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Virgin birth in a hammerhead shark

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Parthenogenesis has been documented in all major jawed vertebrate lineages except mammals and cartilaginous fishes (class Chondrichthyes: sharks, batoids and chimeras). Reports of captive female sharks giving birth despite being held in the extended absence of males have generally been ascribed to prior matings coupled with long-term sperm storage by the females. Here, we provide the first genetic evidence for chondrichthyan parthenogenesis, involving a hammerhead shark (*Sphyrna tiburo*). This finding also broadens the known occurrence of a specific type of asexual development (automictic parthenogenesis) among vertebrates, extending recently raised concerns about the potential negative effect of this type of facultative parthenogenesis on the genetic diversity of threatened vertebrate species.

Keywords: asexual reproduction; automictic parthenogenesis; Chondrichthyes; Sphyrnidae; microsatellite DNA profiling; genomic imprinting

1. INTRODUCTION

The direct development of an embryo from an egg without male genetic contribution (i.e. parthenogenesis) has been documented in all jawed vertebrate lineages (bony fishes, amphibians, reptiles and birds) except mammals and cartilaginous fishes (class Chondrichthyes: sharks, batoids and chimaeras). The absence of parthenogenesis in placental mammals is due to genomic imprinting (Kono 2006), but it remains unknown whether it is similarly absent in chondrichthyans or has simply never been detected. Although there are increasing reports of female sharks producing living offspring in captivity despite extended isolation from males, these cases have been attributed to long-term sperm storage by the females with later fertilization, and have never been investigated further (Castro *et al.* 1988; Voss *et al.* 2001; Heist 2004).

In a widely publicized case that occurred on 14 December 2001, one of the three captive adult female

bonnethead sharks (*Sphyrna tiburo*, family: Sphyrnidae (hammerhead sharks)) gave birth to a normally developed, live female pup which was apparently later killed by another fish in the aquarium. This birth is significant because the well-documented capture history of these sharks is inconsistent with sperm storage by the mother as the probable explanation. All three-candidate mothers had been held in the absence of males for 3 years, since they were wild caught in the Florida Keys as immature animals less than 1 year old. At least 2 years away from the age of first maturity, it is improbable that they were capable of sexual activity and sperm storage prior to capture (Parsons 1993). Moreover, the duration of sperm storage by adult female *S. tiburo* in the wild is relatively brief (five months; Manire *et al.* 1995). None of the candidate mothers showed any sign of even rudimentary external male copulatory organs (claspers) that are typical of rare cases of intersexuality in sharks (Iglésias *et al.* 2005), eliminating the possibility of self-fertilization. These factors led us to consider the possibility of asexual reproduction.

Vertebrate parthenogenesis is most easily detected and thus best known in unisexual, obligate parthenogenetic species (Dubach *et al.* 1997); however, it has also been documented in species that normally reproduce sexually (Olsen 1975; Schuett *et al.* 1997, 1998; Groot *et al.* 2003; Watts *et al.* 2006). Apomictic parthenogenetic pathways can bypass or subvert meiosis to produce a zygote that is genetically identical to its mother (i.e. the maternal genome is transmitted to the embryo intact; Groot *et al.* 2003). In contrast, automictic parthenogenetic pathways (automixis) documented in diapsids (birds and squamate reptiles) operate by fusion of post-meiotic products in the mother, leading to elevated homozygosity in the offspring (i.e. genetic diversity is lost in transmission; Olsen 1975; Schuett *et al.* 1997, 1998; Watts *et al.* 2006).

Recent studies have suggested the importance of understanding how frequently and under what conditions female reptiles engage in automixis, amidst concerns about its potential negative effects on genetic diversity in small threatened populations and in captive breeding colonies (Watts *et al.* 2006). A better understanding of the evolutionary breadth of this little-known parthenogenetic mode would also be useful to determine whether these concerns could be similarly valid for the management of genetic diversity in other threatened vertebrates. Here, we genetically confirm automictic parthenogenesis as the mechanism underlying the hammerhead shark birth, providing the first evidence for asexual reproduction in the most ancient jawed vertebrate lineage.

2. MATERIAL AND METHODS

Tissue samples were obtained from each of the three-candidate mothers (CM1–3) and the pup. Four moderately to highly polymorphic microsatellite marker loci described elsewhere for *S. tiburo* (6–35 alleles per locus, observed population heterozygosities from 0.50 to 0.87; Chapman *et al.* 2004) were used to genotype all specimens, with the aim of identifying the mother and detecting distinct paternal alleles in the pup's genotype. We also used available genotype data from the microsatellite screening of 119 animals from the source population (West Florida, USA, Chapman *et al.* 2004) to estimate the probability of observing specific genotypes via normal sexual reproduction given the population allele frequencies. Multi-locus, amplified fragment

Table 1. Genotypes of the three *S. tiburo* candidate mothers (CM1–3) and pup at four microsatellite loci. (CM1 and CM3 are excluded as the mother by allelic mismatches at three of the four loci (non-bold) in each case. CM2 is the mother of the pup, as shown by the allelic matches between this pair of individuals at each locus (alleles). The pup is homozygous for a maternal allele at each locus.)

shark	<i>Pgl02</i>	<i>Sti01</i>	<i>Sti04</i>	<i>Sti10</i>
CM1	124/124	181/189	101/098	374/278
CM3	121/130	181/189	107/107	315/291
CM2	124/127	181/187	107/107	327/304
Pup	124/124	187/187	107/107	304/304

length polymorphism (AFLP) fingerprinting was employed to further survey the pup's genome for possible paternal genetic contribution. AFLP screening (on a Li-Cor dual-laser system) was carried out using the AFLP Core Reagent kit (Invitrogen) following manufacturer's instructions using two selective *EcoRI* primers (E-ACA and E-ACG). Resulting fragments were scored and analysed (band sharing) using the GENEPROFILER software (Scanalytics, Inc.).

3. RESULTS AND DISCUSSION

The microsatellite genotypes of the pup and three-candidate mothers at the four loci unambiguously identified CM2 as the mother; no allelic mismatches were observed between CM2 and the pup, whereas CM1 and CM3 were clearly excluded by allelic mismatches at three of the four loci (table 1). Despite the collectively high allelic diversity and heterozygosity of these four markers in the source population (Chapman *et al.* 2004), the pup was uniformly homozygous for one of its mother's alleles. The composite pup microsatellite genotype strongly supports the absence of paternal genetic contribution (i.e. asexual reproduction occurred) for two reasons. First, the pup had no unique (paternal) alleles at these four loci. Second, the probability of the observed homozygous genotype at all four loci assuming biparental reproduction is vanishingly small ($p < 1 \times 10^{-7}$) given the population rarity of alleles possessed by the pup at two of the loci (*Sti01* (allele 187, expected population homozygote frequency 0.0009), *Sti10* (allele 304, expected population homozygote frequency 0.002)). Furthermore, although the probability of biparental allelic inheritance is not theoretically eliminated (i.e. extremely small but not zero), none of the wild 119 *S. tiburo* screened were homozygous at all four loci. In addition, any such theoretically possible individuals would be expected to exhibit homozygous combinations of the most common alleles in the source population, rather than some of the rare ones seen in this pup.

AFLP fingerprinting analysis also confirmed the identity of the CM2 as the mother because it shared a higher percentage of AFLP fragments with the pup (84%) than did the other two females (less than 69%; not shown). More importantly, all AFLP fragments observed in the pup were also found in CM2, with no evidence of any unique paternal bands. Finally, 16% of the bands observed in the mother were absent in the pup, which is consistent with the complete homozygosity observed in the pup's composite microsatellite genotype. Based on these observations, the

alternative hypothesis that the pup's very unusual, all homozygous microsatellite composite genotype coupled with an absence of non-maternal AFLP fragments could have resulted from sexual reproduction is extremely improbable.

The pup's homozygosity at all four microsatellite loci and reduced number of AFLP fragments compared with its mother is consistent with an automictic rather than an apomictic parthenogenetic pathway. Automixis also produces homozygosity for sex chromosomes, and the documented cases in vertebrates (birds and reptiles) all have heterogametic females (ZW), and so only produce viable ZZ males and an equal proportion of inviable WW zygotes (Olsen 1975; Schuett *et al.* 1997, 1998). The contrasting heterogametic male system (XX females, XY males) should only produce viable females by automixis. The female sex of the *S. tiburo* pup is therefore consistent with automixis and female homogamety (XX) in carcharhiniform sharks as proposed from karyotyping (Maddock & Schwartz 1996).

With this discovery of parthenogenesis in a cartilaginous fish, asexual reproduction has now been demonstrated in all major jawed vertebrate lineages except mammals (Spurway 1953; Olsen 1975; Schuett *et al.* 1997, 1998; this study), where its absence is due to genomic imprinting. The maternal and paternal genomes in the mammalian zygote are imprinted and differentially expressed, thus both genomes are required for normal foetal development (Kono 2006). This imprinting is believed to have evolved in response to conflicts that develop between the embryonic maternal and paternal genomes with regard to maternal resource allocation in lineages where there is a direct maternal–embryonic connection, such as a placenta (Moore & Haig 1991; Haig 2004). The same intergenomic conflict and selection for imprinting could reasonably be hypothesized to operate in placental sharks with their long evolutionary history of this mode of development (Hamlett & Koob 1999; Feldheim *et al.* 2004). Our finding of successful parthenogenesis in the placentally viviparous *S. tiburo* argues that genomic imprinting in this species is absent, or at least does not occur to the extent that development of a gynogenetic embryo is prevented. This observation raises questions about whether genomic imprinting is absent in sharks generally, despite relatively common placental viviparity in this lineage. Given the wide range of reproductive modes from oviparity to placental viviparity in elasmobranchs (Hamlett & Koob 1999), further investigation into the occurrence of parthenogenesis across this lineage could provide valuable insights into the role of reproductive mode in the evolution of genome imprinting.

Parthenogenesis is difficult to detect in ordinarily sexually reproducing vertebrate species, and its prevalence and potential effects on population genetic diversity are poorly understood. Our results suggest that accumulating cases of female sharks producing healthy offspring in the absence of males (Castro *et al.* 1988; Voss *et al.* 2001; Heist 2004) warrant genetic evaluation to determine how common asexual reproduction, especially automixis, is among these ancient

fishes. In some of these cases, females have produced several viable offspring over multiple reproductive cycles (Castro *et al.* 1988; D. Sweet 2005, Detroit Aquarium personal communication), suggesting that parthenogenesis may be facultative in situations where female sharks have difficulty encountering suitable mates (e.g. a possibility in the wild due to low population densities caused by overexploitation or in emerging captive breeding programmes for endangered sharks). A similar recent discovery of automictic parthenogenesis in Komodo dragons (*Varanus komodoensis*) raised concerns about the possible negative effects of this form of asexual reproduction on the genetic diversity in small natural or captive populations of this and other endangered reptiles (Watts *et al.* 2006). Our finding for a shark extends the known evolutionary occurrence of automictic parthenogenesis to a major basal vertebrate lineage, indicating that these concerns about the conservation of genetic diversity could apply to threatened species over a much broader range of vertebrate taxa.

All research carried out in this investigation strictly followed guidelines provided by Queen's University Belfast Research Ethics Committee.

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