, 2001). This may be especially prevalent in viviparous species in which the developing embryo creates a postfertilization environment where genomic conflicts between the female and her offspring or among multiply sired offspring may occur. A special case of the genetic incompatibility hypothesis is inbreeding avoidance, where postcopulatory mechanisms diminish the cost of inbreeding when females cannot avoid mating with relatives (Zeh and Zeh 2001). If females cannot avoid such matings, then we would expect a negative correlation between level of relatedness of mated individuals and number of offspring produced by these individuals. Physiological interactions and potential genomic conflicts between mother and offspring are extreme in many viviparous sharks because there is direct transfer of nutrients from mother to embryo through a yolk-sac placenta.

Lemon sharks (*N. brevirostris*) range in western Atlantic waters from the northeast coast of the United States south to Brazil. Relict populations also exist off the West African coast and the eastern Pacific from Baja California to Colombia (Bigelow and Schroeder 1948). Lemon sharks are viviparous and rely on nurseries for successful recruitment of young. Juvenile lemon sharks use shallow lagoon nurseries for the first several years of their lives until they venture out to deeper waters as subadults (Morrissey and Gruber 1993; Sundström et al. 2001).

We have been tagging and sampling populations of lemon sharks at western Atlantic nurseries for the past several years. A long-term field and genetic study of a relatively undisturbed population of lemon sharks has been in progress at one of these sites, Bimini, Bahamas, since 1995. Bimini serves as a nursery ground for approximately 300 juvenile lemon sharks (Morrissey and Gruber 1993). The Bimini lagoon also likely serves as a mating ground for these sharks. Although we have never witnessed an actual mating event at Bimini, we have captured females in the lagoon with fresh mating wounds. Previously, we were able to assign a subset of young sharks to five sampled adult females and to show these particular females mated polyandrously and returned to Bimini biennially for parturition (Feldheim et al. 2002).

Because adult sharks (the parental generation) are difficult to sample, we focus here on reconstructing the mating behavior of the adult generation from the genotypes of several hundred offspring sampled over six breeding seasons.

The objective of this study was to characterize the mating system of a population of sharks. Such studies in viviparous sharks are needed, along with comparative studies across diverse species, to begin to assess models of sexual selection, in particular the role that genetic incompatibility may play in the evolution of mating systems in elasmobranchs. The varying potential for maternal/offspring incompatibilities among shark species provides a unique opportunity to test for relationships between viviparity and polyandry. The high level of genetic variability at microsatellite loci allowed us to place these young sharks into sibling groups and thus characterize the genetic mating system of adult sharks at our study site. Genetic characterization of females and their successful mates allowed us to test the inbreeding avoidance hypothesis for polyandrous mating.

MATERIALS AND METHODS

Study Site

Bimini, Bahamas, is a mangrove-fringed island cluster located approximately 85 km east of Miami, Florida. The Bimini islands enclose a shallow lagoon of approximately 21 km² (Morrissey and Gruber 1993). We have sampled lemon sharks at Bimini continually since 1995 in two adjacent areas of the Bimini lagoon, North Sound and Sharkland (Gruber et al. 2001). Annual sampling at these two sites takes place in late spring, just after pupping of the adult females (S. Gruber, pers. obs.). As there are several concurrent projects at Bimini, sharks are sampled from other locales of the north and south islands opportunistically throughout the year.

Historically, the Bimini population has been relatively undisturbed. Recreational fishing of subadults has been the only human disturbance on this population. Recently, however, the southwest corner of the North Sound has been dredged for construction of a new casino. As most of the work for this study was done before dredging, our sampling represents a pristine lemon shark population. This study includes sharks captured between 1995 and 2000. A litter of 18 sharks and their mother (mama) were sampled in 2001. These 19 individuals are included here and represent the only sharks sampled in 2001.

Sampling Techniques

A remove-and-hold technique (Manire and Gruber 1993) employing gill nets (180 m × 150 cm) was used to capture newborn and juvenile sharks. Nets were checked when a splash was heard or every 15 min. All sharks were brought to a central tagging location, where they were weighed, measured, sexed, and implanted with an electronic passive integrated transponder (PIT) tag. Sharks were held in a holding pen and fed fresh or frozen fish every other day until released.

Subadult and adult sharks were captured using long line fishing. These sharks were also PIT tagged, measured, and sexed. A small fin clip was taken from every captured shark for subsequent DNA extraction and genetic analyses.

Age Classification of Sharks

We grouped captured sharks into four age categories: newborns (young of the year), juveniles, subadults, and sexually mature adults (Feldheim et al. 2002). Newborns had an open umbilical scar. Juvenile sharks had a fully closed umbilical scar and did not exceed 70 cm precaudal length (PCL), and subadult sharks had a PCL 70–175 cm (for males) or 70–185 cm (for females). Mature males had a PCL greater than 175 cm, whereas mature females had a PCL greater than 185 cm, following Compagno (1984).

We did not record umbilical data in 1995 and 1996, so we used a cut-off technique to determine their year of birth. This technique compares the PCL of an individual of an unknown year class (1995 and 1996) to the distribution of PCLs of known year classes (1997–2000). PCLs for known young of the year sharks ranged 45–52 cm. As the majority of newborns had PCLs less than 51 cm, we used this value as a maximum PCL to define newborns from 1995 and 1996. Similarly, for sharks that were captured as juveniles in all years, we determined year of birth using an average annual growth range at Bimini of 5.2–7.1 cm (S. Gruber, unpubl. data) and extrapolated back to approximate year of birth. Using this method, we narrowed the year of birth to one of two years for juvenile sharks. We included these juveniles in the within-year sibship analysis for both possible years and then assigned year of birth based on sibship results (see below). For example, if we estimated that shark 76B5F was born in either 1996 or 1997, we included it in both the 1996 and 1997 sibship analyses. If 76B5F belonged to a sibling group of newborns in 1996, then we assigned year of birth as 1996. If 76B5F did not belong with newborn sibling groups from either 1996 or 1997, then we entered year of birth as undetermined.

For sharks that were captured in 1995 as one- and two-year olds and in 1996 as two-year olds, we again used the 5.2–7.1 cm range and extrapolated back to estimate year of birth. However, because we did not sample prior to 1995, we had no newborns with which to group these sharks into sibling groups and subsequently assign a year of birth. For these sharks, we used the fact that most adult females that use Bimini as a nursery ground do so on a two-year cycle (Feldheim et al. 2002, see results). Thus, once we assigned these sharks to a female based on parentage analysis (see below), we assigned year of birth based on the adult female's two-year reproductive cycle. This method allowed us to add a portion of the newborns that were born in 1993 and 1994 to our dataset.

Genetic Techniques

DNA was extracted from fin samples using a salting-out protocol (Sunnucks and Hales 1996). Every individual was scored at nine lemon shark microsatellite loci (details in Feldheim et al. 2001b, 2002). The forward primer from each primer pair was fluorescently labeled with one of three dyes (TET, HEX, or 6-FAM, PE Biosystems, Foster City, CA). Fluorescent polymerase chain reaction (PCR) products were run on polyacrylamide gels in an ABI 373a automated sequencer (Applied Biosystems, Foster City, CA). Accurate and consistent scoring of alleles was accomplished with the aid

of Genescan software (PE Biosystems) and an allelic ladder (Feldheim et al. 2001b).

Sibship Assignment

Relatedness among newborns within each year class was examined using Kinship 1.3 (Goodnight and Queller 1999). Kinship tests hypotheses of relatedness, based on Queller and Goodnight's r (1989), between pairs of individuals in a population. Kinship calculates the ratio of a primary hypothesis (in our case full-sib relatedness between newborns) to the null hypothesis (no relatedness between newborns). Significance of the ratio is then determined by simulation. Three significance levels, $P = 0.05$, $P = 0.01$, and $P = 0.001$, are generated by the simulation for each pair of newborns. We tested sibling relationships between all pairs of newborns within a year class for all years (1995–2000).

To determine if females were philopatric, and if so, their return frequency to Bimini, we tested for relatedness across years as well. For example, if the reproductive cycle of females was biennial, we would expect to find maternally related half-sibs in odd years (1995, 1997, and 1999) and even years (1996, 1998, and 2000). We performed across-year Kinship analyses for every combination of newborns from 1995 to 2000, for a total of 15 separate analyses (95–96, 95–97, 95–98, 95–99, 95–00, 96–97, 96–98, 96–99, 96–00, 97–98, 97–99, 97–00, 98–99, 98–00, and 99–00). We could not run the entire dataset together in one large analysis because of limitations of the output matrix size in Microsoft Excel.

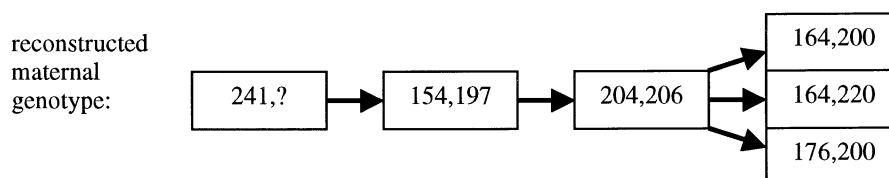
The range of relatedness values calculated by Kinship is such that there is overlap for different levels of relatedness (e.g., full-sibs, half-sibs, and unrelated individuals; Blouin et al. 1996), so results must be interpreted carefully. We considered a group of young to be full-sibs when each of the pairwise comparisons for all members of the putative sibling group had a $\geq 99\%$ chance of being full-sibs. Often, half-sib groups fell out side by side in the Kinship matrix output, making the interpretation of half-sibship between full-sib groups relatively straightforward. Alternatively, some members of half-sib groups appeared to be full-sibs based on the $\geq 99\%$ confidence level. All putative sibling groups were further explored by manually examining the genotypes of all members of a putative sibling group. When a maximum of four alleles were found across all loci, full-sibship was assigned to the group. When five or more alleles were seen at a minimum of two loci, then two or more groups of half-sibs were present.

Parentage Assignment

We used the likelihood approach implemented in Cervus 2.0 (Marshall et al. 1998) to assign parentage of sampled and reconstructed adults (see below) to all subadult, juvenile, and newborn sharks. Cervus calculates likelihood ratios for each candidate parent-newborn pair over all loci given the genotypic data of the population and calculates δ , the difference in LOD scores between the most likely parent (i.e., the candidate with the highest LOD score) and the second most likely parent. Confidence levels of δ are estimated through simulations; we used the default levels of 80% and 95% confidence. Our initial input parameters for Cervus included a 1%

A.

1998 litter	microsatellite locus			
	LS11	LS15	LS48	LS52
7703A	241,315	154,219	206,218	164,200
F5878	241,249	197,199	204,206	200,220
67205	241,315	154,219	204,218	200,220
75747	241,309	154,217	186,204	164,176
65C57	241,241	189,197	206,208	164,176



B.

1996 litter	microsatellite locus			
	LS11	LS15	LS48	LS52
C2267	241,241	154,207	206,208	200,220
46136	241,259	154,207	204,206	164,204
D3D2A	241,259	154,197	204,208	200,204

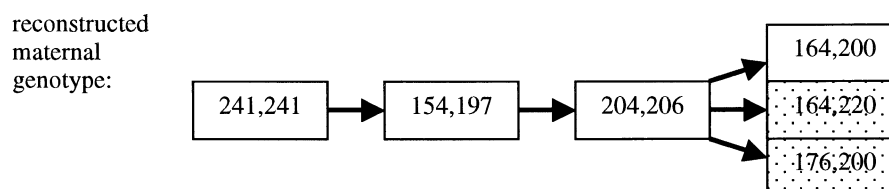


FIG. 1. Example of a reconstruction of an unsampled female genotype using genotypes of half-sibs. In (A), there are three possible genotypes for the female. By adding a litter from 1996 in (B), we confirm loci LS11, LS15, and LS48 and determine the genotype at LS52. Only four of the nine loci and partial sibling groups are presented for simplicity. Homozygosity was accepted at LS11 once eight young exhibited the maternal allele (see text).

error rate, 10,000 cycles, and 33% of candidate mothers sampled (5% for males). We increased percent of adults sampled as parental genotypes were reconstructed and added to the dataset (see below).

Reconstructing Unsampled Parental Genotypes

To reconstruct parental genotypes, we used within-year and across-year results from Kinship. Maternal genotypes were reconstructed first, based on two or more groups of half-sibs. This approach is based on the assumption that we were much more likely to sample littermates than to sample offspring from different females that happened to mate with the same male. Half-sibs within years are likely to be related through polyandrous females and half-sibs found across years are likely related through philopatric females. To reconstruct maternal genotypes, all members of a sibling group from one year (from the within-year Kinship results) were used to reconstruct the mother's genotype as completely as possible (Fig. 1). If the reconstructed genotype was incomplete after

examining a within-year sibling group, we found a sibling group from other years (using the across-year Kinship results). Maternal genotypes were reconstructed from the two alleles (or one in the case of homozygosity) at each locus found in all members of the sibling group. Homozygosity at a locus was accepted when at least eight putative offspring exhibited one possible maternal allele (see Fig. 1 for an example). There is only a one in 128 chance ($2^8 \times 2$) of the female transmitting only one allele if she is truly heterozygous.

Reconstruction was performed using the most parsimonious explanation of allelic inheritance. An example is shown in Figure 1. Often, there was more than one possible genotype for a reconstructed female at a locus. For example, at LS52 in Figure 1, there are three possible genotypes for the reconstructed female based on the 1998 litter: 164/200, 164/220, or 176/200. In these cases, we searched for related newborns in our across-year Kinship results. Across-year sibling groups were used to confirm fully reconstructed female ge-

notypes and to complete partially reconstructed ones. In our example, three newborns from 1996 were used to complete the genotype of the reconstructed female at LS52 (Fig. 1). These three individuals support a 164/200 genotype for the reconstructed mother at LS52. For loci with common alleles and relatively low heterozygosity (LS54 and LS75), often only one maternal allele per locus could be inferred. As a ground-truthing exercise, we employed our method to reconstruct a previously sampled female (E172A, see Feldheim et al. 2001a, 2002) using only the genotypes of her offspring (Fig. 1). E172A's reconstructed genotype (partially shown in Fig. 1) matched her true composite genotype (241/241, 154/197, 147/147, 206/206, 204/206, 164/200, 164/166, 212/218, 169/169 for LS11, LS15, LS22, LS30, LS48, LS52, LS54, LS75, LS82, respectively).

In some cases, we found full-sib groups within years (i.e., a maximum of four alleles across all loci), but we did not find a related half-sib group in any other year. In these cases, parental genotypes are impossible to reconstruct due to Mendelian assortment of independent loci (see fig. 1 in DeWoody et al. 2000), but we assigned a number for the unreconstructed maternal genotype of that group. This helped us track the number of females using Bimini as a nursery.

Male genotypes were reconstructed by splitting maternally related half-sib groups into full-sib groups based on shared alleles (see DeWoody et al. 2000; Feldheim et al. 2001a). This was done easily by eye as, barring mutation, full-sib groups will have no more than four alleles per locus. Male genotypes were then fully or partially reconstructed based on shared nonmaternal alleles. Because some of these genotypes were often reconstructed from relatively few newborns, many male genotypes were only partially reconstructed.

Once we had reconstructed male and female genotypes, we determined if reconstructed adults parented other newborns in our sample. For newborns within a full-sib group, Cervus did not always assign the same level of confidence to all candidate parent-newborn pairs of the group. This is to be expected due to varying genotypes of the offspring and differences in allele frequencies. For example, when a newborn and mother share rare alleles, confidence in parentage is higher than when mother and newborn share common alleles. Thus, in cases where Cervus identified the parent of a newborn at the >95% or >80% level, Kinship was then used to examine the sibling group to which the newborn belonged. All newborns from that sibling group and the reconstructed female in question were examined by eye. If all newborns in the sibling group contained one of the reconstructed female's alleles at eight of nine loci, then parentage for that sibling group was accepted for that female. Accepting parentage for eight loci enabled us to allow for occasional mutations occurring between parent and offspring. Most offspring matched putative parents at all nine loci. We repeated the entire process for all years until nearly all of the newborns and juveniles were assigned to either a reconstructed adult female or a sampled one.

Mating Behavior of Adult Females

For litters of three or more young, we determined the number of males that sired the brood. For many of the litters, we

were able to assign a reconstructed male genotype to most of the siblings in a group based on Cervus analyses. Often, paternity was skewed with one or two males siring the majority of pups in a litter (Feldheim et al. 2002, see results). In these cases, not every sibling in the group was assigned to a reconstructed male genotype, and we estimated the minimum number of males needed to explain the remaining siblings' genotypes based on number of nonmaternal alleles. In addition to litters of three or more, we were able to determine polyandry in two litters in 1994 that were comprised of two young. For these litters, one of the adult male genotypes was previously reconstructed (RECMale8 was reconstructed from litter 49 and RECMale39 was reconstructed from litter 4; see Results) and assigned to one of the 1994 offspring from a Cervus analysis. Because these reconstructed males did not match the other offspring in the litter genetically, an additional unreconstructed male was needed to account for paternity of the other sibling in the group.

Relatedness of Mated Individuals

To test for genetic incompatibility avoidance, specifically inbreeding avoidance, we examined the relatedness of mated individuals using their microsatellite genotypes. If inbreeding avoidance is occurring, mating pairs should be genetically less similar than the average for the population, and genetically similar mates should have fewer offspring than more distantly related mates. Using Kinship, a matrix of relatedness values was generated between all reconstructed and sampled adults. A regression analysis was performed between number of offspring (dependent variable) and relatedness of mated individuals (independent variable) using Microsoft Excel. Because it is likely that they were not sampled completely, litters from 1993 and 1994 and those sampled from South Bimini were excluded from this analysis.

RESULTS

Age Distribution of Sampled Sharks at Bimini

Of the 910 sharks we sampled at Bimini, 13 were adults, 130 were subadults, 222 were juveniles, 532 were newborns, and 13 were of unknown age (i.e., length data were not recorded). Annual sampling of newborn and juvenile lemon sharks over the course of the study represented the vast majority of lemon sharks born annually at Bimini (Gruber et al. 2001). By placing juveniles into sibling groups with newborns of known year of birth (see Materials and Methods), we were able to determine year of birth for 137 of the 222 juveniles. By assuming a two-year reproductive cycle of females and an annual growth range of 5.2–7.1 cm, we estimated year of birth for 66 additional juveniles born prior to 1995. This left 19 juveniles for which we were not able to infer year of birth. Only newborns and juveniles, for which we determined (or inferred, in the case of pre-1995 newborns) year of birth ($N = 735$) are discussed in subsequent sections and hereafter referred to as "young."

Reconstructed Adult Genotypes

We inferred 40 different adult female genotypes, of which 34 were fully reconstructed. The six remaining females

(RECFemale29, RECFemale30, RECFemale33, RECFemale35, RECFemale38, and RECFemale51) gave birth at Bimini only once and to relatively small litters (Table 1; Appendix available online at <http://dx.doi.org/10.1554/04-023.1.s1>). Thus, their genotypes could not be fully reconstructed. These six females are included with the 34 fully reconstructed females in discussions below. Eighty-one distinct adult male genotypes were inferred and partially or fully reconstructed. Of the eight adult females and five adult males that we caught, sampled, and genotyped, five females and three males parented young found in our sample (Feldheim et al. 2002), and we include those adults below for completion. Thus, we have full or partial genotypes for 45 adult females and 84 adult males that parented young from 1993 to 2001. Of the 735 young, we assigned 707 to a reconstructed or sampled adult female (96.2%) and 485 (66.0%) to a reconstructed or sampled adult male (see online Appendix).

Female Reproduction at Bimini

Of the 45 genetically identified mothers, 32 returned to give birth while 13 females only gave birth at Bimini once (online Appendix; Table 1). Of the 32 females that returned, almost all returned on a two-year cycle. The exceptions were RECFemale17, RECFemale27, RECFemale5, RECFemale31, and RECFemale14 (online Appendix, Table 1). RECFemale17 used Bimini in 1995 and then again in 1999. RECFemale27 and RECFemale5 had litters in 1995, 1998, and 2000. RECFemale31 gave birth in 1996 and had another litter in 1999. RECFemale14 had litters in 1994, 1996, and 2000.

More females used Bimini for parturition in odd years compared to even years, resulting in more newborns in odd years (Table 2). In addition to the 45 female genotypes, a minimum of 13 female genotypes was needed to account for 27 young that were not assigned to a female (Table 2). Thus, over the course of the study, at least 58 females used the Bimini lagoon for parturition.

Average annual sampled litter size for the 45 adult females was 6.7 (range = 1–18, 1995: 6.7; 1996: 6.2; 1997: 6.55; 1998: 9.1; 1999: 5.9, 2000: 5.6). Average number of young per female over the course of the study was 16.1 (range = 4–58, Table 1).

We were able to determine mating behavior of females for 97 litters (Fig. 2). Litters comprised of two young were excluded as it is impossible to infer multiple paternity from two individuals (see Materials and Methods for exceptions). Eighty-four (86.6%) of the litters were the result of polyandrous females. Polyandrous females mated with at least two to four males to produce their litter (see online Appendix). Eleven females produced the 13 singly sired litters. RECFemale8 and RECFemale14 both had two singly sired litters each.

Male Reproduction at Bimini

We assigned 485 of the 735 young (66%) to one of 84 male genotypes. The remaining 250 young required a minimum of 120 different unreconstructed male genotypes to account for paternity (see the summation of unreconstructed fathers for litters from 1993–2001 in online Appendix). Thus, although the average number of offspring sired for the 84

reconstructed males was 5.8, when we include the 120 non-reconstructed males, the average number of young sired is 3.6 per male.

Of the 84 reconstructed adult males, 57 (68%) males mated with only one of the reconstructed females (see online Appendix). Twenty-seven males sired more than one litter. Five of the 27 (RECMale29, RECMale84, RECMale67, RECMale40, and RECMale41) either mated with the same female twice (see online Appendix) or females used stored sperm from these males to fertilize multiple litters in subsequent breeding seasons. Twenty-four of the 27 polygynous males mated in multiple years, and some of these males sired young at Bimini at fairly long intervals. RECMale34 first sired young in 1993 with RECFemale19 and then again in 1998 with RECFemale1 (see online Appendix). Similarly, RECMale4 sired young in 1993, 1995, and 2001 (see online Appendix). Three males (RECMale39, RECMale57, and RECMale67) sired young in four separate years (online Appendix). Only six males had litters with two females in the same year (online Appendix): 07E12 (1994), RECMale37 (1995), RECMale60 (1995), RECMale57 (1997), RECMale70 (1997), and RECMale27 (1999 and 2000).

Inbreeding Avoidance

The average relatedness value between mated individuals was 0.0109 (range = -0.369 to 0.3707 , Fig. 3). While this represents a wide range of relatedness values, no evidence of inbreeding avoidance was found. In fact, males that successfully sired offspring with a female were slightly more related, on average, to their mates than a randomly selected individual from the parental pool (average relatedness = -0.0192). There was also no relationship between relatedness of mated individuals and number of offspring produced by mated individuals (Fig. 3, $r^2 = 0.001$, $P = 0.73$).

DISCUSSION

The use of highly variable microsatellite loci has enabled us to reconstruct parental genotypes based almost exclusively on genotyping offspring. Based on these reconstructed genotypes, we were able to elucidate mating system, recruitment patterns, and habitat use of lemon sharks at Bimini. This study is one of the first to reconstruct parental genotypes with very limited sampling of the parental generation (Benzten et al. 2001; Strausberger and Ashley 2003). Reconstruction was based on the assumption that half-sibship through females is more likely than relatedness through fathers, and thus was the starting point for genotype reconstruction. This assumption may bias our results to some extent and possibly overestimate the level of female polyandry at Bimini and underestimate male fertility. However, there are several considerations that help validate this assumption and suggest that the bias introduced by this approach will be small. We felt that in our sampling of newborn cohorts, it was much more likely that littermates were sampled, rather than half-sibs from different females who happened to mate with the same male and use the Bimini North Sound for parturition. If, for example, four newborn half-sibs belonged to an individual male, rather than a female, this would require that a male successfully mated with four different females, and those four

TABLE 1. Female reproduction at Bimini lagoon, including years of parturition at Bimini, return cycle, and total number of young assigned to each female. Mothers inferred through genotype reconstruction (see text) are identified by REC, while those that were caught and genotyped are identified by the last five characters of a PIT tag or by name (in the case of "mama"). Years of parturition were either determined by reconstructing females' genotypes from young of the year half-sib groups (for females 1REC–52REC) or by assigning parentage to young of the year groups (for females 34556, 57E19, A4D11, E172A, and mama). Years of parturition for litters in 1993 and 1994 were assigned assuming a two-year reproductive cycle of adult females (see text). Total number of offspring include subadult sharks (pre-1993 under years of parturition) captured throughout the study and are not included in discussions of "young" in the text.

Mother	Years of parturition at Bimini	Return cycle	Total no. offspring
1REC	pre-1993, 1994, 1996, 1998, 2000	2	48
3REC	1998, 2000	2	25
4REC	1996, 1998, 2000	2	21
5REC	pre-1993, 1993, 1995, 1998, 2000	2, 3	25
6REC	1997, 1999	2	10
8REC	pre-1993, 1994, 1996, 1998, 2000	2	23
9REC	1994, 1996, 1998, 2000	2	25
10REC	1997, 1999	2	16
11REC	pre-1993, 1993, 1995, 1997, 1999	2	34
12REC	1991, 1993, 1995, 1997, 1999	2	38
13REC	2000	—	4
14REC	1994, 1996, 2000	2, 4	8
15REC	pre-1993, 1994, 1996, 1998, 2000	2	32
16REC	1993, 1995, 1997, 1999	2	21
17REC	1995, 1999	4	7
18REC	1999	—	6
19REC	1993, 1995, 1997, 1999	2	28
20REC	1995, 1997, 1999	2	8
21REC	1995, 1997, 1999	2	10
22REC	1995, 1997	2	15
24REC	1993, 1995, 1997	2	23
25REC	1997	—	9
26REC	1996	—	8
27REC	1995, 1998, 2000	2, 3	26
28REC	1995, 1997, 1999	2	11
29REC	1999	—	7
30REC	1999	—	5
31REC	1996, 1999	3	13
32REC	1999	—	8
33REC	1999	—	4
35REC	1998	—	5
36REC	1995, 1997, 1999	2	18
38REC	1996	—	7
39REC	2000	—	4
42REC	1995, 1997	2	10
48REC	pre-1993, 1994, 1996, 1998, 2000	2	15
49REC	1995, 1997, 1999	2	12
50REC	1995, 1997, 1999	2	4
51REC	1997	—	4
52REC	1993, 1995	2	7
34556	1995, 1997, 1999	2	22
57E19	2000	—	4
A4D11	1997, 1999	2	12
E172A	pre-1993, 1994, 1996, 1998	2	29
Mama	pre-1993, 1993, 1995, 1997, 1999, 2001 ¹	2	58

¹ The young from this litter were the only 2001 sharks that we included in our analyses.

females all gave birth at Bimini to a *single* sampled offspring. What became of the rest of the pups from those females' litters? Our collecting occurs just after parturition of females in the spring, and we collect the vast majority of each newborn cohort (Gruber et al. 2001). Lemon shark females have litters of four to 18 pups (Compagno 1984; Feldheim et al. 2002), and previous work at Bimini suggested an average of 10 per litter (Feldheim et al. 2002). Extensive half-sib paternity within years would require unrealistically small litter sizes of female lemon sharks accompanied by an unrealistically

high mortality for newborns. In addition, the pattern of finding cohorts of half-sibs in odd and even years further suggests maternal relatedness because a biennial reproductive pattern is expected for female but not male sharks (Castro 1996). If we had frequently reconstructed male parental genotypes and mistakenly designated them as females, the pattern of biennial reproduction would have been much weaker. Furthermore, our approach does not preclude the identification of paternal half-sibs. There were indeed some males that mated over several years and were identified by paternally

TABLE 2. Maternity assignment success, number of young per year, and number of females giving birth at Bimini annually. The number of offspring in this table does not match the total number of offspring in Table 1 and the online Appendix, as some subadults were included in Table 1 and the online Appendix. "Assigned" are those young for which we either caught the mother or reconstructed the mother's genotype. "Unassigned" are those young that we were not able to assign to a sampled female or a reconstructed female. "Non-REC females" represents the minimum number of females needed to account for unassigned offspring each year. Number of adult females represents all females (sampled, reconstructed, and Non-REC) using Bimini for parturition in a particular year.

Year of birth	Assigned	Unassigned	Non-REC females	No. of adult females
pre-1995 ¹	62	6	3	n/a
1995	125	3	2	21
1996	68	1	1	12
1997	131	6	2	22
1998	99	2	1	12
1999	130	8	3	25
2000	74	2	1	14
2001	18 ²	n/a	n/a	n/a
Total	707	28	13	58 ³

¹ Year of birth for young prior to 1995 was determined assuming a two-year reproductive cycle of females (see text). The six pre-1995 unassigned young were likely born in 1994, based on size at time of capture.

² The 18 young from 2001 represent a litter we sampled from mama.

³ Total number of adult females represents all females (sampled, reconstructed, and Non-REC) that used Bimini for parturition over the course of the study.

related half-sib groups. Finally, the pattern revealed by our approach is consistent with a more limited study using parentage assignment to sampled adults, rather than genotype reconstruction (Feldheim et al. 2002). In that study five adult females reproduced at Bimini. All mated with multiple males and four returned to Bimini on a biennial cycle. In summary, we feel our approach may result in some incorrect parental

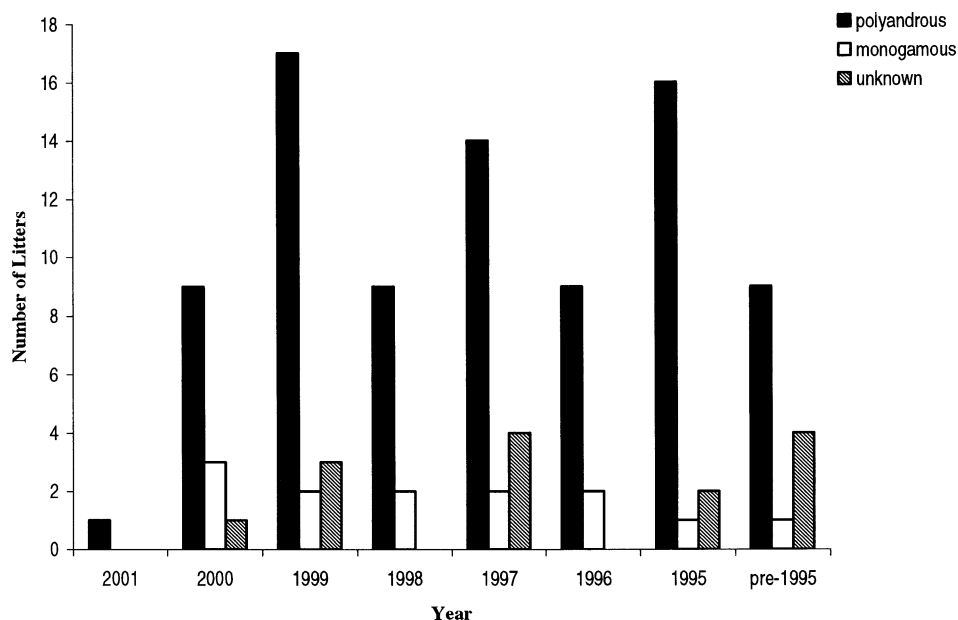


FIG. 2. Litters from polyandrous or monogamous mating by females by year. Unknown behavior was assigned when only one or two offspring were assigned to a female.

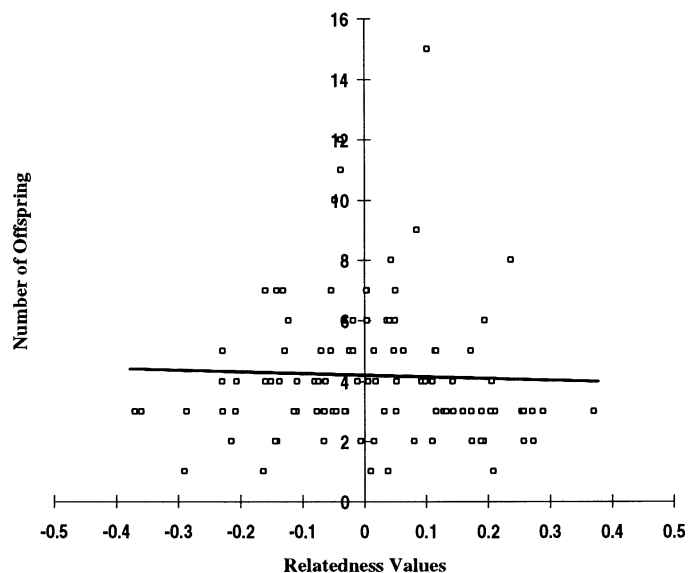


FIG. 3. Regression of number of offspring of mated individuals (y-axis) against relatedness of mated individuals (x-axis). No significant relationship was observed ($r^2 = 0.001$, $P = 0.73$).

assignments, but these would be relatively few and would not obscure the general patterns we aimed to reveal.

We were able to address many aspects of the breeding biology of a large, free-swimming shark. A two-year cycle has been proposed for several other species of sharks, including silky sharks, *Carcharhinus falciformis* (Branstetter 1987); sandbar sharks, *C. plumbeus* (Joung and Chen 1995); blacktip sharks, *C. limbatus* (Castro 1996); and nurse sharks, *Ginglymostoma cirratum* (Castro 2000). These studies, however, relied on necropsies of many adult females at different stages of the reproductive cycle to infer a reproductive time

line for each species. Here, we used reconstructed parental genotypes to determine breeding cycle of individual animals. Overall, we found the biennial reproductive pattern to be pervasive at Bimini, with many gravid females returning to Bimini every other year to give birth to their litters. Of the philopatric females, only five did not exhibit a two-year cycle (online Appendix; Table 1). Three of these females (RECfemale5, RECfemale27, and RECfemale31) had a three-year gap between litters, and two (RECfemale17 and RECfemale14) had a four-year gap. Perhaps these females did not acquire enough resources for vitellogenesis and skipped reproduction. Alternatively, they may have mated with infertile or genetically incompatible males, not mated at all, or we failed to collect their offspring in a particular year. Another explanation, particularly for the females with a four-year return cycle, is that these females used a different nursery for parturition (see below).

Thirteen females used Bimini as a nursery only once. For the eight females that were first seen in either 1999 or 2000 (online Appendix; Table 1), it is possible that these females recently matured and gave birth for the first time. The other females are either on a longer reproductive cycle, died before coming back to Bimini to give birth, or gave birth at a different nursery. Once lemon sharks reach maturity, they suffer very low mortality rates and live at least 20 years (Brown and Gruber 1988) but likely live much longer, so the death of all 13 females seems unlikely. There are several mangrove-fringed islands in close proximity to Bimini lagoon that may have been used by these females. North Bimini is currently being dredged for a new casino (Gruber and Parks 2002). It will be important to examine what effects this extreme habitat degradation has on female philopatry.

Based on the number of young born annually (Table 2), we initially hypothesized that 15–20 females use Bimini for parturition. Instead, we found that 58 females used Bimini for parturition over the course of the study, and there was substantial variation in reproductive success among females. Lemon sharks were previously reported to have litters ranging from four to 18 pups (Compagno 1984; Feldheim et al. 2002); a range of one to 18 was observed here. The low end of our range probably does not reflect natural variation in litter size. With the exception of two litters that were delivered (E172A in 1998 and mama in 2001), young sharks were sampled up to several weeks after birth. A portion of each litter may have died before sampling. In addition, some areas of the lagoon, specifically the south island, are not as exhaustively sampled as are other areas of the lagoon. Females who gave birth in these areas (RECfemale48, RECfemale49, RECfemale50) likely did not have all of their young represented in our sample. Indeed, these three mothers had comparatively few offspring annually (online Appendix; Table 1).

Although our method of genotype reconstruction may have slightly underestimated male fertility, male reproductive success at Bimini was clearly much lower than that seen for females. Most males only sired one or two young and did not reproduce regularly at Bimini. However, our study cannot allow us to make any generalizations regarding male fertility, as males may reproduce over broader areas than females. Low differentiation in microsatellite markers throughout much of the western Atlantic (Feldheim et al. 2001b) suggests gene

flow among lemon shark populations, which may be mediated primarily by males if female philopatry is common across the range of this species. Male-mediated gene flow between shark populations has also been suggested for white sharks (*Carcharodon carcharias*, Pardini et al. 2001) and shortfin makos (*Isurus oxyrinchus*, Shrey and Heist 2003).

One of our most striking findings was the prevalence of polyandrous female mating (online Appendix; Fig. 2). Most litters (86.6%) had multiple sires. Many of the seemingly monogamous females had small litter sizes (online Appendix, Fig. 2). Thus, it is possible that potential half-sibs in these litters were not sampled, and 86.6% represents a minimum estimate of polyandry in this population. Because male sharks do not provide resources to females or exhibit parental care, the prevalence of multiple mating together with the high cost of copulation in sharks suggests that females must gain some form of indirect genetic benefit from such behavior. There is growing evidence in other groups of organisms that females incur fitness gains from polyandry. In a review of polyandry in insects, Arnqvist and Nillson (2000) found females increase their lifetime fitness 30–70% by polyandrous behavior. Polyandrous female guppies had larger broods, and their young were better able to avoid predators compared to monogamous females (Evans and Magurran 2000).

In viviparous sharks, there is the potential for female choice to operate at both pre- and postcopulatory stages, as well as for differential investment in embryos following fertilization. The extent to which female sharks exercise precopulatory mate choice is unclear. In the nurse shark, females used depth gradients in the shallow lagoon of the Dry Tortugas to choose males with which they mate (Carrier et al. 1994; Pratt and Carrier 2001). Nurse sharks may choose their mates based on experience or size (Pratt and Carrier 2001), retreating to the shallows when approached by smaller, less experienced males. It is unknown why females come to Bimini or other lagoons to mate, but perhaps they similarly use the depth gradients.

Females may also employ postcopulatory mechanisms to choose sperm to preferentially fertilize her eggs. Carrier et al. (1994) witnessed lost sperm after copulation in nurse sharks. It is possible that this lost sperm is the result of females ejecting the sperm after mating with a male, but could also be the result of sperm overflow due to mating several times in the same day (J. C. Carrier, pers. comm.). The female reproductive tract of sharks may provide a rich arena for sperm competition and help assure fertilization by high-quality or competitively superior sperm. Many female carcharhinid sharks can store viable sperm in the oviducal gland for several months (Pratt 1993). Genotyping sperm from oviducal glands could determine if females store sperm from multiple males and if there is differential migration or localization of spermatozoa from different males in the oviducal gland. Sperm competition in lemon sharks clearly does not efficiently select for sperm from a single, superior donor, given the high levels of multiple paternity observed. Multiple paternity could reflect constraints on the effectiveness of postcopulatory sexual selection, such as mating order effects. Other genetic-benefit hypotheses contend that multiple paternity per se and its resulting genetic diversity provide benefits, either by increasing the geometric mean fitness of fe-

males (e.g., bet-hedging), through environmental uncertainties (e.g., genetic diversity; Yasui 1998), or by reducing competition (sibling competition avoidance; Ridley 1993). Because young sharks remain in their natal lagoon for several years, the possibility for competition among siblings does exist. Thus, the sibling competition avoidance hypothesis may warrant further investigation.

In viviparous species such as sharks, females may preferentially invest resources in embryos sired by certain males. The genetic incompatibility hypothesis (Zeh and Zeh 1996, 2001) predicts females will use postcopulatory mechanisms to avoid having eggs fertilized by genetically incompatible males. Parental genomes may vary in genetic compatibility due to dominance, overdominance, intra- and intergenomic conflict, immune system function, and fetomaternal interactions (Zeh and Zeh 2003 and references therein). Such incompatibilities are especially likely in viviparous species because internal development of the embryo provides a physiological arena for genomic conflicts (Zeh and Zeh 2001). Inbreeding is one potential source of genetic incompatibility. Bimini appears to serve as both a mating and nursery ground (Feldheim et al. 2002). This, combined with female philopatry, raises the possibility that closely related adults may gather at Bimini and a risk of inbreeding may exist. We used relatedness estimates based on microsatellite genotypes to test whether some females employ pre- or postcopulatory mechanisms to avoid mates that are genetically similar. We found that successful males were no less related to females than a randomly selected individual. Interestingly, successful males spanned a wide range of relatedness values (Fig. 3), but there was no relationship among number of offspring and relatedness.

Genetic incompatibility may arise from many factors other than inbreeding, including intragenomic conflicts such as cellular endosymbionts, transposable elements, segregation distorters, and genomic imprinting (Zeh and Zeh 1996, 2001). Little is known about any of these factors in elasmobranchs, but our demonstration of high levels of polyandry in a viviparous shark species is consistent with the predictions of the genetic incompatibility avoidance hypothesis. Other possibilities, including intrinsic benefits to genetically diverse offspring cannot, however, be ruled out. The findings reported here do suggest that sharks may provide an intriguing system for assessing models of sexual selection. In particular, assessment of levels of polyandry in oviparous and ovoviparous sharks should contribute to our understanding of the evolutionary origins of genetic incompatibilities and polyandry in vertebrates. With long-term field studies and the use of highly polymorphic markers such as microsatellites, previously inaccessible species such as sharks can become intriguing subjects for such studies.

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LITERATURE CITED

- Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* 60: 145–164.
- Bentzen, P., J. B. Olsen, J. E. McLean, T. R. Seamons, and T. P. Quinn. 2001. Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. *J. Hered.* 92: 127–136.
- Bigelow, H. B., and W. C. Schroeder. 1948. Fishes of the western North Atlantic. *Mem. Sears Found. Mar. Res.* 1:59–545.
- Blouin, M. S., M. Parsons, V. Lacaille, and S. Lotz. 1996. Use of microsatellite loci to classify individuals by relatedness. *Mol. Ecol.* 5:393–401.
- Branstetter, S. 1987. Age, growth, and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the northwestern Gulf of Mexico. *Environ. Biol. Fish.* 19:161–173.
- Brown, C. A., and S. H. Gruber. 1988. Age assessment of the lemon shark, *Negaprion brevirostris*, using tetracycline validated vertebral centra. *Copeia* 1988:747–753.
- Carrier, J. C., H. L. Pratt, and L. Martin. 1994. Group reproductive behavior in free-living nurse sharks, *Ginglymostoma cirratum*. *Copeia* 1994:646–656.
- Castro, J. I. 1996. Biology of the blacktip shark, *Carcharhinus limbatus*, off the southeastern United States. *Bull. Mar. Sci.* 59: 508–522.
- . 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. *Environ. Biol. Fish.* 58:1–22.
- Castro, J. I., P. M. Bubucis, and N. A. Overstrom. 1988. The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. *Copeia* 1988:740–746.
- Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe. 2003. Sexual conflict. *Trends Ecol. Evol.* 18:41–47.
- Compagno, L. J. V. 1984. *FAO species catalogue: sharks of the world*. Food and Agriculture Organization of the United Nations, Rome.
- Dawkins, R., and T. R. Carlisle. 1976. Parental investment, mate desertion and a fallacy. *Nature* 262:131–133.
- DeWoody, J. A., D. E. Fletcher, S. D. Wilkins, W. S. Nelson, and J. C. Avise. 2000. Genetic monogamy and biparental care in an externally fertilizing fish, the largemouth bass (*Micropterus salmoides*). *Proc. R. Soc. Lond. B* 267:2431–2437.
- Evans, J. P., and A. E. Magurran. 2000. Multiple benefits of multiple mating in guppies. *Proc. Natl. Acad. Sci. USA* 97:10074–10076.
- Feldheim, K. A., S. H. Gruber, and M. V. Ashley. 2001a. Multiple paternity of a lemon shark litter (Chondrichthyes: Carcharhinidae). *Copeia* 2001:781–786.

- . 2001b. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Mol. Ecol.* 10:295–303.
- . 2002. Breeding biology of lemon sharks at a tropical nursery lagoon. *Proc. R. Soc. Lond. B* 269:1655–1662.
- Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationships using genetic markers. *Mol. Ecol.* 8:1231–1234.
- Gross, M. R., and R. Shine. 1981. Parental care and mode of fertilization in ectothermic vertebrates. *Evolution* 35:775–793.
- Gruber, S. H., and W. Parks. 2002. Mega-resort development on Bimini: Sound economics or environmental disaster? *Bahamas J. Sci.* 9:2–18.
- Gruber, S. H., J. R. C. de Marignac, and J. M. Hoenig. 2001. Survival of juvenile lemon sharks at Bimini, Bahamas, estimated by mark-depletion experiments. *Trans. Am. Fish. Soc.* 130:376–384.
- Harvey, P. H., and R. M. May. 1989. Out for the sperm count. *Nature* 337:508–509.
- Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* 75:21–64.
- Joung, S. J., and C. T. Chen. 1995. Reproduction in the sandbar shark, *Carcharhinus plumbeus*, in the waters off Northeastern Taiwan. *Copeia* 1995:659–665.
- Keller, L., and H. K. Reeve. 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. *Adv. Stud. Behav.* 24:291–315.
- Kichler, K., M. T. Holder, S. K. Davis, R. Marquez-M., and D. W. Owens. 1999. Detection of multiple paternity in the Kemp's ridley sea turtle with limited sampling. *Mol. Ecol.* 8:819–830.
- Landry, C., D. Garant, P. Duchesne, and L. Bernatchez. 2001. "Good genes as heterozygosity": the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proc. R. Soc. Lond. B* 268:1279–1285.
- Manire, C. A., and S. H. Gruber. 1993. A preliminary report on the rate of natural mortality of juvenile lemon sharks, *Negaprion brevirostris*. NOAA Tech. Rpt. NMFS 115:65–71.
- Marshall, T. C., J. Slate, J. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7:639–655.
- Morrissey, J. F., and S. H. Gruber. 1993. Home range of juvenile lemon sharks, *Negaprion brevirostris*. *Copeia* 1993:425–434.
- Ohta, Y., K. Okamura, E. C. McKinney, S. Bartl, K. Hashimoto, and M. F. Flajnik. 2000. Primitive synteny of vertebrate major histocompatibility complex class I and class II genes. *Proc. Natl. Acad. Sci. USA* 97:4712–4717.
- Pardini, A. T., C. S. Jones, L. R. Noble, B. Kreiser, H. Malcolm, B. D. Bruce, J. D. Stevens, G. Cliff, M. C. Scholl, M. Francis, C. A. J. Duffy, and A. P. Martin. 2001. Sex-biased dispersal of great white sharks. *Nature* 412:139–140.
- Petrie, M., and B. Kempenaers. 1998. Extra-pair paternity in birds: explaining variation between species and populations. *Trends Ecol. Evol.* 13:52–58.
- Pratt, H. L. J. 1993. The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. *Environ. Biol. Fish.* 38:139–149.
- Pratt, H. L. J., and J. C. Carrier. 2001. A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. *Environ. Biol. Fish.* 60:157–188.
- Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- Ridley, M. 1993. Clutch size and mating frequency in parasitic Hymenoptera. *Am. Nat.* 142:893–910.
- Saville, K. J., A. M. Lindley, E. G. Maries, J. C. Carrier, and H. L. Pratt. 2002. Multiple paternity in the nurse shark, *Ginglymostoma cirratum*. *Environ. Biol. Fish.* 63:347–351.
- Shrey, A. W., and E. J. Heist. 2003. Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*). *Can. J. Fish. Aquat. Sci.* 60:670–675.
- Simmons, L. W. 2003. The evolution of polyandry: patterns of genotypic variation in female mating frequency, male fertilization success and a test of the sex-sperm hypothesis. *J. Evol. Biol.* 16:624–634.
- Strausberger, B. M., and M. V. Ashley. 2003. Breeding biology of brown-headed cowbirds characterized by parent-offspring and sib-group reconstruction. *Auk* 120:433–445.
- Sundström, L. F., S. H. Gruber, S. M. Clermont, J. P. S. Correia, J. R. C. de Marignac, J. F. Morrissey, C. R. Lowrance, L. Thomassen, and M. T. Oliveira. 2001. Review of elasmobranch behavioral studies using ultrasonic telemetry with special reference to the lemon shark, *Negaprion brevirostris*, around Bimini Islands, Bahamas. *Environ. Biol. Fish.* 60:225–250.
- Sunnucks, P., and D. F. Hales. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol. Biol. Evol.* 13:510–524.
- Tregenza, T., and N. Wedell. 2000. Genetic incompatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* 9:1013–1027.
- . 2002. Polyandrous females avoid costs of inbreeding. *Nature* 415:71–73.
- Watson, P. J. 1991. Multiple paternity as genetic bet-hedging in female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae). *Anim. Behav.* 41:343–360.
- Williams, G. C. 1975. Sex and evolution. Princeton Univ. Press, Princeton, NJ.
- Worthingham Wilmer, J., P. J. Allen, P. P. Pomeroy, S. D. Twiss, and W. Amos. 1999. Where have all the fathers gone? An extensive analysis of paternity in the grey seal (*Halichoerus grypus*). *Mol. Ecol.* 8:1417–1430.
- Yasui, Y. 1998. The "genetic benefits" of female multiple mating reconsidered. *Trends Ecol. Evol.* 13:246–250.
- Zeh, J. A., and D. W. Zeh. 1996. The evolution of polyandry. I. Intra-genomic conflict and genetic incompatibility. *Proc. R. Soc. Lond. B* 263:1711–1717.
- . 1997. The evolution of polyandry II: post-copulatory defences against genetic incompatibility. *Proc. R. Soc. Lond. B* 264:69–75.
- . 2001. Reproductive mode and the genetic benefits of polyandry. *Anim. Behav.* 61:1051–1063.
- . 2003. Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. *Ethology* 109:929–950.

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