

PROCEEDINGS B

A father effect explains sex-ratio bias

Journal:	<i>Proceedings B</i>
Manuscript ID	Draft
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Malo, Aurelio; University of Oxford, Department of Zoology; Chicago Zoological Society; National Zoological Park, Conservation Biology Institute Martinez-Pastor, Felipe ; University of León, Spain Garcia-Gonzalez, Francisco; Doñana Biological Station, Spanish Research Council CSIC, Garde, Julian; castilla-La Mancha University, ; Ballou, Jon; National Zoo Lacy, robert; Chicago Zoological Society, Conservation Science;
Subject:	Evolution < BIOLOGY, Physiology < BIOLOGY, Ecology < BIOLOGY
Keywords:	Sex-ratio, Trivers and Willard hypothesis, male effects, inbreeding, sperm nucleus size, Genetic quality
Proceedings B category:	Evolution

SCHOLARONE™
Manuscripts

A father effect explains sex-ratio bias

Aurelio F Malo,^{* 1, 2, 3} Felipe Martinez-Pastor,^{4, 5} Francisco Garcia-Gonzalez,^{6, 7} Julián Garde,⁴ Jonathan D Ballou,³ Robert C Lacy²

¹Department of Zoology, University of Oxford, OX1 3PS, UK

²Chicago Zoological Society, Brookfield, Illinois, USA

³Smithsonian Conservation Biology Institute, Washington DC, USA

⁴SaBio IREC (CSIC-UCLM- JCCM), Campus Universitario s. n. 02071 Albacete, Spain

⁵INDEGSAL and Molecular Biology (Cell Biology), University of León, Spain

⁶Estacion Biológica de Doñana, CSIC, Sevilla, Spain

⁷Centre for Evolutionary Biology, The University of Western Australia, Crawley, WA, Australia.

*Author for correspondence:
A.F. Malo
e-mail: aurelio.malo@zoo.ox.ac.uk

Running title: Fathers drive sex-ratio bias

Keywords: Sex-ratio, Trivers and Willard hypothesis, male effects, *Peromyscus leucopus*, inbreeding, sex allocation, sperm cell nucleus, sperm nucleus size, sperm morphometry.

Word count: 3622 (excluding references)
Figures: 3
Tables: 2
Text box: 1
Electronic supplementary material: 1 file

Abstract

Sex ratio allocation has important fitness consequences, and theory predicts that parents should adjust offspring sex ratio in cases where the fitness returns of producing male and female offspring varies. The ability of fathers to bias offspring sex ratios has traditionally been dismissed given the expectation of an equal proportion of X and Y-chromosome bearing sperm (CBS) in ejaculates due to segregation of sex chromosomes at meiosis. This expectation has been recently refuted. Here we used *Peromyscus leucopus* to demonstrate that sex ratio is explained by an exclusive effect of the father, and suggest a likely mechanism by which male-driven sex-ratio bias is attained. We identified a male sperm morphological marker that is associated with the mechanism leading to sex ratio bias; differences among males in the sperm nucleus area (a proxy for the sex chromosome that the sperm contains) explain 22% variation in litter sex ratio. We further show the role played by the sperm nucleus area as a mediator in the relationship between individual genetic variation and sex-ratio bias. Fathers with high levels of genetic variation had ejaculates with a higher proportion of sperm with small nuclei area. This, in turn, led to siring a higher proportion of sons (25% increase in sons per 0.1 decrease in the inbreeding coefficient). Our results reveal a plausible mechanism underlying unexplored male-driven sex-ratio biases. We also discuss why this pattern of paternal bias can be adaptive. This research puts to rest the idea that father contribution to sex ratio variation should be disregarded in vertebrates, and will stimulate research on evolutionary constraints to sex ratios—for example, whether fathers and mothers have divergent, coinciding, or neutral sex allocation interests. Finally, these results offer a potential explanation for those intriguing cases in which there are sex ratio biases, such as in humans.

1. Introduction

Trivers and Willard influential work on sex ratio allocation [1] predicts that parents should adjust offspring sex ratio in cases where the fitness returns of producing male and female offspring depend on parental condition. According to Trivers and Willard, mothers in better condition, who can afford the investment, are expected to invest more in the sex that has the potential to provide higher fitness returns, which typically is the sex that is more costly to produce. Mothers in worse condition should invest in the sex that is less costly to produce but has less variance in reproductive success. The Trivers and Willard hypothesis has successfully explained observed sex ratio patterns in some organisms [2, 3], but its power to explain adaptive sex-ratio variation in vertebrates, and more specifically in mammals, has remained limited [4, 5]. For instance in ungulates, the most studied group, female condition accounts for 3-6% of variation in offspring sex-ratio [6].

In taxa where males are the heterogametic sex, males can theoretically influence offspring sex-ratio during sperm production. In mammals, however, the expectation of an equal proportion of X and Y-chromosome-bearing sperm (CBS) in ejaculates has led to most research on sex-allocation being focused on females [2, 7] and to be interpreted in the light of maternal fitness returns [2, 3]. Nevertheless, as with females, high quality males should invest in the more costly sex (usually males). If high quality males are more likely to produce high quality offspring – either through contribution to the parental investment in offspring or via heritability of male quality – then it is adaptive for them to produce more of the sex that has the opportunity for higher reproductive success. Importantly, recent research shows that unbalanced proportions of X vs. Y-CBS pre-ejaculation are not rare [7-9].

Three studies conducted in sexually dimorphic ungulates with high variance in male reproductive success support the idea that fathers can bias sex ratio [10, 11] [12]. First, Gomendio et al. [10] used 14 red deer stags — a strongly sexually dimorphic species and a

classic example for large variance across males in reproductive success— to show that fathers can bias sex ratio at birth [10]: more fertile fathers produce more sons and less fertile males produce more daughters, underscoring for the first time the effects of father quality on sex ratios. Second, Saragusty et al. [11] used 7 pygmy hippos to show that variation in the ratio of X- and Y-chromosome bearing sperm in the ejaculate was associated with variation in the sex of the offspring produced. Third, Douhard et al. [12] recently used a large data set on another sexually dimorphic species, the bighorn sheep, to show, first, that males with higher reproductive success have a higher proportion of male offspring, and second, that such sex ratio bias is adaptive. These three studies suggest that sex allocation is not an exclusive ability of mothers. High quality fathers could accrue higher fitness benefits by biasing sex ratio towards sons who would inherit their quality and go on to produce more grand-offspring. Despite this seemingly straightforward argument, the adaptive meaning of sex ratio bias by males has not been well established. Moreover, we do not know whether the adaptive value of sex ratio adjustments varies depending on the species' life history [2]. Furthermore, regardless of the adaptive mechanism, whether this father effect is an exclusive feature of ungulates or whether it is common across the mammalian tree of life is not known.

Here we investigate the possibility of fathers driving sex-ratio bias in a species lying at the opposite extreme of the mammalian life-history spectrum. We used a non-domestic rodent model, the white footed mouse (*Peromyscus leucopus*), to test three hypotheses related to sex allocation. First, we tested whether a male-specific sperm trait (the size of the sperm nucleus) explains variation in sex ratio bias. Second, we tested if male genetic quality [13], as measured by inbreeding [14], predicted offspring sex-ratio at birth. Third, we tested whether the size of the sperm nucleus is the trait that potentially links father inbreeding levels with offspring sex ratios at birth.

We also discuss the adaptive value of paternal sex ratio bias in rodents. We have previously

104 shown that, in *Peromyscus leucopus*, males have a higher fertility genetic load (lethal
105 equivalents) than females [15]. High quality fathers (those with relatively low inbreeding)
106 would presumably have a higher probability of fertilization in promiscuous contexts (e.g. see
107 [16]). Inbreeding is heritable in some systems [17] [18] (see S2), — with parental levels
108 correlating with offspring inbreeding under non-random mating — allowing for paternal sex
109 ratio bias driven by inbreeding to be adaptive. In addition, even in cases where inbreeding is
110 not heritable, if males assess their quality and adjust offspring sex ratio accordingly, and if
111 inbreeding depresses phenotypic quality [14], then more inbred fathers would be expected to
112 shift the offspring sex ratio toward the less costly sex. On the other hand, as sons inherit their
113 fathers' quality, high quality fathers would be expected to bias sex ratio towards sons because
114 high quality males have a higher probability of successfully fertilizing females and having
115 more offspring than low quality males. Thus, assessments of quality that might drive adaptive
116 sex ratio bias would be influenced by inbreeding. Note that the mechanism proposed above
117 dependent on quality assessment could rely on self-perceptions of quality, as well as on
118 comparisons with nearby males that would be competing for mates.

119 Our study system needed to meet some critical requirements to properly test the predictions
120 above. First, to make our results generalizable to other naturally evolved species, and relevant
121 in the context of the evolution of sex ratios, we used a wild rodent model system where
122 domestication has not depleted natural variation. Second, to have certainty of paternity when
123 linking males to their offspring, and to account for maternal and paternal effects on sex-ratio
124 bias, we used a captive facility and controlled pairings. Third, to ensure that the paternal
125 effects on offspring sex ratio were not confounded with maternal effects, we identified a
126 male-only trait (nucleus sperm area) as the candidate variable mediating the male sex ratio
127 bias. Sperm head size is used as a discrimination criterion for sorting X and Y-CBS in a
128 variety of mammalian species, including humans and bovines [19-23], where it has been

shown that X-CBS are larger than Y-CBS (Box 1). To further minimize the effect of factors contributing to maternal sex-ratio bias, such as maternal diet or female body condition, food was provided at *libitum*. Also, constant food and environment conditions, together with consistent housing, minimize the effects of drivers such as seasonality, density or social interactions on female sex ratio bias [24]. This also reduces the scope for local resource competition, local mate competition and local resource enhancement [25, 26] driving the observed results. Lastly, we recorded and included in the statistical models other confounding variables, such as male age and litter size, known to influence sex ratios [27, 28].

2. Materials and Methods

(a) Study population

We used a population of white-footed mice (*Peromyscus leucopus*) at the Chicago Zoological Society's research animal facility, derived from wild individuals trapped at Volo Bog State Natural Area (Illinois) (see S1.1. for details). Individuals from the 10th generation were used for this study. This population is expected to have retained, on average, 97.5% of the wild genetic diversity and, thus, nearly all additive genetic variance available in the natural population. The animal care protocols and experiments described here comply with all current laws and were approved by the Animal Care and Use Committee of the Chicago Zoological Society (see S1.1 for details on the set up of the founder population). In generation number one, experimental populations were established following three different breeding protocols. Given that inbreeding accumulated in them at different rates (see S1.2. for details) [15, 29], we checked that offspring sex ratio was not affected by breeding protocol (*anova*; $F_{2,35} = 2.40$, $p = 0.11$), and further included this three-level categorical variable in the relevant models below.

(b) Sampling, sperm collection and sperm nuclei area

This study used 58 males. Each male was paired with a single female and allowed to reproduce for up to 70 days. To standardize the conditions of males before sperm collection, after separation from the female, and given that spermatogenesis takes 7-8 days, every male was left in an individual cage for at least 7 days to allow the sperm stores to be replenished. Immediately after euthanasia, the testicles were removed and sperm was collected and processed (see S1.3 and S1.4 for details on sperm sampling). We used the Feulgen stain and a microscope with a $\times 60$ bright-field objective linked to a video camera to visualize the sperm nuclei and carry out the sperm head measurements using computer-assisted sperm head morphometric analysis (CASMA). The software automatically identifies sperm heads and calculates nucleus length and area (figure S1). The nuclei of a minimum of 200 sperm were analyzed per individual (see S1.5. for details on sperm nuclei measurements). We present full statistics for sperm nucleus area, and show the consistency of the results using another relevant measure of the nucleus (length), with which it is strongly correlated (figure S2).

(c) Genetic quality, sex ratio at birth and offspring number

We used the Wright's F coefficient of inbreeding as an indicator of genetic quality [13]. The coefficient of inbreeding of the father and the mother (*sire f* and *dam f*) was calculated from the pedigree of the population. Offspring sex ratios at birth (secondary sex ratios; calculated as the proportion of sons, $N_{\text{sons}}/[N_{\text{sons}}+N_{\text{daughters}}]$), were recorded for each father after his impregnated mate gave birth, as well as the number of pups produced. Male age was recorded to account for this factor in the models.

(d) Data analysis

Means and standard deviations are used to describe the variables included in the models (table 1). We used GLMs with either a Gaussian (offspring sex ratio as a proportion) or binomial (sons vs. daughters, using a logit link function) to test for the predictors of secondary sex ratios. We also used Akaike's Information Criterion (AIC) to assess model

support and to select the best model. Given that males from different breeding protocols [15, 29] varied in the rate of inbreeding accumulation through the 10 generations (see section S1.2 for details), we accounted for this variable in the relevant models. The models vary in sample sizes because the total number of males was 58, whilst the total number of fathers siring offspring was 40. Two of those males were outliers for sperm nucleus area ($>2.85 \times \text{SD}$ away from the mean), so they were excluded from the relevant models prior to analysis. Data were analysed using R software (version 3.3.2, <http://www.R-project.org/>) and STATISTICA (version 7.0, StatSoft Inc., Tulsa, OK, USA).

3. Results

We independently tested the effects of inbreeding (sire f , mean \pm SD = 0.136 ± 0.021) and sperm nucleus size (see table 1) on sex ratio (0.43 ± 0.21) using different GLM models.

(a) Male-specific trait (sperm nucleus size) and sex ratio bias

Results showed that fathers with smaller sperm head nuclei sired a higher proportion of sons than daughters ($\beta \pm se = -0.18 \pm 0.05$, $t = -3.22$, $df = 38$, $p = 0.002$; figure 1).

(b) Father genetic quality (inbreeding) and sex ratio bias

Likewise, a model conducted to test independently the association between *sire f* and offspring sex ratio showed that fathers with lower inbreeding sired a higher proportion of sons than daughters ($\beta \pm se = -2.86 \pm 1.33$, $t = -2.15$, $df = 38$, $p = 0.038$; figure 2).

(c) Sperm nucleus size mediates the effects of paternal inbreeding on sex ratio

We tested whether father inbreeding effects on litter sex ratio were mediated through its effects on sperm nucleus area. We first ascertained whether *sire f* and sperm nucleus area had independent effects on sex ratio by running a GLM including these two predictors, plus other potentially explanatory variables (table 2). After stepwise deletion of non-significant terms, only sperm nucleus area remained as a significant predictor of mean sex ratio, explaining

22% of variation ($\beta \pm se = -0.18 \pm 0.06$, $t = -3.22$, $df = 38$, $p = 0.003$; figure 1). We then included inbreeding in the same statistical model as a predictor. In this model, inbreeding did not explain variation on sex ratio ($\beta \pm se = 1.46 \pm -1.30$, $t = -0.88$, $df = 38$, $p = 0.38$). This result was also confirmed in a different set of models run using an information theory approach (table S1). Finally, we tested whether male inbreeding leads to an increase in sperm nucleus area, and showed that was indeed the case ($\beta \pm se = 11.55 \pm 2.75$, $t = 4.198$, $df = 38$, $p < 0.0001$; figure 3). This result was also confirmed in a different set of models run using an information theory approach (table S2).

Overall, our results show that inbreeding does not have a direct effect on litter sex ratio, but instead, an indirect one operating via its effects on sperm nucleus area. Males of higher genetic quality (i.e., lower inbreeding coefficients) have sperm with smaller nuclei (suggesting higher proportion of Y-CBS) that go on to produce more male-biased litters.

4. Discussion

Here we show, first, that fathers account for over 20% of variation in offspring sex ratio. Second, an increase in father's genetic quality (reduced inbreeding) translated into the production of higher proportion of sons through changes in the proportion of X- and Y-bearing sperm (which we determined by a sperm morphological trait). Third, a father's individual genetic quality (level of inbreeding) explained 8% of the variance in offspring sex ratio. As explained below, the higher costs of increased inbreeding for males than for females (given their higher genetic load for fertility traits) suggest that highly inbred fathers can reduce the costs by having relatively more daughters, while outbred fathers can increase their fitness benefits by biasing sex ratio to sons.

A previous study in red deer, a species having singleton births, experimentally showed that males with higher fertility levels produce more males [10]. That bias could have been due to

either a direct male-driven bias of the proportion of X and Y-CBS in the ejaculate [30], or due to higher competitive ability of Y-bearing sperm in the more fertile males [31]. However, which of the two factors was responsible for the observed male-driven sex ratio bias could not be resolved in that study. Our present results on a woodland rodent suggest that offspring sex ratio biases are due to a direct effect of father genetic quality on the proportion of Y vs. X-CBS, and that such bias can be adaptive given its expected fitness benefits. Notwithstanding, other alternative explanations are possible, such as Y-CBS being more vulnerable, with their vulnerability further exacerbated under inbreeding. Similarly, the possibility of an X drive system that damages Y-CBS—suppressed in fit males, those with lower coefficient of inbreeding—could potentially cause more inbred males to produce more female-biased broods [32], so research on these possibilities is warranted. In any case, by identifying a male-only trait such as the sperm nucleus area—sperm size is a reliable marker of X and Y-CBS [19-23](Box 1)—as the main driver of sex ratio, we can be confident that a male effect drives this result. Claiming that the observed effect is driven by mothers (e.g., due to female allocation based on mate quality assessment, or post copulatory sexual selection including sperm choice) would not be parsimonious as the sperm trait is independent and lies first in the chain of events leading to sex ratio variation. Furthermore, the sperm was extracted from the epididymis post-mortem, which excludes the possibility of a maternal effect explaining the links that we have uncovered. Nevertheless, the possibility that after insemination or during gestation maternal effects also moderate sex ratio cannot be ruled out. However, these effects would impact sex ratios beyond the primary paternal effects that we have uncovered here.

The individual- and population-level mechanisms underlying the effects of inbreeding on sex ratio allocation in vertebrates remain far from being understood [33]. Partly, this may be because males' role on sex ratio bias has been consistently dismissed. Here, we show that

father inbreeding—an indicator of genetic quality— influences sex ratio through its effects on the area of the sperm nucleus – an indicator of X vs. Y-CBS. However, inbreeding only accounts for roughly 1/4 of variation in sperm head (27%), and for 8% overall on sex ratio, so a remaining ~14% of paternal (sperm-driven) variation in sex ratio is not related to inbreeding ($22\% - 8\% = 14\%$). The identification of the area of the sperm nucleus narrows down the possible underlying mechanisms of sex-ratio bias, strongly pointing towards the existence of distortions in the proportions of X- vs Y-CBS before ejaculation. These could emerge after meiosis due to differences in longevity between X and Y sperm, which has been shown previously [34].

In species with high variation in reproductive success between males there is a relative higher scope for the evolution of father sex ratio bias [10, 12]. In contrast, the lack of strong sexual dimorphism in rodents and the expected absence of large differences between sexes in intra-sexual variance in reproductive success could argue against the expectation of adaptive sex ratio bias in this group. However, *Peromyscus leucopus* are polygamous, and males will aggressively exclude other males from home ranges that overlap those of up to several females [35]. In an ecologically equivalent woodland rodent species [36], variance in reproductive success was over two times higher in males than in females (Godsall, Coulson, Malo, unpublished results).

We argue that father influences on sex ratio should be taken into account in sex allocation studies. In monotocous species (producing a single offspring at a time), high ranking females could produce more males not only because they themselves may be biasing sex ratio towards sons, but also because they mate with high quality males, who may be biasing sex ratio in the same direction. In polytocous species (producing multiple offspring at a time), such as mice [27, 37, 38], the failure to acknowledge that fathers can contribute to sex ratio bias can explain the lack of support of the Trivers and Willard hypothesis [4, 39, 40].

Mice present a fast life history compared to the slow life history exhibited by species such as red deer or pygmy hippopotamus [41]. The finding that rodent fathers, which lay at the opposite extreme of the mammalian life history continuum, can also bias sex-ratio at birth suggests that this is a common feature to eutherian mammals. Our results stress the need for a formal extension of sex-ratio theory to include the drivers and consequences of paternal sex ratio allocation in vertebrates.

We hope our paper will help stimulate research on whether fathers and mothers have divergent (antagonistic), coinciding, or neutral sex allocation interests. This question has remained largely unexplored in the study of evolutionary constraints to sex ratios [10, 11]. By identifying the area of the sperm cell nucleus —indicator of the proportion of X- vs. Y- CBS in their ejaculates—as the morphometric trait responsible for the observed sex-ratio bias we show that the first causal factor for biases in offspring sex ratio lies in males. This finding points towards paternal distortions in the proportions of X- vs. Y-CBS at meiosis, or at sperm maturation, as an important driver underlying sex ratio variation in nature, although the underlying mechanism is largely unknown. This work challenges the traditional view in sex allocation theory, which largely disregards the role of fathers in sex ratio allocation and offers a new potential explanation for those cases in which there is sex ratio bias, such as in humans [42]. A shift from only maternal-oriented research to both paternal- and maternal-oriented research — both empirical and theoretical — will provide new insights into the evolution of sex ratio and sex-ratio allocation.

Data accessibility. Raw data will be uploaded before publication in the Dryad Digital Repository.

Author contributions. A.F.M. conceived the project, analysed the data and wrote the

manuscript, with contributions from all authors. A.F.M. and F.M.P initiated the research, analysed sperm samples and wrote the supplementary materials section. R.C.L., F.M.P., J.G., J.D.B. provided equipment, laboratory space and facilities. R.C.L. provided the study system, contributed data and insights on the study system. All co-authors contributed valuable discussions and relevant edits to the manuscript.

Competing Interests. We declare we have no competing interests.

Funding. AFM was supported by a MEC/Fulbright fellowship (FU2005-0893), a travel grant from the Ministry of Education and Science, a Marie Curie fellowship (PIEF-GA-2008-220322) and an ERC grant (249872). FMP was supported by the Juan de la Cierva program (Spanish Ministry of Education and Science), Ramon y Cajal program (RYC-2008-02560, MICINN) and two travels grants from the University of Castilla-La Mancha (Spain). FGG was supported by the Ramon y Cajal program, the Spanish Severo Ochoa Program (SEV-2012-0262), and by a grant (CGL2016-76173-P, co-funded by the European Regional Development Fund) from the Spanish Ministry of Economy.

Acknowledgements. We dedicate this paper to Professor JoGayle Howard (deceased). We are grateful to Glen Alaks for assistance with data collection, and to Jean Dubach for her help with sample storage and shipping. We also thank Stuart West, Tom Pizzari and Alex Kacelnik for their comments on a previous draft.

Footnotes. Electronic supplementary material is available online.

References

1. Trivers R.L., Willard D.E. 1973 Natural-selection of parental ability to vary sex-ratio of offspring. *Science* **179**, 90-92. (doi:10.1126/science.179.4068.90).
2. West S.A. 2009 *Sex Allocation*. Princeton University Press.
3. Charnov E.L. 1982 *The Theory of Sex Allocation*, Princeton University Press.

- 324 4. Festa-Bianchet M. 1996 Offspring sex ratio studies of mammals: Does publication
325 depend upon the quality of the research or the direction of the results? *Écoscience* **3**,
326 42-44.
- 327 5. Hardy I.C.W. 1997 Possible factors influencing vertebrate sex ratios: an introductory
328 overview. *Appl. Anim. Behav. Sci.* **51**, 217-241. (doi:10.1016/S0168-1591(96)01106-
329 9).
- 330 6. Ferriere R., Belthoff J.R., Olivieri I., Krackow S. 2000 Evolving dispersal: where to
331 go next? *Trends Ecol. Evol.* **15**, 5-7.
- 332 7. Edwards A.M., Cameron E.Z. 2014 Forgotten fathers: paternal influences on
333 mammalian sex allocation. *Trends Ecol. Evol.* **29**, 158-164.
334 (doi:10.1016/j.tree.2013.12.003).
- 335 8. Berry D.P., Kearney J.F., Roche J.R. 2011 Evidence of genetic and maternal effects on
336 secondary sex ratio in cattle. *Theriogenology* **75**, 1039-1044.
337 (doi:10.1016/j.theriogenology.2010.11.011).
- 338 9. Chandler J.E., Taylor T.M., Canal A.L., Cooper R.K., Moser E.B., McCormick M.E.,
339 Willard S.T., Rycroft H.E., Gilbert G.R. 2007 Calving sex ratio as related to the
340 predicted Y-chromosome-bearing spermatozoa ratio in bull ejaculates.
341 *Theriogenology* **67**, 563-571. (doi:10.1016/j.theriogenology.2006.09.006).
- 342 10. Gomendio M., Malo A.F., Soler A.J., Fernandez-Santos M.R., Estes M.C., García
343 A.J., Roldan E.R.S., Garde J. 2006 Male fertility and sex ratio at birth in red deer.
344 *Science* **314**, 1445-1447.
- 345 11. Saragusty J., Hermes R., Hofer H., Bouts T., Goritz F., Hildebrandt T.B. 2012 Male
346 pygmy hippopotamus influence offspring sex ratio. *Nat. Commun.* **3**:697.
347 (doi:10.1038/ncomms1700.).
- 348 12. Douhard M., Festa-Bianchet M., Coltman D.W., Pelletier F. 2016 Paternal

- 349 reproductive success drives sex allocation in a wild mammal. *Evolution* **70**, 358-368.
 350 (doi:10.1111/evo.12860).
- 351 13. Neff B.D., Pitcher T.E. 2005 Genetic quality and sexual selection: an integrated
 352 framework for good genes and compatible genes. *Mol. Ecol.* **14**, 19-38.
 353 (doi:10.1111/j.1365-294X.2004.02395.x).
- 354 14. Keller L.F., Waller D.M. 2002 Inbreeding effects in wild populations. *Trends Ecol.*
 355 *Evol.* **17**, 230-241.
- 356 15. Malo A.F., Martinez-Pastor F., Alaks G., Dubach J., Lacy R.C. 2010 Effects of genetic
 357 captive-breeding protocols on sperm quality and fertility in the white-footed mouse.
 358 *Biol. Reprod.* **83**, 540-548. (doi:10.1095/biolreprod.110.085316).
- 359 16. Michalczyk L., Martin O.Y., Millard A.L., Emerson B.C., Gage M.J.G. 2010
 360 Inbreeding depresses sperm competitiveness, but not fertilization or mating success in
 361 male *Tribolium castaneum*. *Proc. R. Soc. Lond. B* **277**, 3483-3491.
 362 (doi:10.1098/rspb.2010.0514).
- 363 17. Reid J.M., Arcese P., Keller L.F. 2006 Intrinsic parent-offspring correlation in
 364 inbreeding level in a song sparrow (*Melospiza melodia*) population open to
 365 immigration. *Am. Nat.* **168**, 1-13. (doi: 10.1086/504852).
- 366 18. Nietlisbach P., Keller L.F., Postma E. 2016 Genetic variance components and
 367 heritability of multiallelic heterozygosity under inbreeding. *Heredity* **116**, 1-11.
 368 (doi:10.1038/hdy.2015.59).
- 369 19. Johnson L.A., Flook J.P., Hawk H.W. 1989 Sex preselection in rabbits - live births
 370 from X-sperm and Y-sperm separated by DNA and cell sorting. *Biol. Reprod.* **41**, 199-
 371 203. (doi:10.1095/biolreprod41.2.199).
- 372 20. Zhuang X.J., Lu Y.Q., Zhang M., Lu S.S., Lu K.H. 2011 Microisolation and
 373 microcloning of bovine X-chromosomes for identification of sorted buffalo (*Bubalus*

- 374 *bubalis*) spermatozoa. *Anim. Reprod. Sci.* **126**, 32-36.
 375 (doi:10.1016/j.anireprosci.2011.04.021).
- 376 21. Parati K., Bongioni G., Aleandri R., Galli A. 2006 Sex ratio determination in bovine
 377 semen: A new approach by quantitative real time PCR. *Theriogenology* **66**, 2202-
 378 2209. (doi:10.1016/j.theriogenology.2006.07.007).
- 379 22. Cui K. 1997 Size differences between human X and Y spermatozoa and
 380 prefertilization diagnosis. *Mol. Hum. Reprod.* **3**, 61-67.
- 381 23. Penfold L.M., Holt C., Holt W.V., Welch G.R., Cran D.G., Johnson L.A. 1998
 382 Comparative motility of X and Y chromosome-bearing bovine sperm separated on the
 383 basis of DNA content by flow sorting. *Mol. Reprod. Dev.* **50**, 323-327.
- 384 24. Lambin X. 1994 Sex-ratio variation in relation to female philopatry in Townsend
 385 voles. *J. Anim. Ecol.* **63**, 945-953.
- 386 25. Lambin X. 1994 Natal philopatry, competition for resources, and inbreeding
 387 avoidance in Townsend's voles (*Microtus-townsendii*). *Ecology* **75**, 224-235.
- 388 26. Armitage K.B. 1987 Do female yellow-bellied marmots adjust the sex-ratios of their
 389 offspring. *Am. Nat.* **129**, 501-519. (doi:10.1086/284654).
- 390 27. Rosenfeld C.S., Roberts R.M. 2004 Maternal diet and other factors affecting offspring
 391 sex ratio: A Review. *Biol. Reprod.* **71**, 1063-1070.
 392 (doi:10.1095/biolreprod.104.030890).
- 393 28. Kruuk L.E.B., Clutton-Brock T.H., Albon S.D., Pemberton J.M., Guinness F.E. 1999
 394 Population density affects sex ratio variation in red deer. *Nature* **399**, 459-461.
- 395 29. Lacy R.C., Alaks G., Walsh A. 2013 Evolution of *Peromyscus leucopus* mice in
 396 response to a captive environment. *Plos One* **8**. doi:10.1371/journal.pone.0072452).
- 397 30. Chandler J.E., Canal A.M., Paul J.B., Moser E.B. 2002 Collection frequency affects
 398 percent Y-chromosome bearing sperm, sperm head area and quality of bovine

- ejaculates. *Theriogenology* **57**, 1327-1346. (doi:10.1016/S0093-691x(01)00721-X).
31. LaMunyon C.W., Ward S. 1997 Evolution increased competitiveness of nematode sperm bearing the male X chromosome. *Proc. Natl Acad. Sci. USA* **94**, 185-189.
32. Jaenike J. 2001 Sex chromosome meiotic drive. *Annu.Rev. Ecol. Evol. Syst.* **32**, 25-49. (doi:DOI 10.1146/annurev.ecolsys.32.081501.113958).
33. Frankham R., Wilcken J. 2006 Does inbreeding distort sex-ratios? *Conserv. Gen.* **7**, 879-893.
34. Martinez F., Kaabi M., Martinez-Pastor F., Alvarez M., Anel E., Boixo J.C., de Paz P., Anel L. 2004 Effect of the interval between estrus onset and artificial insemination on sex ratio and fertility in cattle: a field study. *Theriogenology* **62**, 1264-1270. (doi: 10.1016/j.theriogenology.2004.01.002).
35. Wolff J.O. 1989 Social behavior. In *Advances in the study of Peromyscus (Rodentia)* (eds. Kirkland G.L.J., Layne J.N.), pp. 271-291. Lubbock, Texas, USA., Texas Tech University Press.
36. Malo A.F., Godsall B., Prebble C., Grange Z., McCandless S., Taylor A., Coulson T. 2013 Positive effects of an invasive shrub on aggregation and abundance of a native small rodent. *Behav. Ecol.* **24**, 759-767. (doi:10.1093/beheco/ars202).
37. Krackow S. 1997 Maternal investment, sex-differential prospects, and the sex ratio in wild house mice. *Behav. Ecol. Sociobiol.* **41**, 435-443. (doi:10.1007/s002650050404).
38. Williams G.C. 1979 The question of adaptive sex ratio in outcrossed vertebrates. *Proc. R. Soc. Lond. B* **205**, 567-580. (doi:10.1098/rspb.1979.0085).
39. Brown G.R., Silk J.B. 2002 Reconsidering the null hypothesis: Is maternal rank associated with birth sex ratios in primate groups? *Proc. Natl Acad. Sci. USA* **99**(17), 11252-11255. (doi:DOI 10.1073/pnas.162360599).
40. Sheldon B.C., West S.A. 2004 Maternal dominance, maternal condition, and offspring

- sex ratio in ungulate mammals. *Am. Nat.* **163**, 40-54.
41. Oli M.K. 2004 The fast-slow continuum and mammalian life-history patterns: an empirical evaluation. *Basic Appl. Ecol.* **5**(5), 449-463. (doi:DOI 10.1016/j.baae.2004.06.002).
42. Austad S.N. 2015 The human prenatal sex ratio: A major surprise. *Proc. Natl Acad. Sci. USA* **112**, 4839-4840. (doi:10.1073/pnas.1505165112).
43. Fawcett D.W., Anderson W.A., Phillips D.M. 1971 Morphogenetic factors influencing shape of sperm head. *Dev. Biol.* **26**, 220-253.
44. Pogany G.C., Corzett M., Weston S., Balhorn R. 1981 DNA and protein content of mouse sperm: Implications regarding sperm chromatin structure. *Exp. Cell Res.* **136**, 127-136.
45. Ward W.S., Coffey D.S. 1991 DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. *Biol. Reprod.* **44**, 569-574.
46. Balhorn R. 1982 A model for the structure of chromatin in mammalian sperm. *J Cell Biol.* **93**, 175-181.
47. Noblanc A., Kocer A., Drevet J.R. 2014 Recent knowledge concerning mammalian sperm chromatin organization and its potential weakness when facing oxidative challenge. *Basic Clin. Androl.* **24**, 1-12.
48. Zalenskaya I.A., Zalensky A.O. 2004 Non-random positioning of chromosomes in human sperm nuclei. *Chrom. Res.* **12**, 163-173.
49. Cacciola G., Chioccarelli T., Altucci L., Viggiano A., Fasano S., Pierantoni R., Cobellis G. 2013 Nuclear size as estrogen-responsive chromatin quality parameter of mouse spermatozoa. *Gen. Comp. Endocrinol.* **19**, 201-209.
50. Silkaitis K., Lemos B. 2014 Sex-biased chromatin and regulatory cross-talk between sex chromosomes, autosomes, and mitochondria. *Biol. Sex Diff.* **5**, 1-14.

Box 1.

Evidence supporting that the presence of either sex chromosome drives variation in sperm nucleus size (area and length)

It has been recognized for a long time that internal and not external forces influence the shape of the sperm nucleus [43]. In sperm, DNA reaches highest degrees of compaction. The fact that there is no unused space inside sperm cells together with the evolution of DNA-packaging mechanisms inside the sperm nucleus is proof for the presence of very strong selective forces for volume reduction. In mice, if the DNA packaging in the sperm cells used nucleosomes (as in the rest of cell types) instead of protamines, it would require 213% of the total nuclear volume [44,45].

Variation in volume of CBS and chromatin can be reflected in the nucleus area. Likewise, differences in the length of the sperm head can reflect differences in X vs. Y-chromosomes and their compacting material because 1) protamine-DNA complex is arranged inside the nucleus lying lengthwise inside the minor groove [46] and 2) sex chromosomes are positioned in repeatable, non-intertwined positions along the antero-posterior axis of the sperm nucleus, which are conserved within individuals [47,48]. Furthermore, there is evidence that chromatin condensation influences the shape of the nucleus [43], and that in mice sperm nucleus size and elongation reflects the chromatin content [49], which in turn varies between X and Y chromosomes [50].

Figures

Figure 1. Relationship between a father’s mean sperm nucleus area (or nucleus length; inlet graph) and its offspring sex-ratio. Each data point reflects the mean calculated using a minimum of 200 sperm per male. Two outlier data points for sperm nucleus area were removed (2.85 SD and 3.12 SD away from the mean sperm nucleus area). N=38.

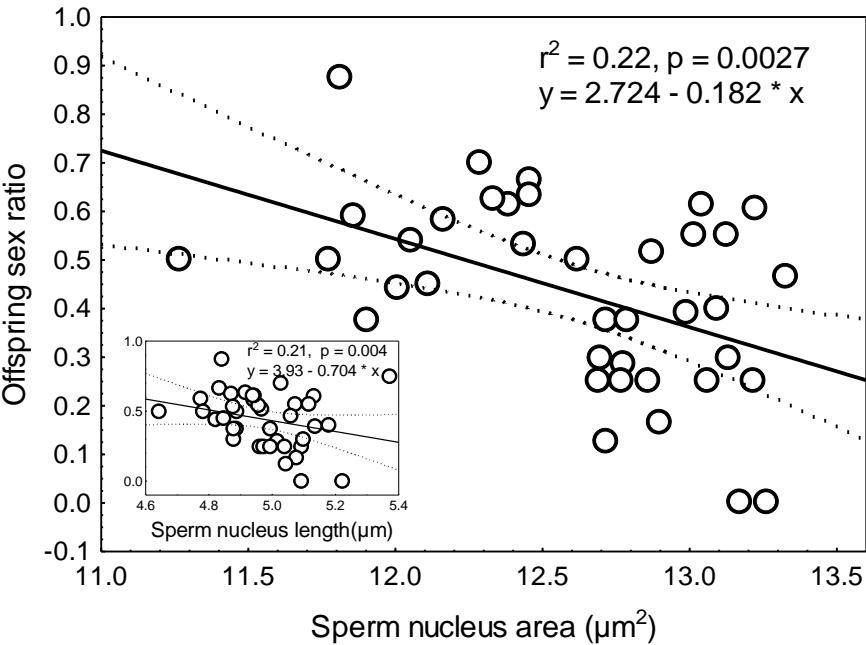


Figure 2. Relationship between the father’s coefficient of inbreeding (f) and offspring sex-ratio. Each data point is weighted by the total number of offspring that a male sired. N=38.

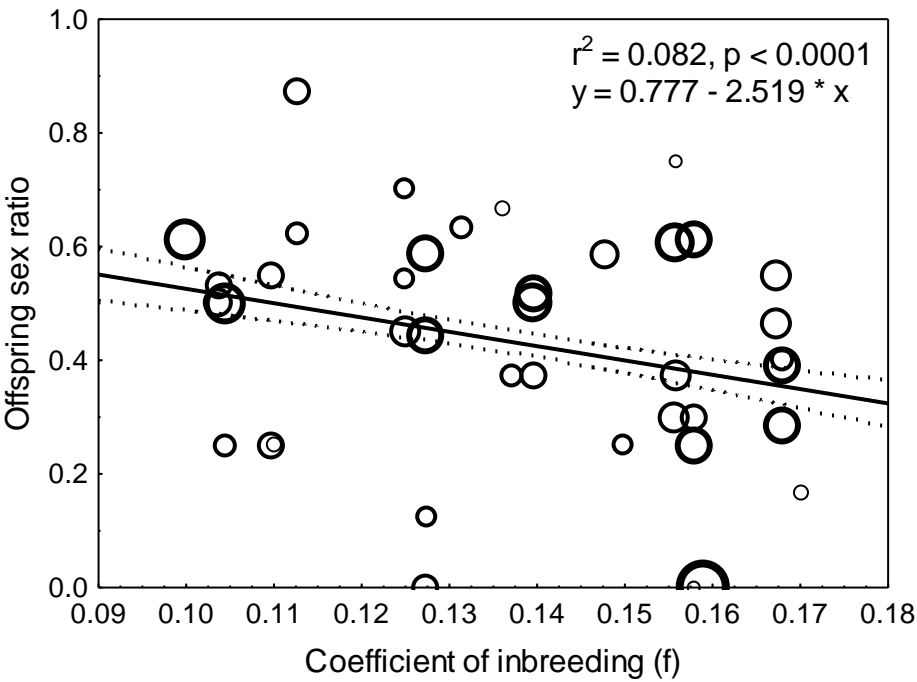
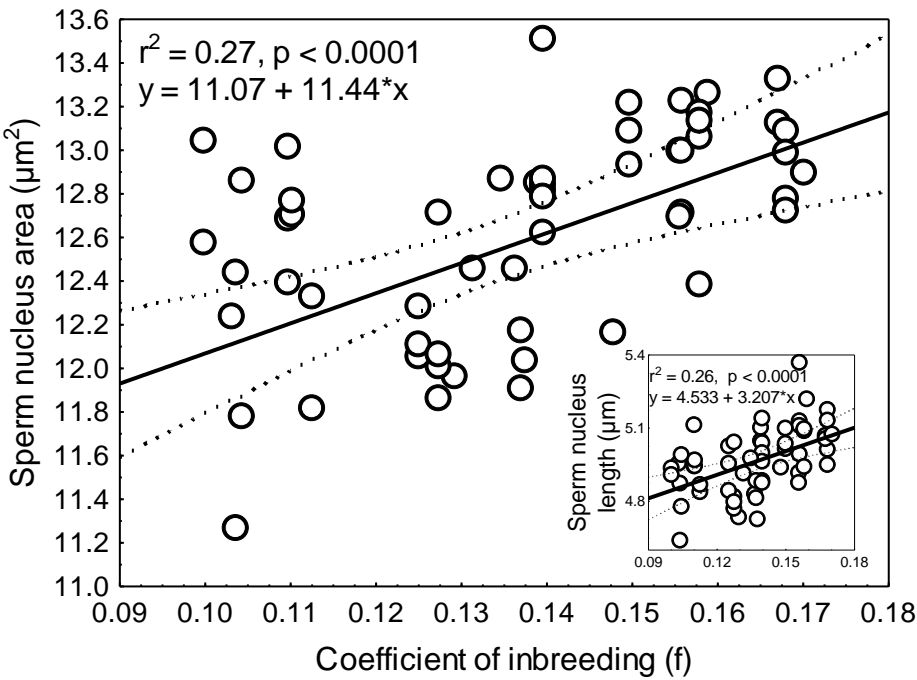


Figure 3. Relationship between the father’s coefficient of inbreeding (f) and the sperm nucleus area (or nucleus length; inset graph). Each data point reflects the mean calculated using a minimum of 200 sperm per male. Two outlier data points for sperm nucleus area were removed (2.85 SD and 3.12 SD away from the mean sperm nucleus area). N=58.



Tables

Table 1. Descriptive statistics for the continuous variables included in the different models conducted. *Sire f* and *dam f* stand for the coefficient of inbreeding of the father and the mother, respectively. The models vary in sample sizes for the following reasons: total number of individuals = 58, total number males producing litters = 40, and two of the males that produced litters were outliers for the variable sperm nucleus area (described in the main text); these males were excluded from the analyses and consequently they are also excluded from the summary statistics.

	Mean	SD	Range min-max	n
Offspring sex ratio	0.43	0.21	0-0.88	38
Sperm nucleus area (µm)	12.54	0.51	11.3-13.5	38
Sperm nucleus length (µm)	4.94	0.12	4.64-5.14	38
<i>Sire f</i>	0.136	0.021	0.099-0.170	58
<i>Dam f</i>	0.134	0.026	0.097-0.170	58
Total number of offspring	9.7	2.7	5-17	40
Age sire (days)	204	20.5	153-249	58

Table 2. Full and minimum adequate (final) models testing the effects of different paternal and maternal drivers on litter sex ratio. *Sire f* and *dam f* represent the coefficient of inbreeding of the father and mother, respectively.

Full model term	Estimate	se	t	p
Intercept	2.275	1.250	1.819	0.078
<i>Sire f</i>	-2.105	3.760	-0.56	0.579
<i>Sperm nucleus area</i>	-0.158	0.073	-2.17	0.038
Protocol	-	-	0.19	0.819
Age	0.003	0.001	1.50	0.143
Number offspring	0.008	0.011	0.72	0.476
<i>Dam f</i>	-1.728	4.543	-0.38	0.706

Final model term	Estimate	se	t	p
Intercept	2.723	0.711	3.826	0.0004
<i>Sperm nucleus area</i>	-0.181	0.056	-3.222	0.0026

Full model: Null deviance: 1.390 on 37df. Residual deviance: 0.959 on 30df. AIC: -13.97.

Final model: Residual deviance: 1.078 on 36df. AIC: -21.502.