Offspring sex ratio increases with male reproductive success in the polygynous southern elephant seals

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Abstract

In polygynous species, most dominant males sire a disproportionate number of offspring and dominance rank is assumed to be age dependent. Yet, extreme inter-male competition and high early male mortality prevent most males from reaching a social status that could guaranty a high reproductive success. Alternative reproductive tactics may have evolved to maximize male reproductive success despite a low social rank. One of them, offspring sex-ratio adjustment, may allow males to produce more offspring of the sex that will provide a higher fitness. If traits influencing dominance in males are heritable and if the average fitness of subordinate males is lower than the average fitness of females, we predict that the probability of producing a son would increase with a male reproductive success as its sons would be more likely to become dominant. We tested this hypothesis on southern elephant seals breeding on the Kerguelen Archipelago. Using 530 pups sired by 52 males, we found that the probability of siring a son increases with a male relative reproductive success. Out finding provide new insights on sex ratio variation can be an important tool in managing population dynamics and structure, which has direct implications on wildlife conservation.

Introduction

In polygynous mating systems, commonly found in mammalian species (Clutton-Brock, 1989), sexual selection favors male phenotypes that promote the monopolization of the access to receptive females (Andersson, 1994). The strength of selection depends on ability of males to defend female groups, which varies, for example, with females' distribution in space and time, predation pressures, costs of social living, and activity of competitors (Emlen & Oring, 1977; Clutton-Brock, 1989). Most dominant males defend and control aggregations of females (also called *harems*), resulting in strong reproductive skew favoring harem-holders (Clutton-Brock, 1985; Hoelzel et al., 1999; Hoffman et al., 2003). The male dominance rank is typically assumed to increase with age as males acquire the needed physical attributes and experience to compete successfully against other males (Heckel & Helversen, 2002; Festa-Bianchet, 2012). However, when inter-male competition is extremely strong combined with a high mortality rate over successive life stages, most of the males in the population fail to hold a harem, which results in a null or very low lifetime reproductive success (Clinton & Le Boeuf, 1993; Loison et al., 1999; Clutton-Brock, 2007).

In this context, the adaptive adjustment of offspring sex ratio (OSR; defined as the % of offspring males) weakens the strength of sexual selection (Fawcett et al., 2011; Booksmythe et al., 2013). Parents should bias the sex ratio of their offspring towards the sex that will have the greatest improvement on their fitness (Trivers & Willard, 1973; Charnov, 1982). The reproductive benefit of producing a male or a female offspring for parents should depend on the relative fitness of sons and daughters, the costs of producing and rearing each sex, and the sex differences in any future competition or cooperation with parents or other kin (Frank, 1986; Emlen et al., 1986; Clutton-Brock & Iason, 1986).

The most influential hypothesis on offspring sex ratio adjustment (OSRA)—the Trivers and Willard hypothesis—predicts that, when variation in reproductive success in higher in males than in females (e.g., in polygynous species) and maternal condition has a stronger effect on the fitness of sons than daughters, females in good condition should produce more sons, whereas females in poor condition should produce more daughters (Trivers & Willard, 1973). The Trivers and Willard hypothesis can be generalized and applied to any factor that has a different effect on the fitness of sons and daughters (West, 2009), such as local density (local resource competition) (Silk, 1983), need for helpers (local resource enhancement) (Komdeur et al., 1997), or male attractiveness (Burley, 1981).

The mate quality hypothesis posits that females can manipulate the sex ratio of their progeny according to their mate's attributes (Burley, 1981; Pen & Weissing, 2001). Females breeding with high quality males (e.g., larger body size, weapons, or ornaments), should capitalize on this advantage by biasing their offspring production in favor of sons, because sons of high-quality males are more likely to have higher fitness than daughters (Fawcett et al., 2007; Cox & Calsbeek, 2010). In contrary, females breeding with poor quality males make the best of a bad job by biasing their progeny in favor of daughters, because sons of low-quality males may have a lower fitness than daughters (Burley, 1981; Fawcett et al., 2007; Cox & Calsbeek, 2010).

Trivers & Willard (1973) originally assumed that mothers influence offspring sex determination. Recent evidence, however, shows that fathers may also adjust offspring sex ratio—especially in mammalian males as they are the heterogametic sex (Edwards & Cameron, 2014; Douhard et al., 2016). For example, red deer (*Cervus elaphus*) hinds, artificially inseminated with no knowledge of male phenotypes, produced twice more sons than daughters when the sire's fertility was higher (Gomendio et al., 2006). Yet, testing the relationship between male phenotypes and OSR has been given little attention, and thus more studies on this topic are needed (Edwards & Cameron, 2014; Booksmythe et al., 2017; Douhard, 2018; Douhard & Geffroy, 2021).

The objective of this study was to test for the relationship between male phenotype and its OSR in a highly polygynous species, the southern elephant seal (*Mirounga leonina*). Elephant seals gather on land once a year for the breeding season. Males arrive before females, and immediately begin interacting with each other to establish dominance hierarchy (Le Boeuf, 1974; McCann, 1981). Pregnant females arrive few weeks later and gather in harems of high density. Females give birth ca. five days after hauling out and nurse their pup for ca. 24 days. Females come to estrus the last ca. four days before returning to sea to forage (Laws, 1956; Le Boeuf, 1972). Dominant males adopt a central position in harems and actively prevent other males from accessing females, while subordinate males stay at the periphery and opportunistically look for copulation attempts. Reproductive success is highly skewed, with harem holders generally siring more than 75% of the pups (Modig, 1996; Fabiani et al., 2004). Variance in lifetime reproductive success is approximately four times greater in males than in females (LeBoeuf & Reiter, 1988). Holding a harem is a highly successful mating tactic. However, inter-male competition is intense and mortality rate is high, and most of the males die before reaching the physical condition and the experience required to hold a harem (Le Boeuf, 1974; Clinton & Le Boeuf, 1993; Lloyd et al., 2020).

We hypothesize that, in a polygynous species where the probability of a male holding a harem (i.e., high reproductive success) is low, natural selection will favor OSRA to increase the fitness of subordinate males. If the average lifetime reproductive success of subordinate males is lower than the average lifetime reproductive success of females, we expect males to adjust the sex-ratio of their offspring depending on their siring probability. As a result, we should find a positive relationship between OSR and a male reproductive success. We tested our hypothesis on the Rivière du Nord southern elephant seal colony, in the north of the main island of the Kerguelen Archipelago. We used genetic markers from skin biopsies to link the paternity of pups to the breeding males.

Methods

Study site, observations, and sample collection

We conducted our study on a colony of southern elephant seals breeding at the *Rivière du Nord* (RdN) site between Sept. 2 and Nov. 10, 2017. The RdN breeding site is located north of the Kerguelen Island (S49° 10’ 33”, E70° 8’ 17”) and characterized by a 450 m long sandy beaches mixed with pebbles. We walked around the colony, almost daily, to record the presence of each male. Individuals were photographed at the first encounter, then identified according to their body scars. We used a 3 m long aluminum pole equipped with a stainless-steel biopsy tip with barb (7 mm diameter and 40 mm length) to sample tissue biopsies from the lateral back area of seals. We sampled 77 breeding males among all the males (n=113) sighted in RdN. In 2018, we returned to RdN and used dissecting scissors to sample tissue biopsies on all accessible weaned pups from the trailing edge of one of the hind flippers (n=977). All tissue samples were preserved in 70% ethanol until laboratory analysis.

Relative body length

We estimated the relative body length of the breeding male seals from photos taken when they were laying straight and flat on the ground (Bell et al., 1997). We used a Canon camera (EOS 5D 12.8 MP DSLR) with a 100-400 mm zoom lens to take the photos. We photographed the seal at a distance about 10 m, perpendicularly to the longitudinal axis of its body, and approximately at the height of the center of its body. We placed a calibrated rope (marked every 50 cm) along the seal's body and used it as a reference for the scale. We disregarded the caudal flippers and the head of the seal as their position varied between the photos. We considered the length of the seal from the eye to the base of the hind flippers. This measure correlates with the total length, i.e., from the tip of the nose to the end of hind flippers (r=0.99, Carlini et al., 2006). We measured the seal body length from the photos using the software ImageJ version 1.53f51. We estimated a unique value of the relative body length for each seal by extracting the best linear unbiased predictors from a univariate linear mixed-effect model fitted in a Bayesian framework using the `brms` R package (Bürkner, 2017). The model included the body length as response variable and the seal identifier as random intercept (for all details about the model specification and fit see the Supplementary Material 1). We calculated the repeatability of body length according to Nakagawa & Schielzeth (2010). The repeatability is used as an indicator of the error in measuring body length of the same individual between photos.

Genetic analysis

DNA extraction

The samples of skin biopsies were digested with proteinase K. We then extracted DNA using the Nucleospin 96 Tissue Kit (Macherey-Nagel), following the manufacturer's instructions, and randomly distributed samples into the plates. We replicated 50 individuals twice at the extraction stage to check the repeatability of the results. We assessed DNA concentration and purity with Qubit DSDNA Assay kit (ThermoFisher) on a Berthold Tristar2 microplate reader.

Microsatellite development and sequence-based microsatellite genotyping

We identified microsatellite markers from a random shotgun sequencing of a DNA pool extracted from ten individuals purified using 1.8 X Agencourt AMPure XP beads (Beckman Coulter, the UK) and quantified with a Qubit 2.0 fluorometer (ThermoFisher). We prepared the DNA library using QIASeq FX DNA library kit (Qiagen) and sequenced it on an Illumina MiSeq sequencer using a v2 nano sequencing kit (2 × 250 bp). We used the software BBmerge v38.87 (Bushnell et al., 2017) to merge paired reads and the software QDD v3.1.2 (Meglécz et al., 2014) to discover microsatellite. We fixed the QDD primer design parameters to target amplicon lengths between 100 and 180 bp and optimized them for multiplex PCR (Lepais et al., 2020). We selected 60 primer pairs based on different criteria to increase polymorphism content and amplification success (Meglécz et al., 2014). We tagged the locus-specific primers at 5′-end with universal Illumina adapter overhang sequences: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG for forward primers and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG for reverse primers (Supplementary Material 2). We tested the amplification of each of the 60 primer pairs in a simplex PCR performed on the DNA pool of the elephant seals. We prepared the PCR in a volume of 10 µL containing 2 µL of 5X Hot Firepol Blend master mix (Solis Biodyne), 1 µL of 2µM primer pairs, 1 µL of DNA pool (10 ng/µL), and 6 µL of PCR-grade water. We performed the PCR on a Veriti 96-Well Fast thermal cycler (ThermoFisher) which consisted in an initial denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 95°C for 20 s, annealing at 59°C for 60 s, extension at 72°C for 30 s, and a final extension step at 72°C for 10 min. We checked the amplification on a 3% agarose gel.

We validated the developed markers by repeated genotyping of a set of 95 samples. We performed a multiplexed PCR amplification of the 60 markers in a volume of 10µL using 2 µL of 5X Hot Firepol Multiplex master mix (Solis Biodyne), 1 µL of multiplex primer mix (0.5 µM of each primer), 2 µL of DNA (10 ng/µL), and 5 µL of PCR-grade water. We performed the PCR on a Veriti 96-Well Fast thermal cycler (ThermoFisher) which consisted in an initial denaturation at 95°C for 12 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 180 s, extension at 72°C for 30 s, and a final extension step at 72°C for 10 min. We performed a second PCR to attach the adapters and sample-specific pairs of indexes (8bp unique sequences) to each side of the amplicons by targeting the universal sequence attached to the locus-specific primers. We carried out this indexing PCR in a volume of 20 µL using 5X Hot Firepol Multiplex master mix (Solis Biodyne), 5 µL of amplicon, and 0.5 µM of each of the forward and reverse adapters. The PCR conditions consisted in an initial denaturation at 95°C for 12 min followed by 15 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 90 s, extension at 72°C for 30 s, and a final extension step at 72°C for 10 min. We then pooled the libraries and purified them with 1.8X Agencourt AMPure XP beads (Beckman Coulter, the UK). We checked quality on a Tapestation 4200 (Agilent) and conducted the quantification using QIAseq Library Quant Assay kit (Qiagen, Hilden, Germany) in a Roche LightCycler 480 quantitative PCR. We sequenced the pool on an iSeq 100 sequencer (Illumina, San Diego, CA, USA) with a 2x150 pb bp kit.

We used the bioinformatics pipeline (Lepais et al., 2020) integrating the FDSTools software (Hoogenboom et al., 2017) to call genotypes from raw sequences. We performed a first analysis on the 95 repeated samples for which we used a negative control to optimize the bioinformatic pipeline to each locus, to estimate the locus-level allelic error rate, and to select the loci that produced repeatable genotypes for the final genotypic dataset.

For the final genotyping, we performed a multiplex PCR on the validated markers in 384 format plates in a volume of 5 µL using 1 µL of 5X Hot Firepol Multiplex master mix (Solis Biodyne), 0.5 µL of multiplex primer mix (0.5 µM), 1 µL of DNA (10 ng/µL), and 2.5 µL of PCR-grade water. We realized the second PCR in a volume of 5 µL using 1 µL of 5X Hot Firepol Multiplex master mix (Solis Biodyne), 1.25 µL of amplicon, and 0.5 µL of each of the forward and reverse adapters (5µM). The PCR conditions for these two PCR are the same as for genotyping validation except the reactions were performed on a Veriti 384-Well thermal cycler (ThermoFisher). We then pooled the libraries from 384 samples, purified them with 1.8 X Ampure beads, and quantified them with QIAseq Library Quant Assay kit. We sequenced each pool on an iSeq 100 sequencer (Illumina, San Diego, CA, USA) with a 2x150 pb bp kit. We performed genotyping analysis with the same bioinformatics pipeline (see above) using optimized parameters determined during the validation phase (Supplementary Material 2).

All the 60 developed primer pairs from the whole genome shotgun sequencing produced specific amplification and were kept in the multiplexed PCR (Supplementary Material 2). Among the 60 loci, 40 produced repeatable genotypes with 368 alleles that showed differences in their sequences (mean: 9.2 alleles per loci) and only 257 alleles that showed differences in their sizes (mean: 6.4 alleles per loci) with an average of 0.7% of allelic error among the 95 repeatedly genotyped samples (Supplementary Material 2).

Among the 40 loci, two were monomorphic (SSRseqMir_057 and SSRseqMir_060, Supplementary Material 2) and one exhibited a too high sequencing error rate (SSRseqMir_039) ; and therefore, eliminated for subsequent analyses. The remaining 37 loci were tested from Hardy-

Weinberg equilibrium and their frequency of null alleles were determinate using the software CERVUS 3.0.7 (Kalinowski et al., 2007). Only one locus (SSRseqMir_002) exhibited significant deviation from Hardy-Weinberg equilibrium and a high frequency of null alleles (0.10, Supplementary Material 2) and was, therefore, eliminated for further analyses.

Paternity analyses

The paternity assignment analyses were conducted using CERVUS 3.0.7 (Kalinowski et al., 2007) for a set of 36 polymorphic microsatellite loci (Supplementary Material 2) typed on 86 males (potential fathers), and 971 pups. The proportion of loci typed was 0.94 and the non-exclusion probability was 2.5×10^{-9}.

A male was considered as a likely father when no mismatches were detected between the pup genotype and the candidate male genotype. When a candidate male exhibited one single mismatch with the pup genotype, we considered this male as the father only if the pair confidence was 95% (or more, i.e., strict confidence). When the pair confidence was too low and/or the number of mismatches higher than 1, we considered that we did not sample the father on this pup.

Statistical analysis

We used a χ^2 test to compare the number of sons and daughters sired by the different males in RdN in 2018. To test for our hypothesis that OSR in southern elephant seals varies with male reproductive success, we used a generalized linear model with a binomial distribution to model the probability of siring a son as a function of the male relative reproductive success. The response variable was the number of sons relative to the number of pups each male sired. We calculated each male relative reproductive success as the number of pups sired by a male divided by the mean number of pups sired by all the breeding males. Reproductive success strongly increases with age in southern elephant seals (Clinton & Le Boeuf, 1993; Lloyd et al., 2020), and age affects OSR (Edwards & Cameron, 2014; Santos et al., 2015; Tanaka et al., 2019). We, thus, added the relative body length to our model as a proxy for age (McLaren, 1993). We found that some males that sired few pups were sighted for a brief period or only at the beginning of the breeding season before the females arrived. We concluded that these fertilizations must have occurred in a neighboring breeding site and that females moved to RdN the following year (2018) when we took the tissue biopsies on the pups. This means that the number of pups sired or the OSR for these males may not be representative of their actual reproductive strategy. To minimize the effect of these off-site copulations, we weighted our model by the number of days individuals spent at RdN.

We used a Bayesian framework to fit our model using the `brms` R package (Bürkner, 2017) (for all details about the model specification and fit see the Supplementary Material 3). We normalized all predictors by diving by the variable standard deviation and shifting the zero to the minimum value such as the intercept is at the minimum value. We ran our analysis on R 4.1.3 (R Core Team, 2021).

Results

Paternity assignments

We found that 52 out of 77 males sired 530 out of 977 pups. The number of pups sired was highly skewed (Figure 1). Approximately 20% of the males were responsible for siring 80% of the pups. The maximum number of pups sired by the same male was 66 pups (i.e., 7%).

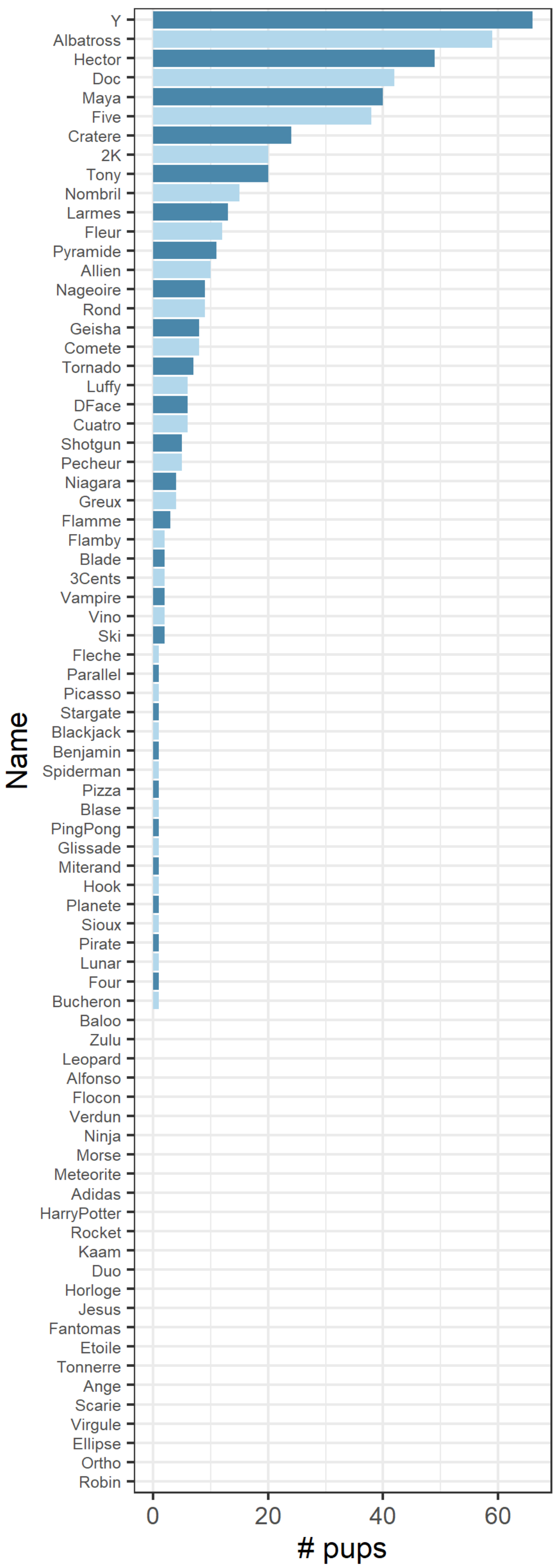

Figure 1: The number of pups sired by each male southern elephant seal.

OSR model

The proportion of sons among all the pups observed at RdN in 2018 did not differ from 0.5 (491 sons and 486 daughters; proportion test: $\chi^2 = 0.02$, P = 0.90). We found a weak positive effect between a male relative reproductive success and its probability of producing a son, and the 95% credible intervals was at the limit of the zero (Figure 2, Figure 3, and Table 1). According to our model, the male with the lowest relative reproductive success has a probability of 45% [credible intervals: 39%, 51%] to produce a son and the one with the highest relative reproductive success has a probability of 54% [48%, 60%]. We did not find any evidence that the effect of the relative body length (used as a proxy for age) on the probability of producing a son was different from zero as the posterior distribution of the effect estimate was centered to zero (Figure 2 and Table 1). The repeatability of body length was 0.94 ± 0.01 [0.91, 0.96].

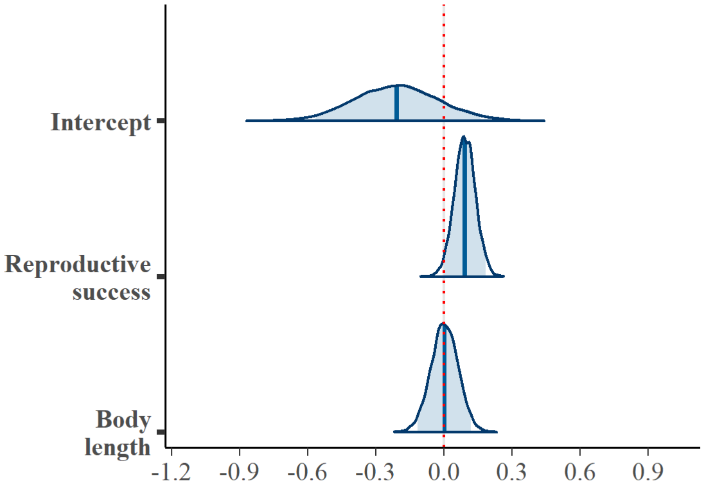

Figure 2: Parameter posterior distributions of the generalized linear model predicting the probability that a male southern elephant seal sires a son as a function of its relative reproductive success and relative body length (a proxy for age). The relative reproductive success is calculated as the number of offspring sired by a male divided by the mean number of offspring sired by all sampled males. The mean of the parameter posterior distribution is marked by a vertical dark blue line and the area representing the 95% credible intervals is colored in light blue.

Table 1: Parameter mean estimates of the generalized linear model predicting the probability that a male southern elephant seal sires a son as a function of its relative reproductive success and relative body length (a proxy for age). The relative reproductive success is calculated as the number of offspring sired by a male divided by the mean number of offspring sired by all sampled males. Parameter mean estimates are presented with standard errors (SE) and 95% credible intervals (CI).

Parameter	Estimate	SE	CI
Intercept	-0.21	0.19	[-0.57, 0.16]
Reproductive success	0.09	0.05	[0.00, 0.18]
Body length	0.00	0.06	[-0.12, 0.12]

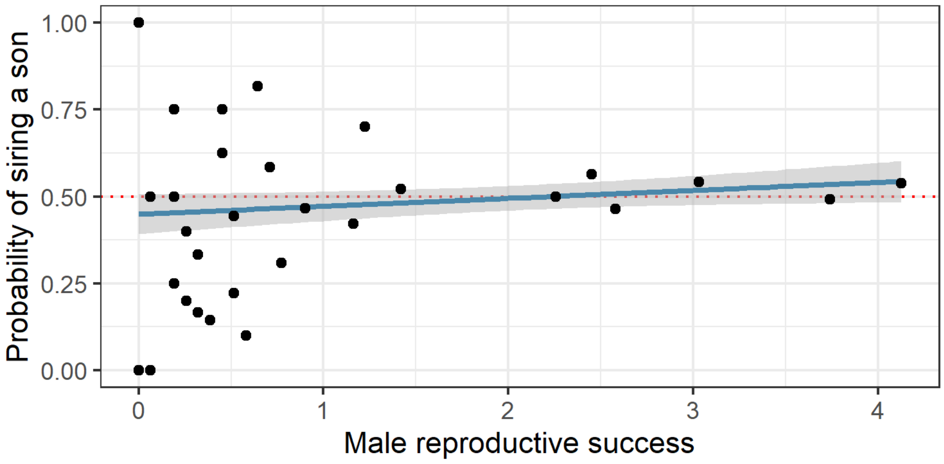

Figure 3: The predictive probability that a male southern elephant seal sires a son as a function of its relative reproductive success. The relative reproductive success is calculated as the number of offspring sired by a male divided by the mean number of offspring sired by all sampled males. 95% credible intervals (grey area) were added around the mean effect (blue line). Points represent the proportion of sons each male sired.

Discussion

We found that OSR increased with the relative reproductive success in male southern elephant seals. This is consistent with previous studies on polygynous species showing a general positive trend between male attributes and OSR (e.g., Gomendio et al., 2006; Røed et al., 2007; Douhard et al., 2016; Malo et al., 2017; Perret, 2018). Nonetheless, to our knowledge, only the study by Douhard et al. (2016) explicitly investigated the relationship between male reproductive success and OSR. The effect size estimated by our model was weak (Table 1) as predicted by theoretical models (Fawcett et al., 2007) and supported by a meta-analysis on empirical studies, albeit mostly bird species (Booksmythe et al., 2017).

The capacity of OSRA to evolve with male reproductive success in polygynous species may depend on the heritability of the competitive ability of males and the differential fitness outcome of sons and daughters (Trivers & Willard, 1973; Clutton-Brock & Iason, 1986). In elephant seals, reproductive success increases with the competitive capacity of males to hold a harem (Hoelzel et al., 1999). We expect traits that influence this capacity, such as large body size, physical stamina, aggressiveness, and boldness, to be heritable (e.g., Kruuk et al., 2000). A harem holder would benefit from producing sons that inherit its competitive ability which will also increase their probability to hold a harem. In contrast, peripheral (subordinate) males should benefit more from producing daughters if the average fitness of daughters exceeds the fitness of sons that never hold a harem. In our study, we did not measure the differential relative fitness between producing sons and daughters, but this was investigated by other studies. For example, the fitness outcome of producing daughters in bighorn rams (*Ovis canadensis*, Douhard et al., 2016) with lower reproductive success and in brown anole lizard males (*Anolis sagrei*, Cox & Calsbeek, 2010) with smaller sizes is greater than of producing sons, supporting the adaptive relationship between male quality and its probability of having a son. Sexually antagonistic genetic variance for fitness may also favour the evolution of OSRA (Blackburn et al., 2010). Variation of some specific alleles may be beneficial to one sex but deleterious to the other, leading to a trade-off between optimal genotypes for males and females (Fedorka & Mousseau, 2004; Foerster et al., 2007; Connallon & Jakubowski, 2009). Given the very small proportion of males that reach the dominant status, and a high reproductive success, biasing OSR in favor of sons corresponds to a high-risk high-reward strategy, whereas biasing it in favor of females corresponds to a bet hedging strategy (Slatkin, 1974; Gillespie, 1974). Bet hedging can help explain the maintenance of these two alternative strategies, even if at first sight lifetime reproductive success in polygynous species can be much higher for males than females (Simons, 2011).

We did not design our study to identify the mechanisms causing OSRA, similarly to most previous studies on this topic (Cameron, 2004; Helle et al., 2008). Although initial hypotheses assumed that mothers exclusively control OSRA (Trivers & Willard, 1973; Burley, 1981), recent evidence shows that OSRA can be driven by fathers, mothers, or both simultaneously (Edwards & Cameron, 2014; Douhard & Geffroy, 2021). In this context, we expect no conflict between females and males. If a male is not competitive, both sexes have an advantage of producing more daughters, whereas if a male is highly competitive, both sexes have an advantage of producing more sons. Hereafter, we discuss some possible causal mechanisms of these different pathways that could explain the results we found in southern elephant seals.

OSRA by females:

Accordingly with the differential allocation and the mate attractiveness/quality hypotheses (Burley, 1981; Sheldon, 2000), mothers may adjust the sex of their offspring in response to fathers' phenotypes in a way to maximize their long-term fitness output (Booksmythe et al., 2017). Mating with a high quality (attractive) male increases the probability of producing sons, as they inherit the phenotypes of their fathers (e.g., ornaments, armaments, or dominance rank, Burley, 1981; Ellegren et al., 1996; Cox & Calsbeek, 2010).

Elephant seal females may perceive the quality of the males through their physical attributes, such as body size, or their dominance status (i.e., harem holder vs. peripheral) (Modig, 1996; Hoelzel et al., 1999; Carlini et al., 2006). For example, elephant seal females resist and protest more against copulation attempts of low rank males than higher rank males (Galimberti et al., 2000a). Dependent on the male quality, various mechanisms of cryptic choice can allow females to skew their offspring sex ratio. This can be done through differential mortality or fertilisation success of the X- and Y-chromosome bearing spermatozoa (CBS) in the reproductive tract (Krackow, 1995; Grant & Chamley, 2010; Navara, 2013). For example, female field voles (*Microtus agrestis*) with higher levels of testosterone and glucose (Helle et al., 2008) and female grey mouse lemurs (*Microcebus murinus*) with lower levels of estrogen (Perret, 2005) produced male-biased litters. In highly dense harems, such as in elephant seals, more centrally located females are more protected from harassment by peripheral males and are more likely to mate with harem holders (Le Boeuf, 1972, 1974). Females reproductive experience and dominance capacity seem to drive their spatial structure (Reiter et al., 1981; McMahon & Bradshaw, 2004). Testosterone levels may play here an important role as it is associated with social dominance (Rada et al., 1976; Williamson et al., 2017) and OSRA (Navara, 2013). Females with higher levels of testosterone may monopolize more central locations in harems and thus more likely to mate with harem holders. At the same time, higher testosterone levels promote the production of sons, for example, via a higher fertilisation probability of Y-CBS (Grant et al., 2008), which benefits centrally located females because their sons may inherit the capacity of their fathers to obtain a high lifetime reproductive success.

Stress may also be an important factor influencing OSRA by mothers (Navara, 2018). Females experiencing higher levels of stress tend to produce more daughters than sons (Geiringer, 1961; Lane & Hyde, 1973). For example, house mouse females (*Mus musculus domesticus*) exposed to higher density of males were more stressed (higher blood corticosterone levels) and produced female-biased offspring sex ratios (Firman, 2020). Stress alters the mother physiology and thus, similarly, may induce a sex chromosome-specific mortality or fertilization (Ideta et al., 2009; Navara, 2018). According to harem characteristics, elephant seal females experience different levels of male harassment (Galimberti et al., 2000b; Galimberti et al., 2000a) resulting in varying stress levels among females. Females that are more subject to male harassment (higher stress levels) are less likely to be fertilized by harem holders and thus benefit to produce more daughters than sons.

OSRA by males:

In mammals, males are the heterogametic sex producing X- and Y-CBS which determine the sex of the offspring. Hence, paternal OSRA represents a parsimonious explanation compared to maternal OSRA in response to male attributes (Edwards & Cameron, 2014; Douhard & Geffroy, 2021). Fathers may adjust offspring sex ratio by varying the proportion of the X- and Y-CBS, where a higher proportion of Y-CBS results in a higher probability of producing a son (Chandler et al., 2007; Saragusty et al., 2012; Edwards et al., 2016).

Variation in the proportion of X- and Y-CBS may be driven by mating frequency variation between harem holders and peripheral males. Harem holders that have a higher mating frequency compared to peripheral males may produce more Y-CBS because they are smaller—i.e., less costly to produce—than X-CBS which contains 4% more DNA (Seidel, 1999). For example, the proportion of X-CBS in humans (*Homo sapiens*) increased with sexual abstinence (Hilsenrath et al., 1997) and mating frequency influences offspring sex ratio in horses, rabbits, rats, and mice (James, 1996).

Variation in the proportion of X- and Y-CBS may also be driven by hormonal variation among males correlated to their breeding status and performance (James, 2008). Social dominance increases with testosterone through more aggressive behaviors (Rada et al., 1976; Williamson et al., 2017). For example, castrated rats showed a decrease in aggressiveness with a loss of social dominance (Albert et al., 1986). Testosterone level is also higher in harem holders in various species of deer (Chunwang et al., 2004) and horse (McDonnell & Murray, 1995). Testosterone levels in blood and semen is correlated to Y-CBS in bulls (Kholghi et al., 2020). Therefore, testosterone levels may link social dominance (reproductive success) with OSRA. For example, in spotless starling females (*Sturnus unicolor*), ibex females (*Capra nubiana*), and grey mouse lemur males, high levels of testosterone were associated with higher social ranks and son biased OSR (Veiga et al., 2004; Shargal et al., 2008; Perret, 2018).

OSRA by fathers may also emerge from the differential competitiveness between X- and Y-CBS (Douhard & Geffroy, 2021). The velocity of sperm and the percentage of normal spermatozoa are the main determinants of male fertility (Malo, Garde, et al., 2005). Differential mobility between X- and Y-CBS among males may thus result in variation in OSR. In red deer, more fertile males sired more sons compared to less fertile males (Gomendio et al., 2006), and fertility is associated to the relative antler size which is an important feature in the competitive ability of males (Malo, Roldan, et al., 2005).

Fathers may also influence the sex of their offspring through the composition of the seminal fluid (Douhard & Geffroy, 2021). The seminal fluid, along with its role in transporting sperm cells, acts on tissues in the female reproductive tract and thus influences implantation success and embryo development (Bromfield, 2014). Males that mate more often such as harem holders may have higher concentrations of glucose in their seminal fluid (Edwards & Cameron, 2017). Since high levels of glucose around conception favor the development and the implantation of male blastocysts (Cameron, 2004; Cameron et al., 2008), this may result in a positive relationship between male reproductive success and son biased OSR.

Conclusion

Our study shows that OSR increased with male relative reproductive success in southern elephant seals. This finding brings an additional support to the adaptive adjustment of OSR by parents. However, we could not identify the underlying mechanisms driving the relationship between OSRA and male reproductive success.

OSRA studies have produced inconsistent and contradictory results in many vertebrate species (Clutton-Brock & Iason, 1986; Cockburn et al., 2002; Silk et al., 2005; Douhard, 2017). This can be explained by the complexity of the factors influencing OSRA (Packer et al., 2000; Brown, 2001; Komdeur & Pen, 2002). For example, two interacting processes such as parental quality (the Trivers-Willard hypothesis) and local density (the local resource competition hypothesis) (Schaik & Hrdy, 1991) may result in an unbiased or biased sex ratio towards either males or females (Wild & West, 2007). This was empirically demonstrated in red deer, where the offspring son-biased production by dominant females declined with population density and winter rainfall, both environmental factors associated with pre-parturition nutritional stress (Kruuk et al., 1999). We, therefore, advocate that future studies should be specifically designed to tease apart the different ecological and evolutionary processes responsible for OSRA, providing important tools for wildlife management and conservation (Clout et al., 2002; Vetter & Arnold, 2018).

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Ethics approval

This study obtained the approval of the CIPA (Comité institutionnel de protection des animaux; protocol #934) at UQAM (Université de Montréal à Québec). This study was also positively evaluated by the ComEth-APAFIS committee (project 19-040 #21375: Adaptations physiologiques et énergétiques des éléphants de mer aux contraintes environnementales au cours de leur cycle de vie) and the Committee for the Polar Environment.

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Conflict of interest disclosure

The authors declare that they have no competing of interest concerning the content of the manuscript.

Availability of data and materials

Data and supplementary materials are available on Zenodo: https://doi.org/10.5281/zenodo.7418941

Authors' contributions

HA and DR conceived the ideas. HA, DR, and CG designed the methodology. HA and CG collected the data. HA processed the data. CR, CB, and OL conducted the genetic analysis. HA conducted the statistical analysis. HA, CR, CB, and OL wrote the first draft of the manuscript. HA, DR, and SP contributed to the discussion of the results. All authors contributed to the revisions of the manuscript.

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