Mother's curse on conservation : assessing the role of mtDNA in sex-specific survival differences in ex-situ breeding programs

Title page

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Short title

MtDNA-induced effects in ex-situ breeding

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Abstract

For captive breeding to be an effective conservation tool, population fitness needs to be guaranteed according to the latest insights. Preserving genetic diversity has been a major pilar in conservation breeding, as it is tightly linked to long term-population viability. Small differences on the DNA level can impact an individual's overall fitness and when situated on the mitochondrial genome might more specifically affect the process of aging and fertility. In general, mitochondrial DNA is exclusively transmitted via the mother. In the process of natural selection variants beneficial for female fitness will be selected for whereas male-harming variants will never be selected against. This biased selective sieve results in the accumulation of male-harming mutations in the mitochondrial DNA, a process labelled "mother's curse". Regardless of the susceptibility of zoo populations to "mother's curse", mitochondrial induced fitness effects have remained unstudied within captive breeding programs. While current conservation breeding strategies focusing on retaining nuclear diversity, studies that specifically concentrate on aberrant extra-nuclear genetic processes might further improve breeding practices. Here we provide empirical evidence on the presence of mtDNA induced sex-specific differences starting from readily available population information. We analyzed whether an individual's mitochondrial background partly affects survival according to a sex-specific pattern using studbook data from captive populations representing 16 species. Our results indicate male survival is affected in five mitochondrial lineages on both the pre- and postnatal level. Additionally, we describe the overall beneficial effects some mitochondrial lineages have over others in terms of survival. These results are of interest in the debate on the maintenance of healthy captive populations and how to further improve safeguarding their genetic diversity for both ex-situ and in-situ conservation.

Keywords: mitochondria; maternal inheritance; survival; conservation breeding; captive populations

Introduction

Human society increasingly impacts the availability of resources for wildlife, resulting in a continuing local decline of animal populations and a global loss of biodiversity. One instrument within a conservationists'

63 toolbox to counter this loss of species is the creation of temporal back-up populations as e.g. captured within
64 zoological or botanical gardens. Robust strategies have been developed to manage conservation breeding
65 programs ensuring demographic stability and genetic health (Fernández & Caballero, 2001; Ivy & Lacy, 2012).
66 These approaches however do not automatically guarantee long-term viability of populations (Lacy, 2013),
67 indicating more research is needed to pinpoint overarching patterns that explain the observed variation in
68 sustainability (Powell, Dorsey, & Faust, 2019). Molecular data is increasingly integrated in breeding
69 management (Ivy & Lacy, 2010) and goes as far as studying gene fitness relationships (Norman, Putnam, & Ivy,
70 2018) . Following current trends in genetics research, the gradual shift from restricted marker studies to
71 genome wide screenings revived interest in how different parts of a genome affect the sustainability of
72 conservation efforts. One genomic region that received conceptional attention in the recent past because of its
73 potential direct role in population viability, but for which more practical evidence remains missing, is to be
74 found with the mitochondrial diversity (Gemmell & Allendorf, 2001). Regardless of its small size, this genomic
75 region is a promising candidate for further optimization of conservation breeding programs (Gemmell &
76 Allendorf, 2001), not only because of its functional role in energy production (Friedman & Nunnari, 2014), but
77 equally important due to its maternal inheritance pattern resulting in aberrant responses to selection.
78 Natural selection describes trends in population fitness as a result of selection upon heritable traits.
79 Changes in allele frequencies are inevitable within this process and well understood for traits encoded within
80 the nuclear genome. Contrary to nuclear DNA, mitochondria and their DNA content (mtDNA) are only
81 transmitted through the maternal lineage in most species (Hutchison et al., 1974). Although natural selection
82 takes action in both males and females, this maternal inheritance implies there is no direct selection against
83 male harming, or for male beneficial, mitochondrial variants towards the next generation (Frank & Hurst,
84 1996). Meanwhile, the process of natural selection induces normal changes in allele frequency within females
85 irrespective of its effects on male fitness. Altogether, mutations beneficial for males but neutral for females will
86 not directly be selected for and therefore will not increase in frequency whereas male-harming but female-
87 beneficial mutations will increase in frequency despite the negative consequences.
88 This sex-specific selection asymmetry should result in the accumulation of a male-harming mitochondrial
89 mutation load, and within this overall rationale the process has been dubbed ‘mother’s curse’ (Frank and Hurst
90 1996; Gemmell et al. 2004). Mitochondria are crucial in cellular energy production (Friedman & Nunnari 2014)
91 and the accumulation of this male-harming mtDNA mutation load can thereby have significant adverse effects

on fitness traits, such as longevity and fertility, in males (Beekman, Dowling, & Aanen, 2014; Vaught & Dowling, 2018). Mitochondrial mutations can affect fitness by producing incomplete formation of the oxidative phosphorylation pathway resulting in impaired energy production (Friedman & Nunnari, 2014). In general such mutations will not affect population viability if they impact both sexes, as these will be selected against in future generations in the maternal lineage. However, they will remain present in the population in females when they exclusively affect males, such as male reproductive traits, or have a substantially larger effect on males when compromised (Frank & Hurst, 1996; Innocenti, Morrow, & Dowling, 2011; Wolff & Gemmell, 2013).

Over the past decades, evidence on the effects of mitochondrial inheritance on fitness traits has grown (as reviewed by Beekman et al. 2014; Vaught & Dowling 2018). Multiple studies show decreased male fertility across a range of taxa (Froman & Kirby, 2005; Dowling, Nowostawski, & Arnqvist, 2007; Smith, Turbill, & Suchentrunk, 2010; Patel et al., 2016; Camus & Dowling, 2018). Recent research has e.g. shown how male-biased mitochondrial mutation loads can affect senescence and longevity in males of different species (Camus, Clancy, & Dowling, 2012; Camus et al., 2015; Đorđević et al., 2015; Milot et al., 2017). Captive breeding populations for which detailed genealogies exist provide a novel opportunity to study the role of mitochondrial inheritance patterns on sex-specific fitness.

Captive breeding programs' long-term viability will benefit from more detailed insights in mitochondrial loads, either in terms of the presence of male-harming mutational loads or from promoting the preservation of mitochondrial lineages that increase overall fitness. Captive populations are in theory more prone to accumulate male harming mitochondrial haplotypes compared to their wild counterparts due to their relative small population size (Whitlock & Bürger, 2004), differences in selective pressures (Lynch & Hely, 2001) and heterogeneity in founding populations (Hvilsom et al., 2013; Soto-Calderón et al., 2015). Moreover, current breeding management focusses on preserving (genetic) diversity to minimize the effects of both drift and directed selection. Selecting underrepresented individuals, as e.g. evaluated by their low kinship values, to generate future generations has proven to be highly effective in reaching this goal and as such has become the method of choice in most breeding programs (Ballou & Lacy, 1995; Ivy & Lacy, 2012; Putnam & Ivy, 2013). Although being rare, there are however specific situations in which these strategies facilitate the spread or even fixation of alleles. Whenever mitochondrial haplotypes harming (early) male survival are introduced in a population via a female founder, these haplotypes will increase in frequency over generations as a result of current breeding practice. A lower survival of male offspring either prebirth or during juvenile stages, results in

a lower contribution of females holding male harming mitochondria in the next generation which is also depicted in a below average kinship. Consequently these individuals or their direct offspring will be selected to produce extra offspring within the next breeding season as such increasing the frequency of this haplotype over generations. This effect is even more pronounced since it will be mainly females surviving and therefore being selected to source the next generation. Depending the selective pressure and in combination with the already lower effective size of mtDNA this might lead to fixation of these “cursed” haplotypes in the scope of a breeding program.

Additionally, there is an increased chance for genomic mismatching in captive populations. Mitochondrial functionality depends on both genes encoded within the mtDNA as well as their interplay with coadapted genes fixed in the nuclear genome (Ryan & Hoogenraad, 2007). Natural populations follow their own evolutionary trajectory as a response to e.g. differential selection, isolation or drift, eventually resulting in population specific mito-nuclear differentiation. Mitochondrial functionality can thus not only be impaired by a mtDNA specific mutational load, but also by disrupting the coadapted mito-nuclear interactions, i.e. genomic mismatch (Wolff et al., 2014). These disruptions of mito-nuclear coadaptation can be caused by mutations, but also through introgression of new or diverged haplotypes or outbreeding (Wolff & Gemmell, 2013; Beekman, Dowling, & Aanen, 2014; Hill et al., 2019). With the origin of captive populations often unknown, unintentional mixing of (premature) evolutionary lineages is expected to be more prevalent ex-situ compared to what is observed under natural conditions (Hvilsom et al. 2013; Milián-García et al. 2014) increasing the chance of disrupting mito-nuclear coadaptation in gene functionality ex-situ (Burton, Ellison, & Harrison, 2006; Beekman, Dowling, & Aanen, 2014; Wolff et al., 2014). This effect will be even more pronounced whenever genetic information is integrated in pedigree based management strategies promoting optimal mixing to minimize loss of genetic diversity or inbreeding (Frankham, 2015).

Here we aimed to determine the occurrence of sex-specific fitness differences in mitochondrial lineages within captive populations, a first step towards further integrating mitochondrial inheritance in conservation breeding management. Our main objective was to investigate whether sex-specific selection asymmetry of mitochondria causes sex-specific differences in fitness. We studied differences in survival between studbook-based maternal lineages, representing mitochondrial haplotypes. Sex ratios at birth was used as a proxy for prenatal differential survival between mitochondrial haplotypes, whereas survival (age in days) was evaluated to study the role of

mitochondrial load between sexes at the postnatal level. We studied a total of 16 different species, two avian species namely the lilacine amazon (*Amazona lilacina*) and the military macaw (*Ara militaris*) and 14 mammal species, namely the okapi (*Okapi johnstoni*), pygmy hippopotamus (*Choeropsis liberiensis*), gaur (*Bos gaurus*), black rhinoceros (*Ceratotherium simum*), Grevy's zebra (*Equus grevyi*), Hartmann's zebra (*Equus zebra hartmannae*), Somali wild ass (*Equus africanus somaliensis*), binturong (*Arctictis binturong*), Southeast African cheetah (*Acinonyx jubatus jubatus*), cotton top tamarin (*Saguinus oedipus*), golden headed lion tamarin (*Leontopithecus chrysomelas*), bonobo (*Pan paniscus*) and the Colombian black spider monkey (*Ateles fusciceps rufiventris*). This study expands our current comprehension of mother's curse as well as genetic management of captive breeding populations.

Methods

This study showcases how mitochondrial inheritance can be studied in captive populations starting from 16 zoo studbooks obtained between August 2015 and May 2019 representing 2 avian and 14 mammal species (Table A1). For studbooks to be included in this study, they required to be founded by at least 2 females, containing over 100 individuals spread over several generations, and with at least 80% of all maternities known. An overview of the studbooks' content can be found in Table A1. We used R (version 3.5.0; R Core Team 2013) within the RStudio environment (version 1.1.453; RStudio Team 2015) to analyze the studbooks.

Starting from standard studbook information i.e. individual identifier, sire, dam, sex, date of birth, date of death, litter sizes, parity and studbook completeness was evaluated. Pedigree gaps (percentage unknown ranged from 0.03 to 0.72; Table A1) for which no multiple parenthood was registered (cfr. MULTs) were accounted for by assigning adults as potential parents based on birthday, reproductive age, gestation time and location (through physical holdings data) and dividing parentage over all recorded potential parents. In the rare cases female founders conceived in the wild and gave birth in captivity, we added additional unrelated male founders to the studbook as being the father of the resulting offspring.

Extra parameters such as maternal lineages, litter chronology (survival might be affected by parental experience), and inbreeding coefficients were assessed starting from available data and following overall conservation breeding management assumptions. We assumed mitochondrial lineages to be homoplasmic, and founders to be unrelated, i.e. every maternal founder represents a unique mitochondrial lineage. Maternal

lineages (mtls) were dropped through the pedigree starting from these female founders. Combining parentage and individual birth dates, litter chronology (individual's litter order, e.g. first-, second- or third-born for both sire and dam) was determined. Inbreeding coefficients were derived from kinship values as described by the standard method Backus and Gilpin (2002), supplemented by weighing kinship based on parental probabilities.

Parental probabilities predetermined by the studbook keeper (cfr. MULTS) were averaged in kinship calculations. In other cases, all (potential) sires and/or dams were given equal parental probability. The kinship of an individual to itself was taken as half the kinship between the individual's mother and father. The inbreeding coefficient (F_{IS}) was set as twice the individual's kinship to itself.

Realized sex-ratios at birth were evaluated for each species on the individual dam level based on the provided pedigree information. Subsequently, we evaluated deviations from population specific sex-ratios on the mitochondrial level using generalized linear mixed models following a binomial distribution. To correct for false positives Bonferroni corrections following Benjamini-Hochberg's false discovery rate were applied on the population level.

Bayesian Generalized Linear Mixed Model (GLMMs) from the MCMCglmm package (v2.26; Hadfield, 2010) were used to determine mitochondrial- and sex-specific difference in survival over time. This approach enables the inclusion of living individuals, i.e. censored data, and the implementation of a pedigree, which detects correlated random effects by taking kinships into account.

Individuals with estimated and therefore unreliable birth dates (e.g. founders which are born in the wild) were excluded from further analysis. Maternal lineages with fewer than 40 individuals and less than 15 females or males were converted into one maternal lineage to act as the reference in the statistical analysis. For the three studbooks that did not have enough individuals to act as a reference (see Table A2) we tested each maternal lineage independently with all other lineages as a reference, rather than using the small lineages only.

We used Markov chain Monte Carlo (MCMC) techniques to draw variables from their posterior distribution. Priors were set as described by Gelman et al. (2008) with nu-values set to 1. Sire and dam were fitted as random terms in all models to account for paternal and maternal effects. In the initial model we included inbreeding coefficient and litter chronology (both paternal and maternal) as fixed terms, all interacting with the variables maternal lineage and gender. We added the variables individual litter size (size of

litter the individual came from) and mean litter size (birthed by the individual) if the mean litter size of the population was larger than 1.5 (see Table A1). The number of iterations, thinning, and burn-in period for the models were set to 65000, 32, and 3000 respectively. After running this initial model, the parameters were increased if necessary, to avoid autocorrelation. We evaluated model fit based on the deviance information criterion (DIC) value and assessed less complex models by removing the most insignificant fixed terms and their interactions one by one.

Survival was analyzed and survival probability graphs were created using the survival package in R (version 2.42-6; Therneau and Grambsch 2015) and modified functions from the package survminer (version 0.4.3; Kassambra and Kosinksi 2018).

Results

All 16 studbooks were analyzed for deviations in sex-ratios and the presence of sex-specific differences in survival within and between maternal lineages. However, for the postnatal survival analysis three studbooks were excluded from the survival analysis as they held too few individuals to be tested. As expected, the size of the analyzed studbooks, number of founders, and generations varied substantially, affecting the number of maternal lineages and the percentage of the population that could be analyzed per studbook (see Table A2).

Two maternal lineages showed a significant deviation from the population average birth sex-ratio within the populations being studied. With a sex-ratio at birth of 0.31 compared to 0.54 on the population level ($Z = 0.331$, $p = 0.0027$) one (mtl 3) out of the two most prevalent maternal lineages within the captive gaur population showed a significant deficit of male offspring compared to females. Additionally, this maternal lineage represents one of the two remaining lineages in the current population (19 females total) and is currently found in 2 fertile females. A similar pattern was found within the Colombian black spider monkey breeding program in which one maternal lineage (mtl 68) had a sex-ratio at birth of 0.28 compared to 0.42 on the population level ($Z = -1.9$, $p = 0.048$). The current sex-ratio for this specific lineage within the breeding program equals 0.18 with many of the 14 females representing this lineage still able to breed. The captive population has 41 remaining maternal lineages and the females from mtl 68 make up 9% of the total fertile female population.

A total of three maternal lineages (mtl) that were part of three studbooks showed a significant difference in postnatal survival between males and females (Table 1). For the remaining thirteen studbooks we noted no differences or significant differences in other directions (e.g. maternal lineages with an overall increased survival compared to the reference population, see Table A2 and A3, and Fig. A4). Survival curves and age density plots (the latter based on deceased individuals only) were produced to determine the magnitude and potential cause of these sex-specific difference (Fig. 1).

Mother's curse was observed in three maternal lineages representing three separate studbooks (Table 1), namely mtl 4 within pygmy hippo (*Choeropsis liberiensis*, Fig. 1a), mtl 12 within binturong (*Arctictis binturong,* Fig. 1c) and mtl 18 within Somali wild ass (*Equus africanus somaliensis,* Fig. 1e*)*. In these lineages male survival was significantly lower compared to females and lower than the overall population mean. For both the pygmy hippo and binturong this pattern was due to a lower survival in the early ages of males (Fig. 1b and d), while for Somalian wild ass there was a gradually accumulating survival difference (Fig. 1f). Male survival probability for pygmy hippo's of mtl 4 male also reaches zero earlier in time (Fig. 1a). The maximum intersexual survival difference within these three lineages ranged from 10-20% (e.g. Fig. 1b). The pygmy hippo population contained an additional maternal lineage where male survival was lower, but this was only nearly significant (post.mean = 1.63 (-0.12 - 3.33); eff.SS = 1675; pMCMC = 0.065).

Discussion

While conservation breeding management mainly focusses on nuclear DNA to reach the general goals of minimizing inbreeding and preserving overall genetic diversity, recently several studies have shown that mitochondrial DNA and more specifically its atypical form of inheritance can explain variances in fitness between individuals and even populations (Gemmell, Metcalf, & Allendorf, 2004; Beekman, Dowling, & Aanen, 2014). Here we present for the first-time empirical evidence for the presence of mtDNA induced sex-specific variation in survival across different species within captive breeding programs. This sex-specific selection asymmetry and its correlation with sex-specific fitness effects is not only of interest for the broader scientific community, but equally important for the conservation breeding community in the ongoing discussion on what should be preserved and how to improve conservation management practices.

Our study shows that mother's curse effects, affecting survival, are present in captive populations and affect survival from conception to adulthood. Here we present differential prenatal survival of females over males of two different species, namely gaur and Colombian spider monkey, and sex-specific differences in postnatal survival with a difference of up to 20% in three species, the Somali wild ass, pygmy hippo and binturong. This finding of a mtDNA induced male-harming effect is in agreement with the evidence available in literature (Camus, Clancy, & Dowling, 2012; Camus et al., 2015; Đorđević et al., 2015).

The reported male specific effects in survival could result from differences in basal metabolic rates between sexes (Beekman, Dowling, & Aanen, 2014). Male metabolic rates are generally higher and since the mitochondria have evolved in response to female demands, this could result in mitochondria to be less adapted to cope with the males' high oxygen demands and make mitochondrial function more susceptible to damage contributing to the longevity difference (Beekman, Dowling, & Aanen, 2014). Additionally, since mitochondria are adapted to female demands, mitochondria in males can be susceptible to increased amounts of ROS-related damage, producing deleterious mitochondrial mutations ultimately leading to inadequate OXPHOS activity and the related mitochondrial diseases (Pak et al., 2003) lowering male survival rates.

In this study two maternal lineages were found to have a skewed sex-ratio and differences for two lineages in the postnatal survival analysis were mainly due to low early survival in males (mtl 4 and mtl 12 within respectively pygmy hippo and binturong). To our knowledge this is the second non-human study that shows a mtDNA induced sex-specific difference in early survival. Đorđević et al. (2015) found a significant female-biased sex ratio in seed beetles (*Acanthoscelides obtectus*), where more females of two disrupted maternal lineages survived the egg-to-adult stage relative to males, alongside a difference in longevity. Both processes were attributed to a mito-nuclear mismatch. As mentioned before, mitochondrial function relies on mito-nuclear coadaptation and disrupting this epistasis by combining mtDNA and nuclear DNA with very little shared evolutionary history can affect fitness if it causes the disruption of coevolved mito-nuclear complexes (Beekman, Dowling, & Aanen, 2014). Other studies including directed crossing experiments have showcased the role of genomic mismatch in explaining observed fitness differences between mitochondrial lineages (Dowling, Nowostawski, & Arnqvist, 2007; Clancy, 2008; Yee, Sutton, & Dowling, 2013; Jelić et al., 2015). At present we cannot directly determine the nuclear environment mitochondrial lines are interaction within the studbooks under study. As such it is currently impossible to evaluate whether sex-specific differences in

292 survival observed within this study are induced through an accumulated mutation load or the mismatch of the
293 mitochondrial lineage with its nuclear background.
294 However, mito-nuclear coadaptation is predicted to be population-specific as populations experience
295 different selection pressures producing different patterns of mito-nuclear coadaptation (Wolff et al., 2014),
296 and that genomic mismatches are more likely to occur when genomes are more divergent (Burton, Ellison, &
297 Harrison, 2006). Although theoretically sound, empirical evidence to support this theory remains scarce and
298 predicted mismatching effects are not always observed (Rand, Fry, & Sheldahl, 2005). Altogether more
299 research is needed, this study points towards a potentially interesting system to focus on. Breeding programs
300 are often founded by individuals originating from divergent populations and hybridization and introgression is
301 known to be present in zoo populations (Witzenberger & Hochkirch, 2011), indicating a potential role for
302 genomic mismatching. More specifically, the captive populations from the three species showing mother's
303 curse are known to originate from different source populations or have a large distribution, thus have a high
304 chance of intraspecific genetic differentiation through isolation by distance (Orsini et al., 2013). As such the
305 disruption of mito-nuclear coadaptation through mixing divergent populations is a plausible explanation for the
306 observed sex-specific differences in survival of these captive populations. The impact of this process could be
307 studied by analyzing and comparing both the nuclear and mitochondrial haplotypes present in wild and captive
308 populations to determine what combinations are common in the wild and which potentially create
309 mismatches.
310 While our study did find maternal lineages affecting male survival, most lineages showed not to be
311 affected. The absence of a clear pattern in most lineages might be in line with low frequencies of mother's
312 curse effects in natural systems or might be context specific (e.g. founding effects and subsequent small
313 effective population sizes). Within the survival analysis we studied all lineages represented by at least 40
314 individuals which may not have been enough for sex-specific differences to be detected. Additionally, this study
315 assumed all maternal founders to be unrelated on the mitochondrial level, thus representing unique
316 mitochondrial lineages. In reality founders often originate from either the same family (Ivy et al., 2009) or
317 populations in which mitochondrial variation is limited (Gemmell, Metcalf, & Allendorf, 2004). Although being
318 highly conservative, this approach might therefore have resulted in over splitting lineages causing many false
319 negatives due to sampling sizes. DNA analyses can identify the haplotypes present in the current captive
320 populations and determine the validity of founder uniqueness/unrelatedness on the mitochondrial level.

There are several mechanisms counterbalancing the accumulation of mitochondrial mutational loads, potentially explaining the low frequency of mother's curse in the studied populations. As explained above compensation for mitochondrial load might come from the nuclear genome (Rand, Haney, & Fry, 2004; Dowling, Friberg, & Lindell, 2008). Additionally, it has been suggested that paternal leakage of mitochondria occurs to counteract deleterious mutations (Gemmell, Metcalf, & Allendorf, 2004). More applicable to the current study is the theory that regardless of their maternal inheritance male-harming mitochondrial lineages can be limited within a population through inbreeding. Inbreeding limits the ability of mutants to spread through the population as it reduces female fitness (Unckless & Herren, 2009; Wade & Brandvain, 2009; Hedrick, 2012). Captive populations tend to carry a relatively high amount of inbreeding (Witzenberger & Hochkirch, 2011) and some high levels of inbreeding are also found within this study (Table A1) indicating that inbreeding could be a cause for the absence of sex-specific differences within the studied populations. Additionally, genetic drift could limit the fixation of maternal lineages. As the uniparental nature of inheritance decreases the effective population size of mitochondrial genomes (Beekman, Dowling, & Aanen, 2014) drift can thereby either remove low frequency male harming mitochondrial lineages, or less frequently result in the fixation of these cursed lineages. This last option might partly explain the historic loss of captive populations. Within this same rational it has to be noted that small populations were excluded from the current analysis due to statistical constraints, yet are vulnerable as they have a higher chance of accumulation or fixation of a mutation load (Rand, Haney, & Fry, 2004).

Lastly, the fact that there are no sex-specific differences in survival does not mean there is no effect of maternal inheritance on fitness. Other traits may be affected but masked by breeding management or undeterminable through studbooks. For a captive colony of European brown rabbits (*Lepus europaeus*) Smith et al. (2010) found no evidence for reduction in average litter sizes when individuals were grouped by mitochondrial haplotype, but their results did show that male reproductive success was affected. Unfortunately, male reproductive success cannot currently be studied through studbooks as breeding attempts are generally not registered.

While the effect of mother's curse on captive populations appear to be small (only five lineages are affected, and male survival is only slightly lower), our study indicates maternal lineages with negative effects on male prenatal survival currently occur at a relatively high frequency within living populations. The establishment and

management of captive populations does not automatically removes mother's curse effects and under some circumstances might increase its prevalence. Premature death of male offspring will lower the contribution of a specific dam which in practice will result in managers giving extra breeding opportunities for this female to equalize its contribution to the next generation. With an above average number of females being born, often in combination with this below average contribution, more females of this maternal lineage will be selected to take part in generating the next generations of offspring as such further increasing the spread of this mitochondrial line. As a general approach, we therefore strongly recommend keeping track of the presence and frequency of mother's curse in captive breeding programs. More specifically (pre- and postnatal) male survival should be evaluated more structured and strategies to manage mitochondrial diversity should be implemented whenever long-term sustainability cannot be guaranteed (Lees & Wilcken, 2009). Maternal lineages that display effects should be followed and managed more closely in terms of preventing them to fixate within the breeding program. Meanwhile, research on the DNA level should be launched for these species to fully comprehend the role of mitochondrial diversity aiming to guide optimal management for these species.

Alongside the need to collect molecular data of captive populations, this study further highlights the need to increase our understanding of genetic differentiation within natural settings due to fragmentation and the need to resolve taxonomic uncertainties with a focus on mito-nuclear genomic mismatches. It is this aspect that brings mitochondrial inheritance effects beyond captive breeding. For example, in-situ conservation strategies use intraspecific mixing of lineages for its overall positive effect in terms of increasing genetic diversity (cfr. genetic rescue) and reconnect isolated populations or restock depleted populations. However, the potential of introducing a harmful haplotype or instigating a mito-nuclear mismatch (Smith, Turbill, & Suchentrunk, 2010) should be taken into account, especially when fragmentation between populations is ancient enough to allow genetic differentiation.

Our study shows that it is possible to determine maternal founder-induced fitness effects within captive populations by using studbook information. Several genomic approaches nowadays enable rapid and consistent screening of full mitochondrial genomes. In combination with the ongoing endeavor to construct conservation breeding biobanks, molecular tools (e.g. targeted enrichment strategies) will become valuable to complement the current studbook analyses approach. Molecular analysis of captive populations will not only

validate assumptions made here but will allow the study of small populations currently excluded due to statistical constraints and might identify genes and mechanisms driving this process, from single point mutations towards mito-nuclear mismatches and other pleiotropic effects. Future studies should focus on how both positive and negative effects of mitochondrial inheritance can be incorporated in existing strategies for captive breeding management.

Supporting information

Detailed information on content and structure of the studbooks (Table A1), the sex-ratio and survival analysis for the studied mitochondrial lineages (Table A2) and the additional results from the statistical analysis (Table A3 and Fig. A4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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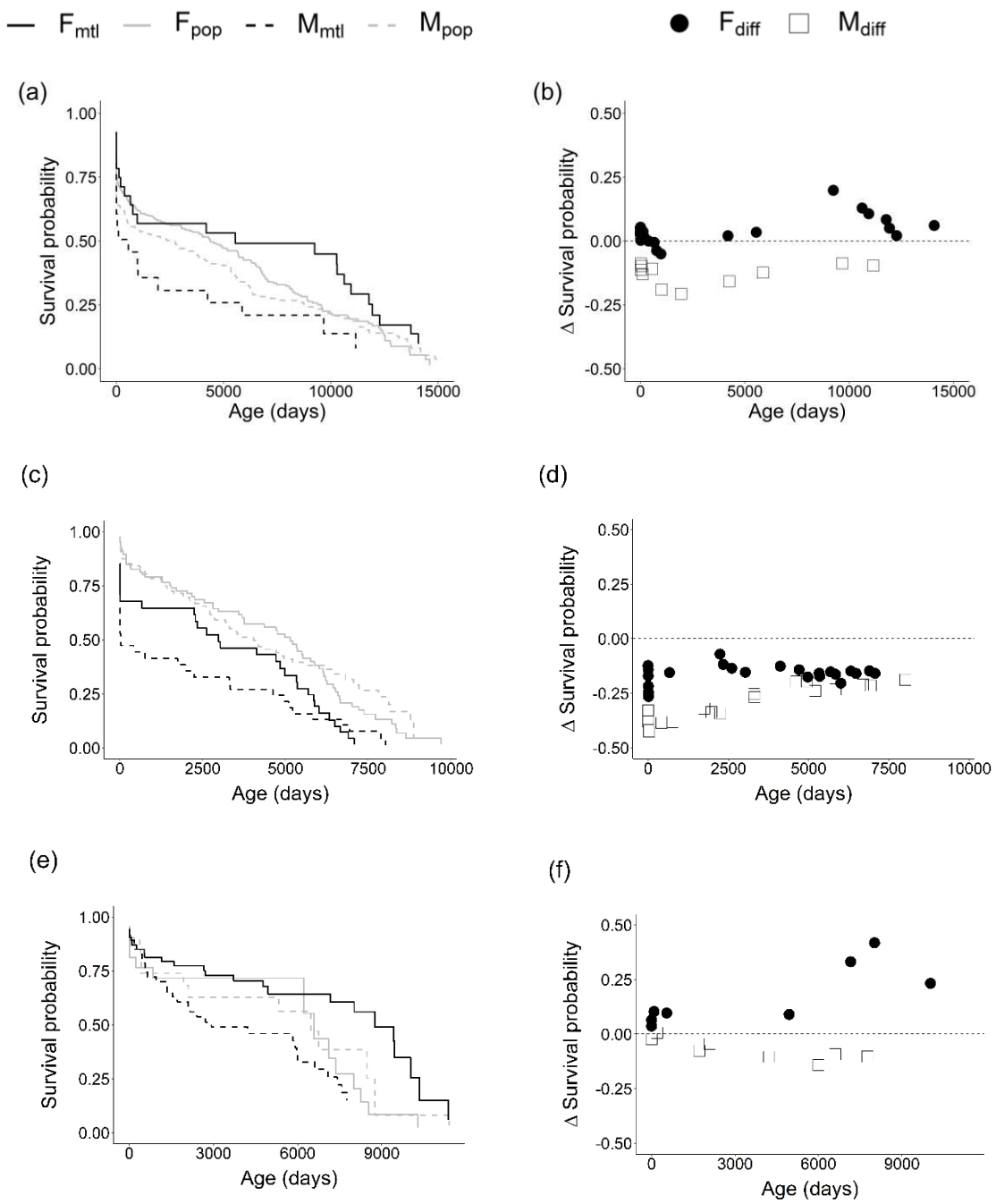
512

Figure and table

Fig. 1 Survival curves (left) of three maternal lineages showing survival probability over time of males (M) and females (F), of the maternal line (mtl) and the population reference (pop), and corresponding survival probability differences (right; based on deceased individuals only) between males and females of the maternal lineages.


F_mtl
F_pop
M_mtl
M_pop
F_diff
M_diff
(a)
(b)
(c)
(d)
(e)
(f)
Survival probability
Δ Survival probability
Age (days)

518 Table 1. Results of the Markov chain Monte Carlo generalized linear mixed model from mitochondrial lineages
519 that show significant effects in survival, including maternal lineage specific information.

520

Species	Mtl-i	N*total*	N*live*	N*itt*	Thin	Eff.SS	Post. mean	L-95CI	U-95CI	pMCMC
Pygmy hippo	4	53 (20.28)	5 (2.3)	64985	32	1938	1.851	0.355	3.438	0.027 *
Somali wild ass	18	101 (46.54)	41 (13.28)	499751	250	1831	1.880	0.048	3.698	0.046 *
Binturong	12	87 (34.34)	0 (0.0)	499751	250	2050	3.264	0.800	5.546	0.007 **

Species, common species name; Mtl-i, mitochondrial lineage identifier; N*total*, total number of individuals in the mitochondrial lineage (male.female); N*live*, number of living individuals at time of extraction of the studbook (male.female); N*itt*, number of iterations; Thin, thinning; Eff.SS, effective sample size; Post. Mean, posterior mean; L-95CI and U-95CI, lower and upper 95% confidence interval; pMCMC, particle Markov Chain Monte Carlo with significant codes (*** < 0.001; ** < 0.01; * < 0.05).