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Experimental evidence for homeostatic sex allocation after sex-biased reintroductions

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First principles predict negative frequency-dependent sex allocation, but it is unproven in field studies and seldom considered, despite far-reaching consequences for theory and practice in population genetics and dynamics as well as animal ecology and behaviour. Twenty-four years of rhinoceros calving after 45 reintroductions across southern Africa provide the first *in situ* experimental evidence that unbalanced operational sex ratios predicted offspring sex and offspring sex ratios. Our understanding of population dynamics, especially reintroduction and invasion biology, will be significantly impacted by these findings.

Fisher's principle[1] for sex ratio parity was the world's first obligate evolutionary stable strategy. It then developed into the first ever facultative sex allocation theory[2,3], and was the precursor to game theory[4]—all fundamental to understanding population dynamics and genetics, ecology and behaviour. Importantly, it has the potential to substantially modify predictions of species invasiveness or recolonization, recovery from catastrophic declines, and resilience to habitat fragmentation and small population size[5,6]. With unprecedented numbers of species facing extinction, testing Fisher's principle and its application is critical, especially *in situ*.

Fisher's principle predicts a negative frequency-dependent, or homeostatic, sex allocation (HSA) response to an unbalanced operational sex ratio (OSR; the ratio of sexually competing males and females) that weakens as the OSR approaches parity[3]. These predictions cannot be tested by observational, single-population studies because the null model—random Mendelian sex chromosome assortment—also predicts fluctuations in OSR that converge on parity, and those fluctuations are insufficiently extreme to initiate an HSA response. Thus, distinguishing the null model from HSA requires that OSRs be manipulated experimentally. But such experiments are notoriously difficult, especially *in situ*, where several sex allocation influences and mechanisms might interact, and especially with large vertebrates, because they require independently replicated OSR treatments across multiple populations[7]. Unsurprisingly, therefore, robust *in situ* experiments of HSA have not yet been achieved (see Supplementary Table 1) and no experiment, either *in* or *ex situ*, has yet tested for the second prediction.

Fortunately, the translocation of organisms for species rescue and restoration are powerful field experiments of evolutionary and ecological theories, including those for facultative sex allocation. Species reintroductions—the release of animals into their historic range from where they had been extirpated—such as those for the critically endangered black rhinoceros (*Diceros bicornis*, Rhinocerotidae), can be replicated manipulations of OSR among released cohorts (different populations) across a large range of environmental conditions (different sites) to test for HSA *in situ*. Moreover, black rhinoceros have comparatively low adult mortality and dispersal distances, and long reproductive lives (senescence after 35 years) relative to the time required for offspring sexual recruitment (~5 years), such that several generations overlap and OSR biases are persistent[8]—meeting the criteria for a test of HSA[9]. Black rhinoceros are asocial and polygynous. Males are 11% heavier than females and the more dispersive sex, while females produce a single offspring per pregnancy[8], thereby negating the need to consider brood-size effects.

The OSRs of 45 reintroductions including 103 female black rhinoceros across southern Africa (1981–2005) varied between the possible extremes, sometimes without adult males or females (breeders of the absent sex were juveniles at release or arrived later as supplementary introductions). The other cohorts' OSRs varied from 17 to 75% adult males. The sexes of subsequent calves were also known. If HSA occurs then a sex bias among breeders after reintroduction should be followed by an equal but opposite sex bias among offspring, with the strength of the effect weakening for OSRs nearer parity.

We report the first *in situ* experimental evidence for HSA from multi-population, independently replicated OSR treatments of varying magnitude. Calf sex ratios were negatively frequency-dependent with OSRs, and the effect was weaker for populations closer to OSR parity. The relationships for populations and individual mothers were statistically different from those derived by randomized Mendelian sex allocation (our null model), despite the number of male and female calves born across all reintroductions being similar (152 males and 134 females; binomial test $P = 0.31$).

The OSRs of reintroduced cohorts were predictive of populations' calf sex ratios (linear regression: co-efficient ± standard error (SE), -0.498 ± 0.202, $F_{1,33} = 6.09$, $P = 0.019$, $R^2 = 0.16$, for all populations with $n \geq 2$ calves; Fig. 1a) and subsequent calf sexes (logistic regression: co-efficient ± SE, -1.706 ± 0.705, $z = -2.420$, $P = 0.013$, Fig. 1b). A similar, but weaker, relationship was found between calf sex and OSRs at time of calf conception (logistic regression: co-efficient ± SE, -1.609 ± 0.894, $z = -1.799$, $P = 0.072$, Fig. 1c) because the OSRs when calves were conceived were less variable than the OSRs of the reintroduced cohorts (that is, the coefficient of variation at conception was 33% as opposed to 48% at reintroduction) and extreme OSRs were more predictive of calf sex, whereas OSRs near parity were not predictive (Fig. 1d). The less variable OSRs at conception can, in part, be attributed to calf recruitment, but deaths or removals, and supplementary introductions, continued to modify OSR in both directions in these small populations after the founding cohort was released, thus preventing a simple OSR convergence with parity over time.

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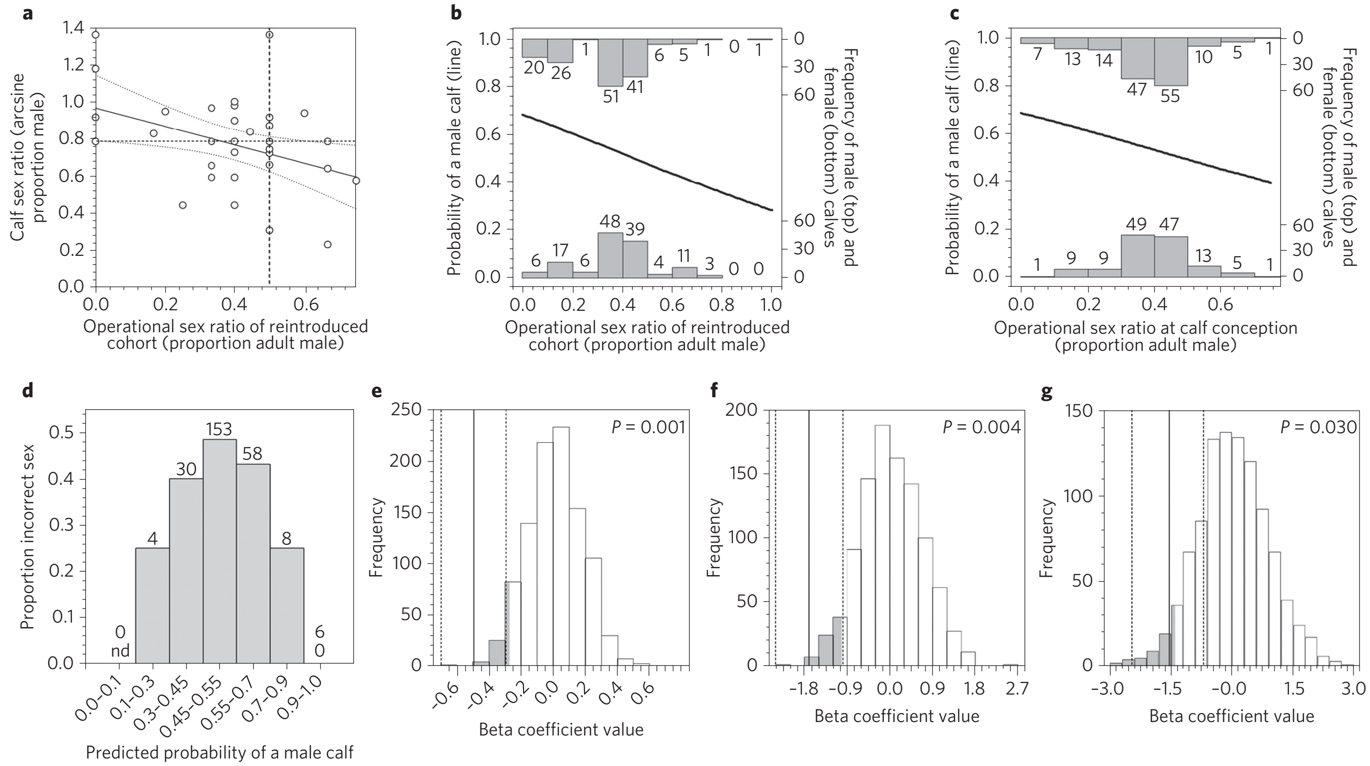

Figure 1 | Sex allocation pattern and effect size. **a**, Calf sex ratios in 35 black rhinoceros populations (that produced at least 2 calves) reintroduced at varying operational sex ratios (OSR). Sex ratio parity for populations and calves is represented by the vertical and horizontal dashed lines, respectively. The linear relationship (linear regression: co-efficient ± standard error (SE), −0.498 ± 0.202, $F_{1,33} = 6.09$, $P = 0.02$, $R^2 = 0.16$) is indicated by the solid line (with the dotted lines indicating its 95% confidence interval). The response variable has been transformed using the arcsine square root transformation for proportionate data. **b**,**c**, The probability of male calves ($n = 286$) in relation to the OSR of the released cohort (logistic regression: co-efficient ± SE, −1.706 ± 0.705, $z = -2.420$, $P = 0.013$) (**b**) and when each calf was conceived (logistic regression: co-efficient ± SE, −1.609 ± 0.894, $z = -1.799$, $P = 0.072$) (**c**). **d**, The predictive error (proportion incorrect sex) of the logistic model in **b** for calf sex (probability of being male) showing the accuracy of the model improves as OSR bias increases in both directions. There were no calves born that had a predicted probability of being a male calf between 0.0 and 0.1, hence 'nd' refers to there being no data for this interval. There were six calves born where the predicted probability of a male calf was between 0.9 and 1.0 and none of those predicted cases were female. **e**–**g**, The frequency distributions (corresponding to **a**–**c**) of 1,000 beta coefficients after the random allocation of calf sex (our Mendelian null model) for the relationship of populations' calf sex ratios (where $n \geq 2$) against the OSR of the reintroduced cohort (**e**), and logistic regressions of calf sex against the OSR of the reintroduced cohort (**f**) and at calf conception dates (**g**). The grey bars indicate the 5% (one-sided test, $\alpha = 0.05$) of beta coefficients that were most negative. The vertical solid lines show the beta coefficients of the relationships from observed data, and dotted lines their 95% confidence interval. The *P* values displayed are the proportions of 1,000 regressions of randomly allocated calf sex whose beta coefficients were the same or more extreme than our observed values.

These population and individual relationships were statistically different from those expected from random Mendelian sex allocation. One thousand simulations in which calf sex was randomized and the linear regressions of population calf sex ratios against OSR of the reintroduced cohort were repeated revealed that almost all (99.9%) of the relationships were weaker than from the observed data (Fig. 1e). Simulations of the logistic regressions of randomized calf sex against the OSR of the reintroduced cohort and at calf conceptions also yielded weaker relationships compared to those observed from the data: 99.6% (Fig. 1f) and 97% (Fig. 1g), respectively.

OSR was the leading and majority influence on individual calf sex in the logistic mixed models without recourse to grouping (random) effects such as site, cohort and birth order. Contrary to the expectations of other condition-dependent sex allocation theories (such as Trivers-Willard and Local Resource Competition), environmental and maternal differences were not important.

The regression of populations' calf sex ratios against reintroduction OSR will be sensitive to sample (population) number and the size of each population (number of calves) because smaller populations of calves introduce greater sex ratio variance. Nonetheless, including the 10 populations with only one calf or reducing population number by excluding the populations with the fewest calves did not change its relationship or statistical support (Supplementary Table 2). Over half of the smaller calf populations (24 (53%) with fewer than 6 calves or 26 (58%) with fewer than 7 calves) had to be removed from the regression before the relationship's statistical support deteriorated substantially — but, even then, randomization tests of the truncated data sets demonstrated that more negative beta coefficients than found from the observed data were still unlikely to occur by chance.

For the first time, we have detected an HSA effect at population and individual-animal scales *in situ*. We also measured a decline in the magnitude of that effect as OSRs approach parity, which explains why HSA has not previously been detected *in situ* in the absence of experimental and extreme OSR treatments that are independently replicated across populations. Nonetheless, the intrinsic mechanisms for HSA are still to be elucidated and the fitness benefits quantified. Although theory predicts fitness benefits from HSA where OSR fluctuates between extremes in space and time[4], even greater longitudinal data than the nine generations presented here are required from the long-lived rhinoceros to confirm theory. Moreover, for HSA to evolve, parents (most likely the female in polygynous species) must have one or more physiological (endogenous) mechanism(s) that respond to OSR. The most likely mechanisms are those in which the OSR determines levels of intrasexual competition and inter-sexual aggression for parents with physiological and ecological consequences for rhinoceros mothers[10,11] (as for lizards and plants[12,13]), especially around or soon after conception and before conceptus implantation[14].

Explaining the allocation of resources by parents among male and female offspring is a leading problem in evolutionary biology with major implications and diverse applications[7]. Extreme OSRs commonly occur[15–17] and so the occurrence of HSA is important for predicting the outcomes of an enormous range of ecological processes, including species invasion, recolonization and survival after catastrophe (for example, climate and disease), habitat fragmentation and population exploitation (harvest), and the influence of small population size, symbionts, parasites and predators, and conservation management that may induce extreme spatial or temporal variation in OSRs[5,6,18,19].

Where HSA is possible, parents capable of producing the rarer sex will achieve greater fitness, and populations of them will be more invasive and resilient because their small populations will have improved establishment and greater viability, especially in species with sex-biased dispersal. Such species will populate habitats faster, and be less susceptible to demographic stochasticity and genetic drift as small populations, because demographic variance is more quickly ameliorated. But almost all population models for invasiveness, viability and extinction risk, even those treating the interaction between OSR and mating systems, assume birth sex ratio is fixed[6,15,16]. Our evidence indicates that this may not be the case.

We have demonstrated that species management, such as reintroductions, can be powerful experiments in life-history and population dynamics at a scale necessary to test theory but ordinarily unachievable. As such, they are a remarkable, largely untapped resource for testing sex allocation theory in particular. The corollary of their use in this way is that reintroduction and invasion biology, theory and practice will be substantially modified by our *in situ* evidence for HSA.

Methods

Data reporting. The sexes of 286 calves born to 103 female black rhinoceros (*Diceros bicornis*) after 45 reintroductions across southern Africa, 1981–2005, and the populations' OSR (sexually mature, >4 years old) at reintroduction and thereafter were known[10]. Individual female calving records ranged from 18 months and one calf to 24 years and nine calves, and post-release conception dates from 52 days to 22.7 years. Thirty-five calves were removed from the data set because their sex had not yet been reported by the end of 2005, or calf death and carcass decay or disappearance occurred before it could be sexed.

Statistical analyses. Two analyses were performed to test for HSA: the ability of population OSR to explain calf sex and populations' calf sex ratios. First, we linearly regressed the arc-sin-square-root transformed sex ratio of each population's calves after reintroduction against the OSR of the reintroduced cohort. HSA predicts a negative relationship between OSR and populations' calf sex ratios. Second, we built two mixed-effects logistic regression models with calf sex as the binary dependent variable ($0 =$ female, $1 =$ male), and (i) reintroduction cohort OSR or (ii) OSR at calf conception as a fixed effect. Conception times for each calf were calculated by back-dating gestation length (460 days[14]) from estimated birth dates at first post-birth observation. Twenty-seven mothers' first calves after reintroduction were conceived before translocation. For those calves, the OSR of the reintroduced cohort is used to represent OSR conditions during calf gestation.

Random effects for maternal identity nested within reintroduced release site and cohort and also calf birth order after reintroduction were included in logistic models because each reintroduction cohort could include from 1 to 8 mothers, and those mothers contributed from 1 to 9 calves. We tested the influence of random effects using calculated repeatability[20]. The variances attributed to random effects for release site and cohort, mother and calf birth order were small (release site and cohort/mother $= 0.07 \pm 0.27$; calf birth order $= 0.02 \pm 0.14$) such that repeatability was low (release site and cohort/mother $= 0.021$; calf birth order $= 0.006$), and inclusion of these random effects did not improve the power of OSR at reintroduction to predict calf sex. The model for calf sex with OSR without random effects was also a substantial improvement on the intercept-only model (logistic regression (intercept only): $z = 1.064$, $P = 0.287$, $\Delta\mathrm{AIC}_c$ (small sample Akaike Information Criterion) $= 4.46$). Thus, we removed random effects and repeated the logistic regression for our final models.

Tests against the null model (random Mendelian sex allocation). The robustness of regression relationships was tested by reallocating calf sex randomly to each mother at a 1.13:1.00 male:female sex ratio (representing the 152 male and 134 female calves born during this study, and the birth sex ratio in wild populations) 1,000 times and repeating the regressions for each reallocation. The frequency distribution of resulting regression (beta) coefficients was compared with our regression coefficients to evaluate the likelihood of the relationships occurring by chance. We also repeated the statistical analyses and randomization tests by including the 10 populations with only one calf, or progressively excluding the smallest populations with only two, three, four, five or six and fewer calves to evaluate the sensitivity of statistical outcomes to reduced sample number and within-population variance.

Data availability. The data sets analysed during the current study are not publicly available to protect the identities and locations of rhinoceros populations that are the subject of illegal hunting and trade. However, the data set without reserve identities or locations is available from the corresponding author on reasonable request.

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References

1. Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon, 1930).
2. Werren, J. H. & Charnov, E. L. *Nature* **272,** 349–350 (1978).
3. Taylor, P. D. & Sauer, A. *Am. Nat.* **116,** 305–310 (1980).
4. Hamilton, W. D. *Science* **156,** 477–488 (1967).
5. Miller, T. E. X., Shaw, A. K., Inouye, B. D. & Neubert, M. G. *Am. Nat.* **177,** 549–561 (2011).
6. Lee, A. M., Saether, B. E. & Engen, S. *Am. Nat.* **177,** 301–313 (2011).
7. West, S. *Sex Allocation* (Princeton Univ. Press, 2009).
8. Owen-Smith, R. N. *Megaherbivores: The Influence of Large Body Size on Ecology* (Cambridge Univ. Press, 1988).
9. Williams, G. C. *Proc. R. Soc. B-Biol. Sci.* **205,** 567–580 (1979).
10. Linklater, W. *et al. PLoS ONE* **7,** e30664 (2012).
11. Linklater, W., MacDonald, E., Flamand, J. & Czekala, N. *Animal Conserv.* **13,** 104–111 (2010).
12. Lopez, S. & Dominguez, C. A. *J. Evol. Biol.* **16,** 1177–1185 (2003).
13. Olsson, M. & Shine, R. *J. Evol. Biol.* **14,** 120–128 (2001).
14. Linklater, W. *Reprod. Fertil. Dev.* **19,** 831–839 (2007).
15. Caswell, H. & Weeks, D. E. *Am. Nat.* **128,** 707–735 (1986).
16. Lindstrom, J. & Kokko, H. *Proc. R. Soc. B-Biol. Sci.* **265,** 483–488 (1998).
17. Ranta, E., Lummaa, V., Kaitala, V. & Merila, J. *Ecol. Lett.* **3,** 30–34 (2000).
18. Gutowsky, L. F. G. & Fox, M. G. *Hydrobiologia* **671,** 27–37 (2011).
19. Hornett, E. A., Charlat, S., Wedell, N., Jiggins, C. D. & Hurst, G. D. D. *Curr. Biol.* **19,** 1628–1631 (2009).
20. Nakagawa, S. & Schielzeth, H. *Biol. Rev. Cambridge Phil. Soc.* **85,** 935–956 (2010).

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Author contributions

W.L.L. conceived the project; P.d.P. administered the programs that gathered the raw data from Namibian populations; W.L.L. and J.V.G. collated and conducted quality assurance of data; W.L.L., P.R.L. and J.V.G. conducted and interpreted analyses; W.L.L. wrote the first draft of the manuscript and all authors contributed to revisions.

Additional information

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Competing interests

The authors declare no competing financial interests.