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Evolutionary biology

The behavioural and genetic mating system of the sand tiger shark, *Carcharias taurus*, an intrauterine cannibal

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Sand tiger sharks (*Carcharias taurus*) have an unusual mode of reproduction, whereby the first embryos in each of the paired uteri to reach a certain size ('hatchlings') consume all of their smaller siblings during gestation ('embryonic cannibalism' or EC). If females commonly mate with multiple males ('behavioural polyandry') then litters could initially have multiple sires. It is possible, however, that EC could exclude all but one of these sires from producing offspring thus influencing the species genetic mating system ('genetic monogamy'). Here, we use microsatellite DNA profiling of mothers and their litters ($n = 15$, from two to nine embryos per litter) to quantify the frequency of behavioural and genetic polyandry in this system. We conservatively estimate that nine of the females we examined (60%) were behaviourally polyandrous. The genetic mating system was characterized by assessing sibling relationships between hatchlings and revealed only 40 per cent genetic polyandry (i.e. hatchlings were full siblings in 60% of litters). The discrepancy stemmed from three females that were initially fertilized by multiple males but only produced hatchlings with one of them. This reveals that males can be excluded even after fertilizing ova and that some instances of genetic monogamy in this population arise from the reduction in litter size by EC. More research is needed on how cryptic post-copulatory and post-zygotic processes contribute to determining paternity and bridging the behavioural and genetic mating systems of viviparous species.

1. Introduction

Animal mating systems can be parsed into the behavioural component, describing copulation patterns, and the genetic component that describes the realized distribution of parentage [1]. Most broods of internally fertilizing animals exhibit multiple paternity, indicating a genetic mating system that includes behavioural polyandry (i.e. females mating with multiple males; [1]). In a few species or populations, however, single paternity of broods is more common ('genetic monogamy' [2–4]). Although multiple paternity provides definitive proof of behavioural polyandry and demonstrates some level of concordance between the behavioural and genetic mating systems, genetic monogamy is ambiguous with regard to the underlying mating behaviour. Genetic monogamy can result from a female only mating with one male, but it can also arise after

post-copulatory competitive or selective processes allow one of several mating partners to monopolize paternity [1,5].

The sand tiger shark, *Carcharias taurus*, has a remarkable reproductive mode in which the behavioural and genetic mating system are decoupled by embryonic cannibalism ('EC'), potentially enabling post-zygotic processes to ultimately determine paternity of the litter. Female sand tigers ovulate for several months, with ova entering the upper oviduct where fertilization occurs [6]. Single or a few fertilized ova are then encapsulated and then settle in either the right or left of the paired uteri. The first embryo in each uterus to 'hatch' from its egg capsule, occurring when it reaches a length of approximately 55–60 mm, is called the 'hatchling' [6]. Soon after, the approximately 100 mm hatchling proceeds to attack, kill and eventually consume all of its younger siblings, achieving exponential growth over this period [6]. The hatchling then has sole access to unfertilized ova accumulated in the uterus, which are consumed for much of the rest of gestation ('oophagy'). Term litters consist of a pair of hatchlings, one from each uterus, that are born at such large sizes (approx. 950–1250 mm) that they have few predators [6].

The unusual developmental mode of sand tigers raises interesting questions about how EC influences the species genetic mating system (i.e. distribution of parentage). The behavioural mating system of female sand tigers is not known, but if behavioural polyandry is common then it is possible that they initially gestate litters sired by multiple males similar to many other animals [1,2,5]. Unlike most other animals, however, the majority of embryonic sand tiger sharks are not carried to term because of EC [6]. This raises the possibility that some sires that initially fertilize ova are excluded because all of their progeny are consumed by the hatchlings. From a mating system perspective, this could frequently result in genetic monogamy despite behavioural polyandry by females. We therefore used microsatellite DNA profiling to conduct the first parentage study of a species with EC, which may be an important determinant of the genetic mating system.

2. Material and methods

Gravid sand tigers were obtained from mortality events associated with protective beach meshing operations in Richards Bay, South Africa (28°48'0" S, 32°6'0" E) from 2008 to 2012. Tissue samples from mothers and their embryos were stored at room temperature in 95 per cent ethanol, as were samples from adult males and females collected in the same area. Microsatellite genotyping of each individual at 10 polymorphic loci (9–32 alleles per locus) is described in the electronic supplementary material. The parental exclusion probability of this panel of markers was over 99.9 per cent if one parent is known as calculated in GERUD v. 2.0 [7] using allele frequencies observed in sampled adults. Genotypes of individuals used in this study can be found in the electronic supplementary material.

We first aimed to characterize the behavioural mating system of this population by estimating the frequency of behavioural polyandry. Like nearly all sharks there are no observations of free-living sand tigers mating. We can therefore only estimate the frequency of behavioural polyandry using genetic parentage analysis. This is necessarily conservative because it only detects polyandry that results in multiple paternity. Since diploid males can pass a maximum of two alleles to their offspring at each locus, more than two paternal alleles in a litter reveals the presence of multiple sires (i.e. the paternal allele count method). This can only be tested in litters composed of three

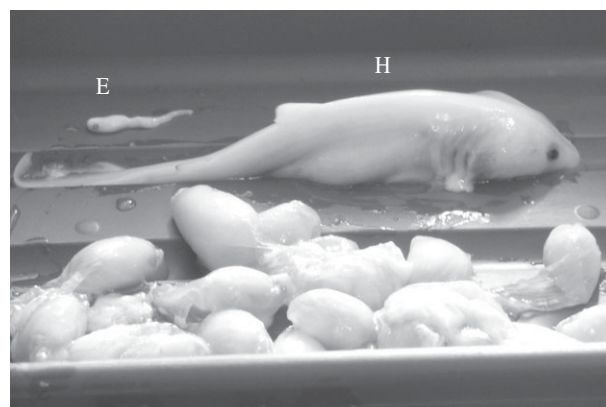


Figure 1. Size differential between the hatchling (H) and an embryo (E) from the same uterus in one of the litters we sampled. In all five litters sampled prior to EC, we observed a similar size differential.

or more embryos (i.e. litters sampled prior to the end of the EC phase of development). For litters composed only of a pair of hatchlings (i.e. litters sampled after EC is completed), we calculated the probabilities that the pair were either full siblings (same father and therefore genetic monogamy) or maternal half siblings (different fathers, proving both behavioural and genetic polyandry) in the program ML-RELATE [8]. ML-RELATE's hypothesis testing option was used to assess the probability of each sibling relationship. These probabilities were generated from 10 000 simulated genotypes using population allele frequencies estimated using adults sampled in the study area. We report which relationship had the highest probability, since these are the only two possibilities. In summary, we considered a female to be behaviourally polyandrous when its litter had more than two paternal alleles or, when there were only two embryos available, if they were maternal half siblings.

The genetic mating system of sand tigers is determined by the paternity of hatchlings, as they are the only embryos that survive to term and contribute to the next generation. As there are only two hatchlings per litter, we used ML-RELATE to determine whether they were full or maternal half siblings as described above. The identity of hatchlings in litters composed of more than two embryos was obvious in the litters we examined because of their larger sizes relative to their siblings, most of which were also still encapsulated (figure 1). In summary, we report two separate mating system parameters. The frequency of behavioural polyandry was conservatively estimated by examining all embryos available in each litter (the behavioural mating system). The frequency of genetic polyandry was estimated based on the relationship of each pair of hatchlings (the genetic mating system).

3. Results and discussion

Gravid females are rarely captured in the beach meshing programme, but we were able to collect 15 litters (mothers and embryos) from 2007 to 2012. Twenty-three additional adult males and females also captured in Richard's Bay were used to estimate population allele frequencies. Five of the 15 litters were collected prior to the completion of EC and were composed of six to nine embryos each (table 1). These were amenable to using the paternal allele count method. All of them exhibited three or four paternal alleles at four to eight of the 10 profiled loci, conclusive evidence that these females had mated with at least two males each (table 1). The remaining litters ($n = 10$) were sampled after EC was finished and the hatchlings were all that remained of the litter. Four hatchling

Table 1. Results of genetic analyses of sand tiger litters (Ct001–Ct015). *N* embryos is the litter size. PAC, paternal allele counts. The relationship between the hatchlings as assessed in ML-RELATE is provided under the following headings: *Put_{FS}*, the simulation-based probability that the alternative relationship (half-siblings) better fits the hatchling genotypes than the putative relationship of full-siblings. If this value is low then the putative relationship is most likely to be true; *Put_{HS}*, the simulation-based probability that the alternative relationship (full-siblings) better fits the hatchling genotypes than the putative relationship of half-siblings. n.a., test not applicable; ?, mating behaviour uncertain. ‘Behavioural polyandry’ was established by either PAC and/or when hatchlings were most likely to be half-siblings. ‘Genetic polyandry’ is established when hatchling were most likely to be half-siblings.

female	<i>n</i> embryos	litter PAC	hatchlings		behavioural polyandry	genetic polyandry
			<i>Put_{FS}</i>	<i>Put_{HS}</i>		
Ct001	2	n.a.	0.84	0.02	yes	yes
Ct002	2	n.a.	0.002	0.65	?	no
Ct003	2	n.a.	0.45	0.034	yes	yes
Ct004	2	n.a.	0.01	0.45	?	no
Ct005	2	n.a.	0.01	0.45	?	no
Ct006	2	n.a.	0.132	0.999	?	no
Ct007	2	n.a.	0.685	0.009	yes	yes
Ct008	2	n.a.	0.79	0.005	yes	yes
Ct009	2	n.a.	0.032	0.34	?	no
Ct010	2	n.a.	0.003	0.85	?	no
Ct011	7	2–4/locus	0.32	0.06	yes	yes
Ct012	8	2–4/locus	0.34	0.05	yes	yes
Ct013	6	2–4/locus	0.007	0.52	yes	no
Ct014	9	2–4/locus	0.005	0.56	yes	no
Ct015	7	2–4/locus	0.001	0.59	yes	no

pairs shared no paternal alleles, which is highly unlikely if they were full siblings (table 1). We conclude that they were maternal half siblings (i.e. had different fathers). We therefore found evidence of behavioural polyandry in nine out of the 15 litters we examined and conservatively estimate that 60 per cent of the females we sampled, including all five of those sampled prior to the completion of EC, were behaviourally polyandrous.

To assess the genetic mating system of sand tigers, we calculated the probabilities that hatchling pairs sampled were full or half siblings for all 15 litters. We found that the hatchling in six of the 15 were maternal half siblings (40% genetic polyandry; table 1). The remaining nine females (60%) were genetically monogamous (table 1). In six of these, we sampled only hatchlings and therefore cannot resolve whether or not the female was behaviourally polyandrous. However, in the three cases where we sampled both hatchlings and smaller embryos paternal allele counts proved that the female was behaviourally polyandrous. These three cases demonstrate that some sires are excluded by EC, and individual cases of genetic monogamy sometimes occur despite behavioural polyandry.

It is widely known that behavioural polyandry can initiate post-copulatory competition between males in internally fertilizing species [1]. The existence of behavioural polyandry suggests there is sperm competition in sand tigers, which corresponds with the observation that males of this species store larger volumes of sperm than other sharks [9]. EC broadens sexual selection beyond post-copulatory competition to simply fertilize ova because it generates competition to fertilize specific ova: those that are most likely to become hatchlings. Fertilizing the earliest ova is likely to be important because

the earliest embryos to settle in each uterus have a head start when it comes to reaching hatchling size [6]. Male sand tigers are reported to engage in mate guarding behaviour in captivity, with a dominant male physically excluding rivals from females until after it had copulated [10]. If this is representative of natural behaviour, then mate guarding could have evolved to maximize the probability of fertilizing the earliest ova and/or to delay subsequent fertilizations by other males. Sexual selection is broadened still further in cases where there are a few similar-sized embryos in each uterus as they approach hatching [6]. In this situation, any trait that enhances the competitive ability of the embryo itself would be subject to positive selection. For example, individual growth rate could determine which of several similar-sized embryos would hatch first and consume its younger siblings, presumably selecting for males to carry genes that promote rapid embryonic growth. It is also possible to envision how this type of selection may have influenced the precocious development of eyes and teeth that occurs in embryonic sand tigers, which enhances their ability to locate and consume their siblings [6].

Female sand tigers make an extremely large energetic investment in two offspring per gestation [6]. Although female sharks, including sand tigers, can either cooperate or engage in avoidance behaviours during copulation attempts it is thought that they do not have absolute control over mate choice or mating frequency [11]. EC may increase the probability that compatible and/or competitively superior males (in terms of behavioural dominance, sperm competition or producing competitive embryos) sire the hatchlings, even if females are unable to control mating frequency or outwardly assess mate quality. The ‘convenience polyandry hypothesis’

postulates that females may accept multiple mating partners simply because the costs of resistance exceed the costs of accepting superfluous copulation [12]. EC may allow female sand tigers to engage in convenience polyandry after mating with preferred males without actually investing in embryos from these superfluous copulations.

The original envisioning of sexual selection was based on the competition that occurred between males for mates [13]. A century later, it was recognized that sexual selection continues after copulation when sperm from different males compete to fertilize ova [5]. While multiple male sand tigers commonly fertilize a single female, EC sometimes excludes all but one of them from gaining offspring. This system

highlights that competition and sexual selection can still occur after fertilization. Although EC is rare in nature [6], competition between embryos is common in viviparous species and may lead to mortality during or immediately following gestation [1]. This competition can play an important and probably underappreciated role in determining male fitness.

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