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Reproductive timing as an explanation for skewed parentage assignment ratio in a bisexually philopatric population

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Abstract

In mammals, reproductive success can often be directly observed for females, but not males. Early-life correlates of female reproductive success can also be easier to observe due to higher rates of philopatry. Though relatively uncommon, populations in which both sexes remain in their natal home ranges can facilitate studies of mate choice and sex-specific drivers of reproductive success. Genetic parentage assessment in these systems should be more complete due to spatial philopatry since the pool of potential mothers and fathers should be equally accessible for sampling. Nevertheless, many studies still report more maternities than paternities even when individuals are randomly sampled with respect to age and sex. This discrepancy is often attributed to unobserved outbreeding. Here, we investigate two potential drivers for biased genetic parentage assignment in a bisexually philopatric community of bottlenose dolphins in which twice as many maternities as paternities are assigned to randomly sampled adults. We examine whether this pattern can best be explained by (1) sex differences in reproductive timing or (2) high levels of extra-community mating. We use long-term data on female calving success to search for biases in our genetic data collection and to constrain simulations of male reproductive timing patterns that could generate our observed data. We find that the majority of the skew in parentage assignment could be explained by differences in reproductive timing, with a smaller putative role of extra-community mating. We discuss how explicitly considering age effects as well as outbreeding can improve our understanding of sex-specific drivers of reproductive success.

Significance statement

In most mammals, mothers are easy to identify because they provide extended parental care to their offspring, but fathers can be absent in space or time. In a resident population of Indo-Pacific bottlenose dolphins, twice as many mothers as fathers are detected with random genetic sampling. We tested whether we failed to detect paternities because fathers were outside of our main study area or if they were simply older than mothers and likely died before they could be genetically sampled. We found evidence that fathers could be much older on average than mothers. We show that comparing maternities to paternities can reveal potential sources of bias when estimating reproductive success from genetic samples, and our results can be used to target more efficient sampling in future studies.

Keywords Life history · Parentage assignment · Reproductive timing · Reproductive success

Introduction

Bisexual philopatry is rare in animal populations, but provides an opportunity to study sex-specific reproductive trade-offs across the entire lifespan of both sexes. Such studies have, for instance, offered insights about the selective forces that shape life history parameters such as age specific mortality (Lemaître et al. 2015), reproductive senescence (Karniski et al. 2018), and menopause (Ellis et al. 2018). Understanding lifespan reproductive strategies for both sexes requires fitness data for both females and males, traits

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that are particularly challenging to acquire in long-lived mammals. Most mammalian studies of reproductive success have focused on females, as female fitness can be more readily determined than male fitness by inferring parentage through observations of maternal care. Since paternal care is rare in mammals, determination of male fitness usually requires genetic data from offspring and potential fathers, as behavioral observations of mating access can be poor correlates of reproductive success (e.g., Soltis et al. 1997; Coltman et al. 1999; Worthington Wilmer et al. 2000; Preston et al. 2001). Determination of male reproductive success is a significant challenge, even in long-term studies, given the ephemeral presence of males spatially and temporally (Blake 2017; this issue). Factors impacting reproductive success for both sexes in a population are rarely understood, or, because male-biased dispersal is the predominant pattern for mammals, can be biased according to the window in which immigrant males are observed (Clutton-Brock and Lukas 2012). Therefore, studies on bisexually philopatric populations that include genetic data on parentage can provide valuable insights into sex-specific reproductive strategies throughout the lifespan.

Even in bisexually philopatric populations, paternities can be systematically undersampled. For instance, a bias in parentage assignment ratios, i.e., the relative number of maternities vs. paternities has been observed in orangutans, which are socially philopatric, but typically breed outside of their natal group (Goossens et al. 2006). In several toothed whale species, males breed outside of their natal pod during brief encounters (killer whale, Johnstone and Cant 2010; long-finned pilot whale, Amos et al. 1993; short-finned pilot whale, Alves et al. 2013). In these species, males benefit from social philopatry (e.g., food-sharing, survival in killer whales, Wright et al. 2016) without demonstrated inbreeding costs (Pilot et al. 2010; Ford et al. 2011; but see Ford et al. 2018). In pinnipeds, individuals may range widely but show philopatry to breeding sites on land or ice where most matings are thought to occur, but recent genetic studies have indicated cases in which substantial numbers of offspring cannot be assigned to the males sampled at these terrestrial breeding sites, suggesting females may also be mating at sea (Nichols et al. 2022). In most of these cases, missing paternity assignments are attributed to females mating with males from outside the study area in question.

In Shark Bay, Australia, male and female bottlenose dolphins are genotyped at similar rates, but genetic paternities are assigned at a significantly lower rate than maternities (Krützen et al. 2004). Several sources of evidence (Krützen et al. 2004; Manlik et al. 2016; Wallen et al. 2016, 2017) suggest that females are unlikely

to breed with outside males at the rates suggested by the low rate of genetic paternity assignment. An alternative explanation may be that male reproductive success is biased toward older males, as has been found in other dolphin species (Green et al. 2011). Additionally, reproductive success toward the end of the lifespan is difficult to capture, due to the minimum 3-year delay between conception and when the offspring reaches an age old enough for biopsy sampling. Therefore, in this study, we investigate an alternative explanation for the missing paternities, and specifically attempt to discern whether putative fathers are primarily undersampled on a temporal or spatial scale.

Indo-Pacific bottlenose dolphins have been studied in Shark Bay since 1984. Neither sex emigrates, so individuals can be observed from birth to death (Tsai and Mann 2013). Ages are known for most individuals born since the 1980s and because *T. aduncus* speckle with age, size, and speckling rate can be used to estimate ages for individuals whose birth years are not known (Krzyszczuk and Mann 2012). Average age at first birth for females is 13 years (Mann 2019), and females successfully wean a calf on average once every 8 years (Mann et al. 2008). Female reproductive senescence is evident, with a linear decline in calf survival and longer inter-birth intervals as females age (Karniski et al. 2018). Over 945 calves born to ~380 mothers have been documented by observation (close association and swimming position), and no maternities identified by observation have conflicted with genetic analyses (Foroughirad et al. 2019). Adult males form stable first-order alliances of 2–3 individuals that cooperate with each other and with other alliances to sequester individual females and prevent them from mating with other males (Connor and Krützen 2015). Alliance formation among age cohorts takes many years, stabilizing in the mid- to late-teens (Galezo et al. 2020; Gerber et al. 2020). Males within alliances are not more closely related than expected by range overlap due to natal philopatry (Krützen et al. 2003; Gerber et al. 2021), but rather exhibit preferences for partners close in age (Gerber et al. 2021). Mating behavior is not a good indicator of paternity as adult male–female copulations are rarely observed (Mann 2006).

Here, we compare observed reproductive histories of females against the data obtained from genetic parentage assignment to investigate the potential biases in data sampling. Female calving data are then used to anchor simulations of possible male reproductive patterns that could explain our observed skew. Specifically, we test two major non-exclusive hypotheses to explain why fewer paternities are identified than maternities from genetic data: (H1) A significant proportion of breeding males comes from outside of our study site; and (H2)

reproductive success occurs much closer to the end of the lifespan for males than females, and subsequently the temporal window in which a male and his offspring are both alive and able to be sampled is much smaller. If H1 is supported, we would expect females generally to breed with males that have minimal home range overlap, and possibly to detect genetic differences between the offspring of local males in our data set to those not assigned a father. If H2 is supported, then most paternities would involve older males, and missing paternities could be explained by a model in which offspring of older fathers were disproportionately sampled after their father's death. While few studies can completely census paternity data due to logistical constraints, we highlight that investigating multiple potential drivers of skewed parentage assignment ratios can be essential to calibrate calculations of reproductive timing and success, and can help improve genetic sampling regimes.

Methods

Demographic data

Demographic data on bottlenose dolphins were collected from an area spanning about 600 km² along the eastern coast of the Peron peninsula of Shark Bay, Western Australia between 1984 and 2019. Both sexes exhibit natal philopatry (Tsai and Mann 2013), allowing birth years to be known for many of the individuals in our sample (48%) through observation of calf characteristics (size, fetal lines, and swimming position, see Mann and Smuts 1999; Mann et al. 2000). For individuals born before the 1980s or first observed after weaning, birth years were estimated based on size or ventral speckling, which begins around sexual maturity (age 10) and increases throughout life (Krzyszczuk and Mann 2012), with individuals in their 20 s and 30 s developing speckling on their dorsal fins. Sex was determined through views of the genital area, the presence of a dependent calf for females, or from detection of sex-linked genetic markers obtained from tissue samples (Gilson et al. 1998; Foroughirad et al. 2019). It was not possible to record data blind (e.g., in order to reduce observer bias) as this study involved observation of animals in the field.

Mortality assignment can be complicated by variable rates of observation, as the study area and individual home ranges are large, and not all animals are photographically recaptured every year. For calves, death dates were assigned based on the midpoint between the last sighting of the calf and the first sighting of the mother without her calf as long separations are rare before weaning. For dolphins older than age 3 (minimum typical

weaning age), death dates were assigned based on the last sighting date of the individual and any gaps in the sighting history of that individual. Unless the dolphin was sighted almost daily or was seen with severe shark bite wounds or obvious illness, a death date was only assigned once the dolphin had gone undetected for at least 3 years. If the individual was not seen for 3 years, we assigned a default death date as 1-May of the following year of the last sighting as our survey effort generally stretches from May to December annually. For dolphins sighted on a less than annual basis, we scaled the waiting time to assign death dates according to the length of the maximum gap between previous sightings. For example, if a dolphin had a sighting gap of 2 years, death would not be assigned until 6 years after the last sighting date, at which point the death date would be set to 1-May 2 years after its last sighting. These rules are designed to be conservative in assigning death and have resulted in low error rates (< 1% false positive death assignment) over the course of the study.

Genetic sampling and parentage assignment

Between 2013 and 2019, skin samples were obtained during boat-based surveys throughout the study site using a remote biopsy system (Krützen et al. 2002). Only individuals who were determined to be at least 2 years of age were deemed eligible for biopsy sampling. Tissue samples were stored in either dimethyl sulfoxide or an RNA-stabilizing buffer and DNA was extracted via isopropanol precipitation and sequenced using restriction-associated digest methods at Diversity Arrays Technology in Canberra, Australia using their proprietary DArTseq™ technology (Jaccoud et al. 2001; Kilian et al. 2012) as described in Foroughirad et al. (2019).

This study includes genetic data from 403 animals over 2 years of age, 194 males and 209 females, which represent 59% of uniquely identified animals greater than 2 years of age encountered in the study site during the sampling period (95% of animals encountered during surveys were uniquely identified).

A quality-filtered panel of 4235 SNPs was used to calculate relatedness coefficients using the dyadic maximum likelihood estimator (Milligan 2003) as implemented in the program COANCESTRY (Wang 2011). For parentage assignments, SNPs were further filtered to only those which had a minor allele frequency greater than 5% and no more than 5% missingness across individuals. The resulting panel of 2748 SNPs was used to assign parentage using the R package *sequoia* v. 2.3.5 (Huisman 2017), with genotyping error rate set to 0.05, and other parameters set to default values. We accepted parent–offspring assignments based on the program's

default minimum log-likelihood ratio (0.5) of parent–offspring to the next most likely relationship. Pedigree-wide confidence intervals were estimated using the *EstConf* function in *sequoia* by simulating 1000 sets of genotypes based on our reconstructed pedigree and with a genotyping error rate set to 0.05, and counting mismatches between reconstructed and simulated data.

Once parentage was assigned, we ran a binomial regression on the probability of an individual being assigned a parent based on the sex of the parent, the birth year of the offspring, and the sighting rate of the offspring. The sighting rate was the number of observations per year that the sampled individual was surveyed during the sampling period, and offspring sighting rate was used as a proxy for maternal sighting rate as these numbers are tightly correlated under maternal home range inheritance (Tsai and Mann 2013; Strickland et al. 2021). We then tested for two possible interactions corresponding to our hypotheses. Under H1, if females were mating outside their home ranges, we might expect an interaction between sighting rate of the offspring and the probability of assigning a father, as sighting rate declines with distance to the center of our study site, and females near the edge of our study site may be more likely to be mating with outside males. Alternatively under H2, we might expect an interaction between birth year and the probability of assigning a father, as calves born more recently may be more likely to have the real father still alive to be included in the dataset.

Genetic maternity assignments were used to calculate a distribution of ages at conception for females and were compared to the same distribution calculated from observation of mother–calf pairs in the field using a Welch's *t*-test. Inclusion of calf births was limited to those calves that survived until 2 years of age, as that is the minimum age at which calves could be biopsied, and calves who reach 2 years of age typically survive until weaning (Mann et al. 2000). A gamma distribution was fitted to the observed data using maximum likelihood in the R package *fitdistrplus* (Delignette-Muller and Dutang 2015). We chose a gamma distribution as it is a relatively tractable two-parameter distribution that provided the best fit to the maternal age at conception data while reasonably constraining the parameter space for simulation of male age at conception distributions.

Mated pair distance

We investigated two potential lines of evidence, spatial and genetic, for females mating with males from outside our study area. First, we examined the spatial distance between a mother and the assigned father of her offspring compared to the set of possible distances between the mother and the set of potential fathers. We calculated geographic

centroids for all adults with more than 5 independent locations available by first calculating a utilization distribution as described in Strickland et al. (2017) using the package *adehabitatHR* (Calenge 2006). We then calculated the 90% kernel contour and the centroid position using *rgeos* (Bivand and Rundel 2021). We calculated euclidean distances between centroids of all mothers and the genetically assigned fathers of their offspring, as well as from each mother and the set of adult males sampled who were alive at the time of conception of each offspring. We compared the distributions of true and potential distances using a permutation-based implementation of a Wilcoxon signed-rank test in the package *coin* (Hothorn et al. 2008).

Second, we investigated whether there was a detectable genetic difference between offspring who were and were not assigned a father from our sample, under the hypothesis that individuals who were not assigned a father disproportionately had fathers who came from the outside of our main study area. We first filtered for a set of unrelated mothers by using genetic relatedness coefficients to remove any putative first and second degree relatives ($r \geq 0.25$). We then compared their offspring in our sample that were assigned local fathers ($n = 22$) to those who were not assigned a father from our sample ($n = 29$). We used the *adonis* routine from the package *vegan* (v. 2.5) to run a permutation-based AMOVA to detect genetic differences between these two groups and visualized the results using a PCA plot of the SNP data.

Age simulations

In order to investigate what distributions of paternal ages at conception could generate our observed data, we conducted a series of simple simulations. We designated all individuals in our sample that were conceived between 1984 and 2017 (assuming 53 weeks gestation; Wallen et al. 2017; Zhang et al. 2021) as potential offspring ($n = 338$), and reassigned them a pair of pseudo-parents selected from the members of the population who were alive and at least 10 years old at the time of conception ($n_f = 531$, $n_m = 490$). The pseudo-parents were drawn from the pool of potential parents based on their age at the time of conception with a probability equal to the expected frequency from a gamma distribution. For pseudo-mothers, this was set to the distribution fit to the observed maternity data. For pseudo-fathers, we varied the parameters of the gamma distribution ($\alpha = (1, 30)$, $\beta = (0.1, 1.2)$) to simulate different probabilities of ages of conceptions. Parameter space was constrained to sets with cumulative distributions of > 0.95 between ages 9 and 55. We then mimicked our biopsy sampling procedure on each iteration of sampled pseudo-parents

to generate a reduced set of pseudo-parents, the pseudo-sampled parents, and compared the attributes of this pseudo-sampled set, averaged over 20 iterations of each parameter combination, to the attributes of our observed data. Specifically, we compared the ratio of fathers to mothers in the pseudo-sampled set to our observed assignment ratio, as well as the mean age at conception for fathers in the pseudo-sampled set and our observed genetic assignment data. We then selected the distributions that best matched our observed data for further discussion. All analyses were conducted in R v 4.1.2 (R Core Team 2021) unless stated otherwise.

Results

Maternity and paternity assignment

Of the 403 individuals from which we successfully obtained genetic data, we assigned 141 maternities and 70 paternities. The lowest log-likelihood ratio for an assigned paternity was 7.95, and pedigree-wide confidence estimates were $> 99\%$. Among individuals which had reached sexual maturity by the end of the sampling period ($n_f = 173$, $n_m = 162$; Fig. 1), females were sampled at a slightly higher rate, 1.07:1, though not a rate high enough to explain the discrepancy in parentage assignment, 2.01:1. The adult sex ratio in this population has been reported as 1:1 (Manlik et al. 2016), though this value varies depending on the age of adulthood onset used, as high juvenile male mortality means early onset definitions are slightly skewed toward females, as demonstrated by the 1.07:1 ratio reported for this study when looking at all individuals above age 10, compared to the 1:1 ratio reported when age 15 was used for the onset of male adulthood in Manlik et al. (2016).

No genetically assigned mother conflicted with our observed mother-calf relationships. Three mother-calf pairs were not assigned despite both being in the sample

pool (2.1%), though we note that in these cases at least one member of each pair had missing allele call rates (5–6%) near our exclusion threshold of 10%. The three pairs all had relatedness coefficients in the expected range for mother-calf pairs ($r = 0.42$ – 0.47). All pairs in which both individuals had $< 5\%$ missing data were successfully assigned, and there was no difference in rates of missingness between males and females, indicating that genetic assignment failure likely does not play a role in imbalanced parental assignments.

The oldest offspring who was assigned a parent was born in 1984 and was 30 years old at the time of sampling, and the mother was 42 years old at the time of her sampling. In total, 361 individuals in our sample were born in 1984 or later, and of those 41% were assigned a mother, and 20% were assigned a father. Eighty seven individuals were assigned a mother but not a father, and 16 individuals were assigned only a father. In those 16 cases, 8 offspring were known to have mothers that died before the sampling period started, 3 died during the sampling period but before a sample could be obtained, 4 are still known to be alive but have yet to be sampled, and one had no known mother.

Mated pair distance

There were a total of 54 offspring who had both parents genetically assigned from the sample. These parent pairs had an observed distance between centroids of 3.6 ± 3.4 km, which was significantly lower than the distance between all mothers and candidate fathers in the dataset (10.3 km, $Z = -7.8773$, $p \leq 0.001$; Fig. 2), indicating that females are more likely to mate with nearby males. The permutation-based AMOVA on calves who were and were not assigned a father in the sample showed no significant difference between groups ($R^2 = 0.0215$, $p = 0.129$; Fig. 3), suggesting that the missing fathers most likely derived from the same population as the assigned fathers.

Fig. 1 Frequency distributions of age ranges for all adult females and males ($n_f = 173$, $n_m = 162$) from which genetic data were obtained (left) and total observed individuals ($n_f = 273$, $n_m = 254$) during the sampling period from 2013 to 2019. The 1.07:1 female:male ratio in the sampled dataset is representative of the 1.07:1 ratio observed in the population, but substantially lower than the 2.01:1 obtained parentage assignment ratio

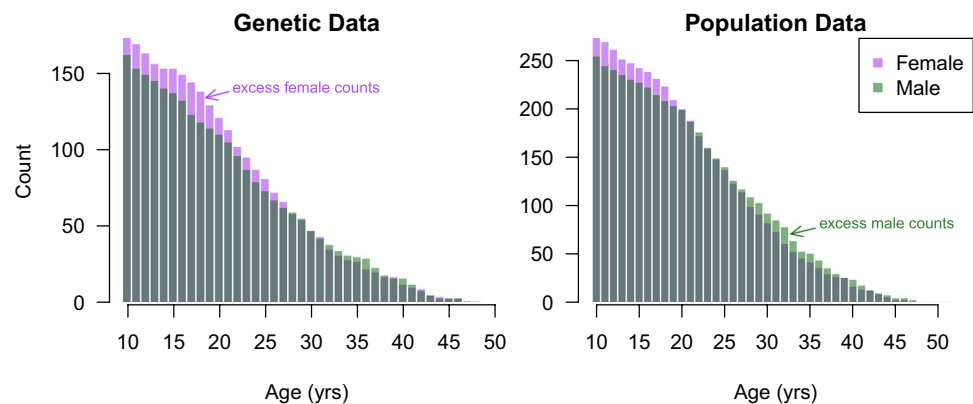


Fig. 2 Distribution of possible distances between the centroids of an offspring's known mother and all candidate sires that were genotyped and alive at conception. The red solid line indicates the observed mean distance between all mated pairs ($n = 54$) and dotted lines enclose the 95% interval of distances between all observed mated pairs. There was a significant difference between the mean observed distance (3.6 km) between the centroids of successfully reproducing pairs and the mean distance between candidate pairs (10.3 km), indicating that within our sample females were more likely to mate with local males

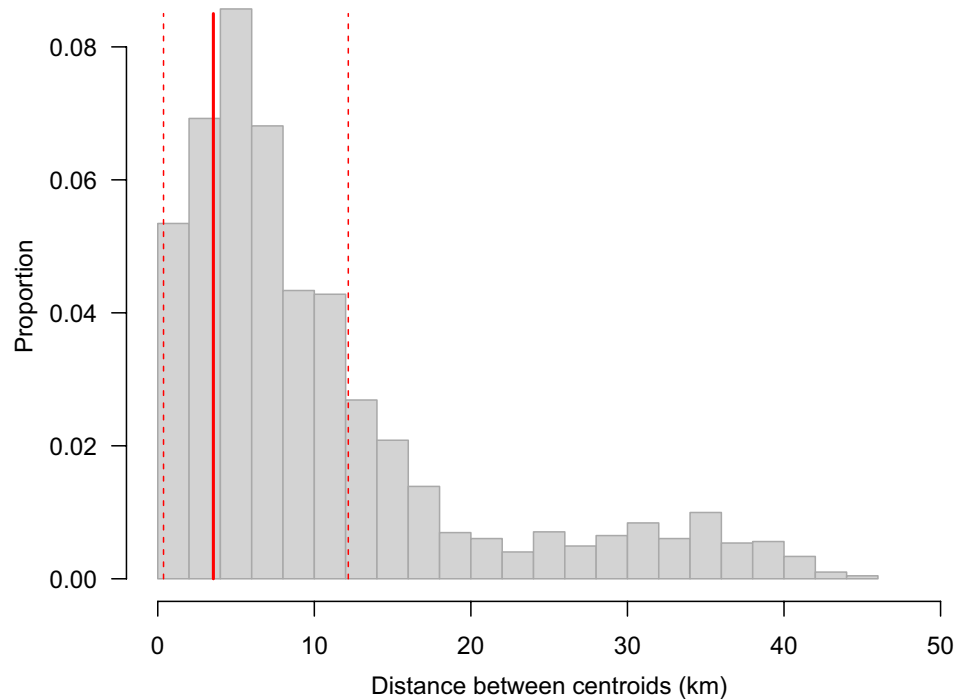
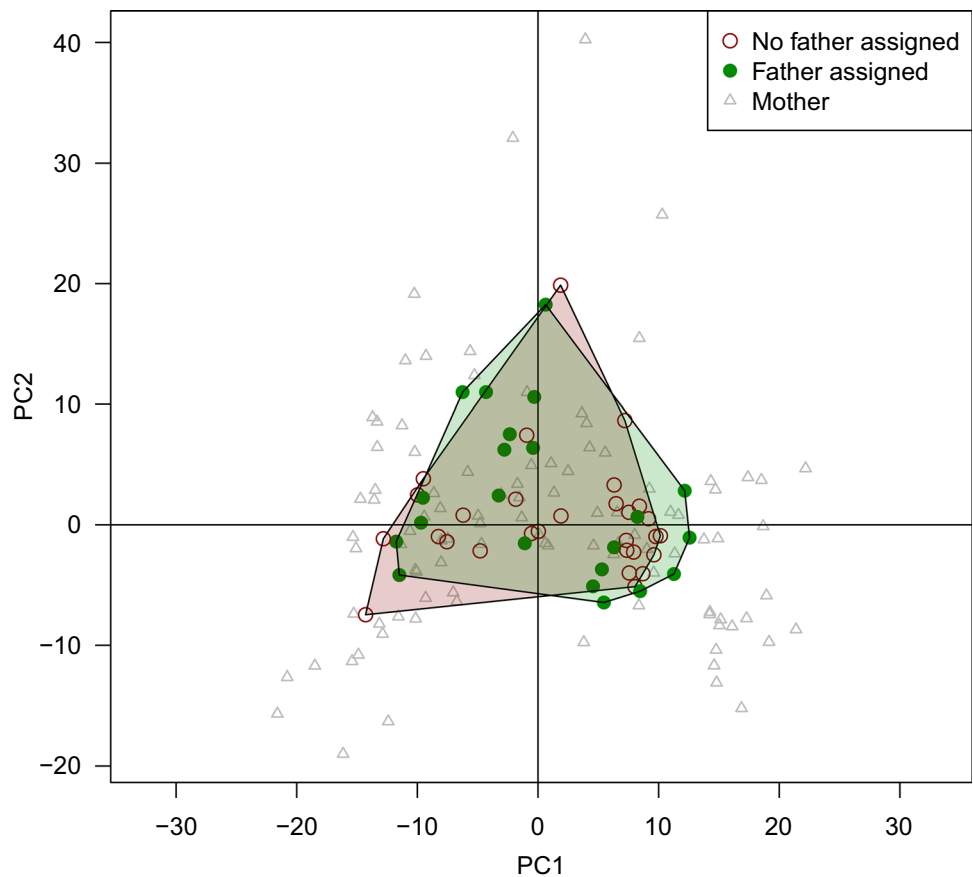


Fig. 3 Genetic PCA on all potential mothers (females born before 2004) after removing close relatives ($n = 99$). Offspring were then added to the PC space, with those assigned local fathers in green ($n = 22$), and those without in red ($n = 29$). A permutation-based AMOVA detected no significant differences between the offspring groups



Age at conception

The youngest successful paternities achieved were at age 11 at conception for two males, about 3 years older than the youngest age documented for females (8 years, Mann 2019) although only a year older than earliest age, 10 years, documented for females within this study. However, both males had birth years that could have been underestimated because they were first observed as adults. The youngest age at conception for a male whose birth year was known to within a year was 16 years.

Probability of parental assignment was positively correlated with both the sighting rate of the offspring and the birth year of the offspring (Table 1; Fig. 4). While fathers were significantly less likely to be assigned relative to mothers, there was no significant interaction between paternal assignment and either sighting rate or birth year, failing to provide support for either the age or location-based bias in parental assignment. We note that the paternity assignment rate was much higher for offspring born during the sampling period (80% for offspring born after 2013), which could indicate support for undersampling driven by paternal age, but this cohort was small ($n=10$) due to the minimum age requirements for sampling and a decrease in births during the study period due to the effects of a heatwave (Mann et al. 2021).

We compared the distribution of age at conception for mothers assigned to offspring genetically to that of all observed births of calves that survived to age 2 from our demographic data ($n=510$), and found no significant differences ($t = -0.835$, $df=448.4$, $p=0.404$, $\bar{x} \pm SD_{\text{sampled}} = 18.5 \pm 6.5$, $\bar{x} \pm SD_{\text{observed}} = 18.1 \pm 6.2$). We fit a gamma distribution to these data with shape = 8.96 ± 0.55 and rate = 0.50 ± 0.03 to use in simulations of male age at conception (Fig. 5).

Male age at conception simulations were able to generate parentage assignment ratios of up to 1.64:1 (F:M; range 1.08:1–1.64:1) when sampled male age at conception was

constrained to within ± 3 years of the mean age observed in our dataset (Fig. 6). The 1.64:1 ratio was produced by a male age at conception distribution ($\alpha = 22.4$, $\beta = 0.71$) in which male probability of reproductive success was maximized between ages 30 and 31 (Fig. 7). This corresponded to a mean age of conception in the simulated dataset of 26.2 years, as even though peak probability of reproductive success may occur later, more younger males are available in the population.

A parentage assignment ratio of 1.64:1 would be equivalent to assigning 86 fathers to our 141 mothers. As we actually assigned only 70, this means that there is a minimum remaining 11% (16/141) of missing fathers that cannot be reasonably explained by age effects under our current simulation framework. However, a 1.64:1 expected assignment ratio would explain 55 out of the 71 missing paternities (77%), supporting the role of age-based mortality in driving a large proportion of the discrepancy in parental assignment rates.

Discussion

Our results demonstrate the value of assessing drivers of skewed parentage assignment ratio in a bisexually philopatric system. By leveraging our extensive observational and genetic dataset on the reproductive histories of female and male bottlenose dolphins in Shark Bay, we found that differences in the age at conception between natally philopatric males and females, rather than outbreeding, could explain the majority of the difference in parentage assignment rate. Female reproductive timing as measured from the genetic data matched that obtained through field observations, indicating that the degree of skew might result from a mismatch between male reproductive timing and our sampling protocol. Together, our results reveal the potential for sex specific trade offs between reproduction and somatic effort in relation to social dynamics that may be underestimated by relying on genetic data alone.

Several challenges in sampling potential fathers, such as outgroup matings, dispersal, and paternal death before offspring can be sampled, can make capturing fathers difficult and result in skewed parentage assignment ratios. We show that neither non-random sampling nor geographic bias is likely to explain the skew in parentage assignment in our system. First, while we sampled only 7% more adult females than adult males, we assigned more than twice as many maternities as paternities, similar to rates reported in a previous study in the same population (Krützen et al. 2004). Second, we have shown that genetic compositions between offspring of assigned and unassigned fathers overlap (Fig. 3), which fails to provide support for a significant number of unassigned fathers coming from a distinct population

Table 1 Logistic regression for the probability of parentage assignment based on sex of the parent, birth year, and sighting rate of the offspring. Parent sex, birth year, and sighting rate were all significant (p -values indicated in bold), but no significant interactions were detected

Predictors	Parentage assignment			
	Odds ratio	Std. error	z-value	p
(Intercept)	0.000	0.000	−11.285	
Birthyear	1.179	0.017	11.267	<0.001
Sighting rate	1.064	0.015	4.380	<0.001
Parent sex [Male]	0.211	0.047	−6.943	<0.001
R ²	0.360			

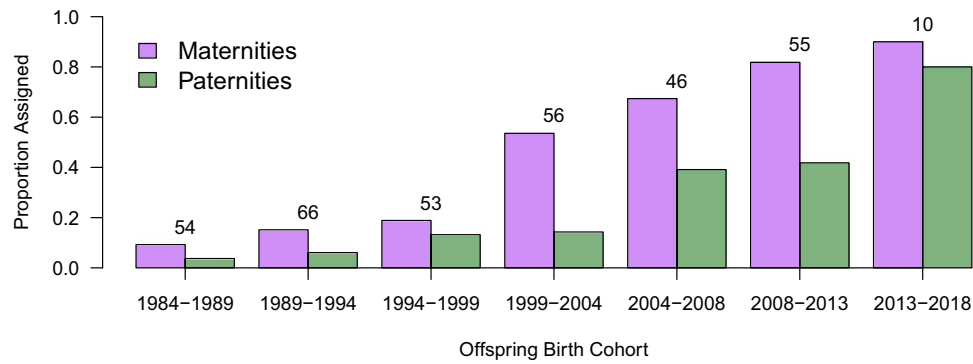


Fig. 4 Proportion of offspring that were assigned a genetic mother or father based on birth year. The numbers above the bars indicate the total number of genetic samples obtained from each cohort. The number of offspring sampled in the 2013–2018 birth cohort is low in

part because of a decline in calf survival related to a marine heatwave and seagrass dieoff (Mann et al. 2021), but also because calves are not biopsied until at least 2 years of age

segment. However, we should note that the sensitivity of our analysis to detect genetic differences at this scale is likely quite low, especially if the exchange of males is happening evenly throughout the study site rather than concentrated near a specific border.

We instead demonstrated that the majority of our unexplained paternities could be due to reproductive success being concentrated nearer to the end of the

lifespan in males than in females. We found compatible distributions of paternal ages at conception that would explain up to 77% of the discrepancy between maternity and paternity assignment rates within a closed population. This pattern of reproductive timing would reduce the probability that father-offspring pairs were simultaneously alive during the sampling period relative to mother-offspring pairs. In a species without paternal

Fig. 5 Proportion of females conceiving a calf that survives to at least 2 years of age at each maternal age ($n=510$ calves), compared with the proportion of just the calves that were assigned a mother in the genetic parentage assignment procedure ($n=141$). Histogram represents all observed mother-calf pairs, with gaussian kernel density estimates (KDE) and a fitted gamma distribution overlaid

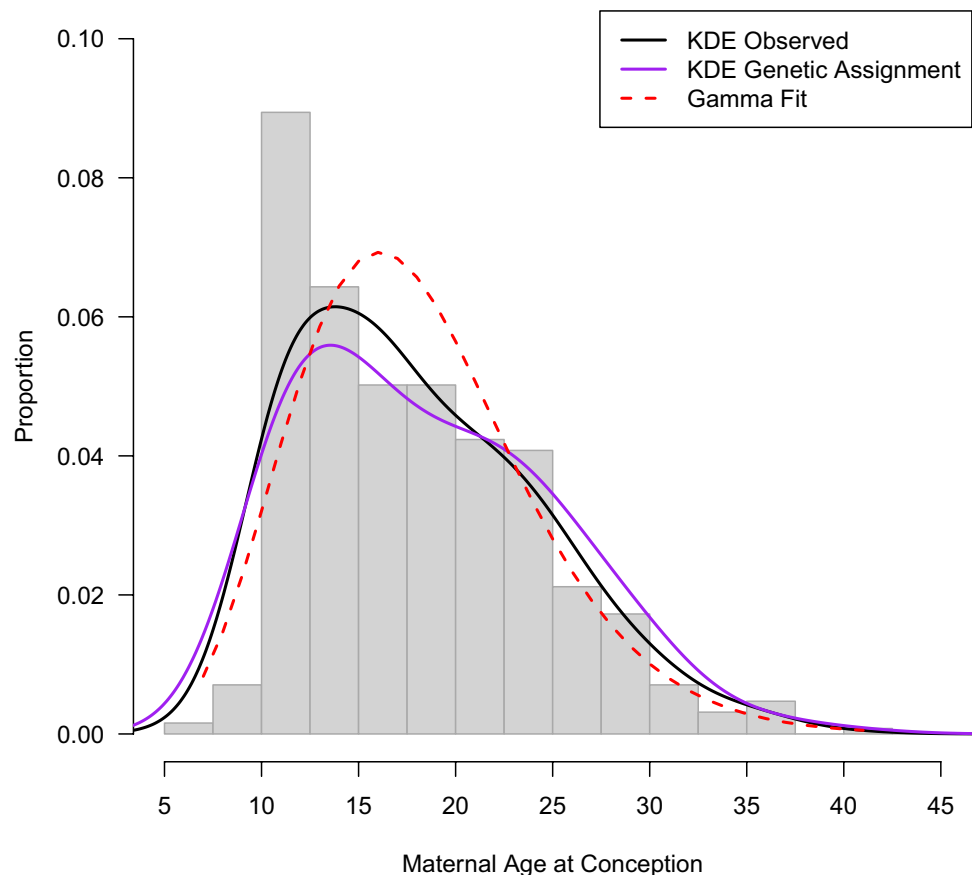
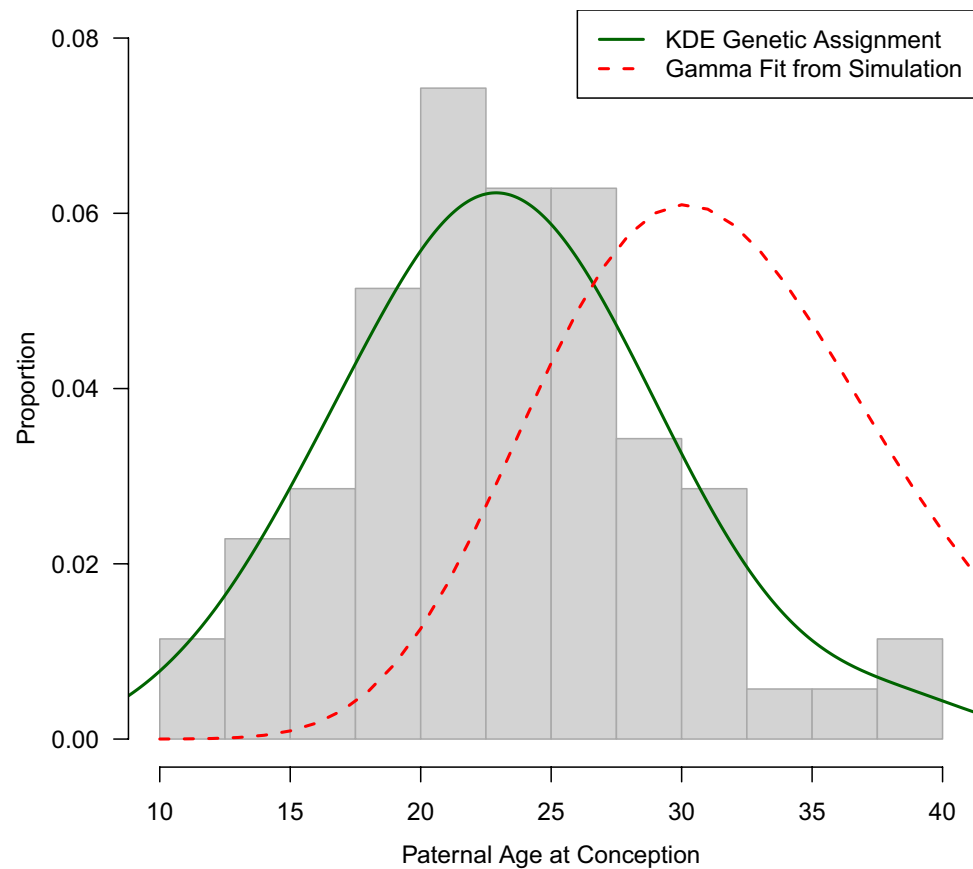


Fig. 6 Proportion of males siring a calf that survives to at least 2 years of age at each paternal age ($n=70$ calves) based on genetic assignment data. The gamma distribution derived from simulations that most closely matches the observed parentage assignment ratio is shown in red. Histogram represents all assigned father-calf pairs, with gaussian kernel density estimate and the gamma distribution fit by simulation overlaid



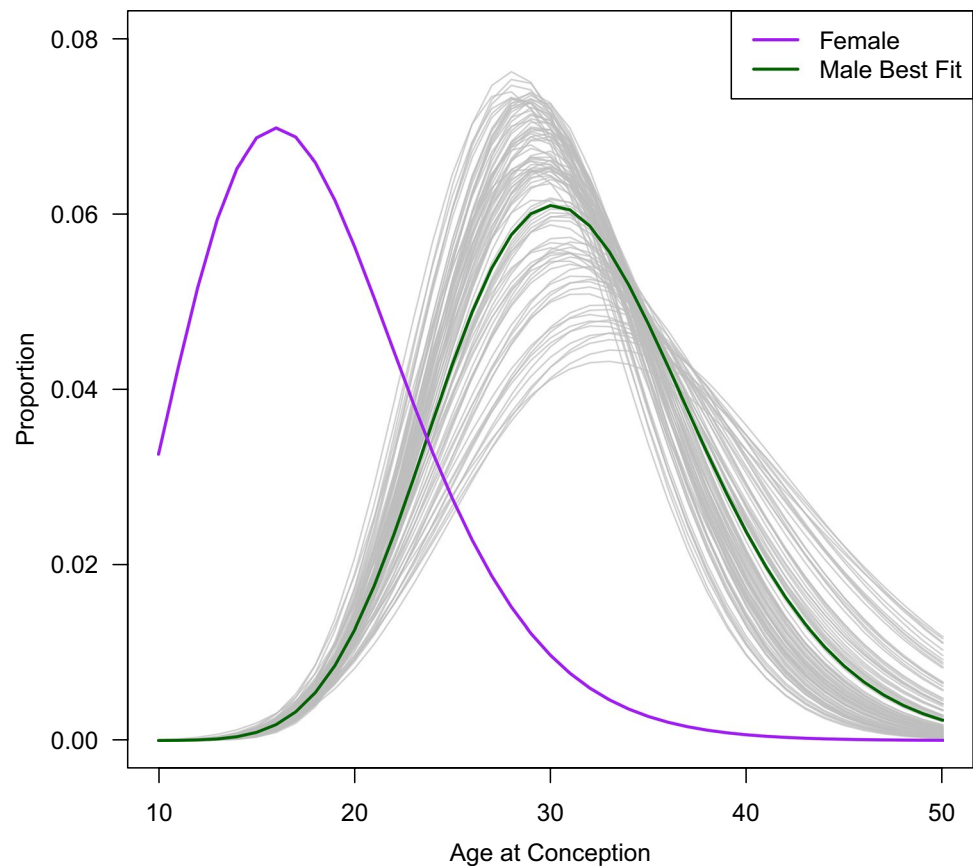
care such as bottlenose dolphins, by definition, mothers of a surviving calf must themselves survive to the calf's minimum weaning age, at least 3–4 years past the date of conception. Males, on the other hand, do not need to survive past the date of conception as they provide no parental care.

Methodologically, our results emphasize the limitations of randomized genetic sampling over short time frames relative to the lifespan of the subjects. This can result in skewed parentage assignments due to sampling designs rather than biological reasons, obscuring factors relevant to male reproductive success. Lower than expected paternity assignment rates have been described for several populations of marine mammals expected to show breeding site fidelity (Kita et al. 2013; Nichols et al. 2022). In these studies, outside gene flow was the most commonly offered explanation, and here, we highlight that the role reproductive timing can play in reproducing these patterns even inside a closed population. Male reproductive success often occurs at later ages than for females, and this has been observed in several species of toothed whales as well (Ford et al. 2011; Green et al. 2011). Failure to account for drivers of imbalanced parentage assignment ratios can lead to biased estimates

of reproductive timing and reproductive skew, impacting biological interpretations.

Biologically, our results suggest that peak male reproductive performance could occur as late as the age of 34, almost 10 years after the peak indicated in the raw sampled data. This result highlights the extensive investment males must make in their social bonds with alliance partners before they are able to compete for access to reproduction (Gerber et al. 2022). These putative age differences in conception between females and males may indicate divergent sex specific reproductive strategies. Alternatively, costs of reproduction may add an additional effect such that some males who invest heavily in reproduction at the expense of survival produce offspring close to the end of their lifespans regardless of their age (Lloyd et al. 2020; Ritchot et al. 2021). This may be unlikely given the alliance structure though, where individuals must coordinate strategies. However, evidence for some reproductive skew among males has been shown in this population (Krützen et al. 2004). If disproportionately successful males are more likely to be undersampled for reasons other than age or location, for instance, if reproductive success is correlated with behavioral attributes like evasiveness, that could be an

Fig. 7 Estimated proportion of all surviving calves attributed to each age at conception for male and female dolphins in Shark Bay. The distribution of female ages is generated from a gamma distribution fit to the observed calving data. The male distributions are generated from gamma distributions selected through simulation that best fit the observed ratio of assigned maternities to paternities and the mean age of conception generated from the sampled genetic data. The green line indicates the distribution that most closely reproduced the observed male parentage assignment ratios, top 95 are shown



alternative mechanism that would explain low paternity assignment rates. Additionally, the distribution of paternal age at conception may not be well-approximated by the simple gamma distributions explored in our analysis.

Our results also indicate that outgroup mating is unlikely to explain most of the lack of paternity assignment. We do find space for some level of outbreeding, as at least 11% of missing fathers were unattributable to simulated age effects and therefore possibly the result of females breeding with males outside our main study area. Previous studies have estimated annual migration rates between this field site and the community in the western gulf of Shark Bay to be $Nm/year \approx 0.5$ (Manlik et al. 2016), which could explain these remaining births. Nevertheless, the mated pairs in our study had home range centroids that were only 3.6 km apart on average, showing that females disproportionately mated with nearby males within our study site. This distance corresponds closely with the observed 3–4 km shifts reported for females during possible consortships (Wallen et al. 2016). However, Wallen et al. (2016) also noted that cycling females shifted from baseline centroid positions even when not in active consortships, suggesting that females may move away from the centers of their home ranges when cycling as a method of inbreeding avoidance (Frère et al. 2010). Alternatively, cycling females might be sighted further from their core home range because they were recently with males. The

majority of our biopsy sampling occurred outside of the breeding season, when we may have been less likely to encounter animals who shift over the boundaries of our study site.

In systems where maternity can be determined by observation, few studies report both the genetic maternities as well as paternities, and even fewer studies examine the causes of imbalanced assignments. Our results indicate that uneven parentage assignment can be driven by sex differences in reproductive timing and highlight the importance of investigating discrepancies in assignment rate before coming to conclusions about sex-specific patterns of reproductive success. When missing paternities are attributed to outbreeding, there can be an unstated assumption that the missing fathers share similar attributes to the captured males in terms of demographic parameters, but if fathers are instead missing due to age-related mortality, this assumption is violated. Continuous long-term studies or studies that prioritize genetic sampling among the oldest cohorts (when ages are known) can help elucidate these effects and calibrate sex-specific predictors of fitness.

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Author contribution VF, MM, and JM conceived the study. VF, AL, and AK collected samples for genetic analysis and AL and VF conducted lab work. JM, VF, and MM collected behavioral and demographic data. JM, CF, and AL provided funding. VF, JM, MM, and CF wrote the first draft. All authors edited and approved the final version.

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Data availability Code and data to reproduce all analyses are available at <https://doi.org/10.5281/zenodo.6970002>.

Declarations

Ethics approval All applicable international, national, and institutional guidelines for the use of animals were followed. Ethics approval was obtained by the University of the Sunshine Coast (AN/S/15/35) and Georgetown University IACUC (13–069, 07–041, 10–023, 2016–1235). Research was conducted under the Western Australian Department of Biodiversity, Conservation and Attractions permit (SF-009876, SF- 010347, SF-008076, SF009311, SF007457).

Competing interests The authors declare no competing interests.

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