Male Fertility and Sex Ratio at Birth in Red Deer

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Efforts to test sex ratio theory have focused mostly on females. However, when males possess traits that could enhance the reproductive success of sons, males would also benefit from the manipulation of the offspring sex ratio. We tested the prediction that more-fertile red deer males produce more sons. Our findings reveal that male fertility is positively related to the proportion of male offspring. We also show that there is a positive correlation between the percentage of morphologically normal spermatozoa (a main determinant of male fertility) and the proportion of male offspring. Thus, males may contribute significantly to biases in sex ratio at birth among mammals, creating the potential for conflicts of interest between males and females.

The Trivers and Willard hypothesis (1) for sex allocation predicts that parents should increase the production of the sex with the higher fitness benefit. This hypothesis has been applied most often to mothers, who have a strong influence on offspring quality through maternal care. It can also apply to any trait that parents transmit to offspring that has a differential effect on the reproductive success of sons and daughters. Thus, among birds, offspring sex ratios may be adjusted in relation to the attractiveness of the father, because sons will inherit large sexual ornaments and will achieve high reproductive success (2). However, it is assumed that such manipulation is under female control, because in birds females are the heterogametic sex.

The possibility that males may also facultatively adjust sex ratio has seldom been considered. In haplodiploid insects, the offspring sex depends on whether the ovum is fertilized or not, and males may constrain sex ratios because males with poor-quality ejaculates fail to fertilize the ovum (3). In mammals, although male fertility may have a great influence on the reproductive success of sons. Ungulates are good models to test sex ratio theory because they are sexually dimorphic in body size, variance in reproductive success is greater among males, and the reproductive success of sons is more strongly influenced by maternal investment. Early studies on red deer (Cervus elaphus) found support for the prediction that high-quality mothers should produce sons (4), but subsequent studies have generated inconsistent results (5). Our previous studies have shown that in natural populations of red deer, males differ markedly in their fertility rates, and more-fertile males have faster swimming sperm and a greater proportion of normal spermatozoa (6). Thus, male reproductive success may not depend exclusively on body size, but also on the ability of males to fertilize females after copulation. Male fertility is advertised by antler size and complexity, so more-fertile males also have larger and more elaborate sexual characters, which may be inherited by their sons (7).

We tested the hypothesis that more-fertile red deer males produce more sons. The key challenge was to disentangle male and female effects by designing an experiment to retain the inter-male variation in fertility rates found in natural populations while minimizing differences between females (8). Thus, our experimental design was aimed at eliminating several female factors known to influence sex ratios: (i) We avoided the possibility that females may bias sex ratio in response to male quality by artificially inseminating females so that they had no direct experience with the males. (ii) We minimized differences in body condition by using a sample of females that were all in good physical condition, were kept under similar environmental conditions, and had access to an unlimited food supply. (iii) All females were inseminated at the same time in relation to ovulation, avoiding the confounding effects of insemination time. In contrast, by using sperm collected during the rut from males living in natural populations, we ensured a representative sample of the large degree of variation in male fertility previously described (6).

When the entire study sample is considered, a similar number of male and female offspring were produced (Table 1). However, among males, differences in fertility rates and in the proportion of male offspring were substantial. Male fertility rates ranged from 24 to 70%, and the proportion of male offspring ranged from 25 to 72% (Table 1).

Table 1. Descriptive statistics [mean, standard deviation (SD), and range] for male fertility rates, proportion of male offspring sired, percentage of normal sperm, sperm swimming-velocity parameters, and number of hinds inseminated per male (n = 14 red deer stags). VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertility rate (%)</td>
<td>50.39</td>
<td>13.06</td>
<td>24–70</td>
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<td>Proportion of male offspring</td>
<td>0.50</td>
<td>0.14</td>
<td>0.25–0.72</td>
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<td>Morphologically normal spermatozoa (%)</td>
<td>80.07</td>
<td>8.78</td>
<td>65–95</td>
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<td>VCL (µm/s)</td>
<td>126.87</td>
<td>28.48</td>
<td>85–163</td>
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<tr>
<td>VSL (µm/s)</td>
<td>67.86</td>
<td>27.31</td>
<td>28–111</td>
</tr>
<tr>
<td>VAP (µm/s)</td>
<td>88.74</td>
<td>26.52</td>
<td>53–122</td>
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<tr>
<td>Hinds inseminated per male</td>
<td>24.57</td>
<td>16.00</td>
<td>11–69</td>
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</table>

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References

25. Materials and methods are available on Science Online.

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The frequency histogram of 1980 to 2000 rain is consistently above that of 1951 to 1970 rain for intensities greater than 70 mm/day (fig. S2B). The number of events larger than 70 mm/day shows a trend significant at the 0.1 significance level.

31. For each year, the calculation of percentiles is based on 122 days (monsoon season) and 143 grid points.
32. We thank the IMD for making the daily gridded rainfall data available, the Department of Ocean Development, Government of India, for partial support for this work, and J. Srinivasan for useful discussions.

Supporting Online Material
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Materials and Methods
Figs. S1 to S3
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There was a significant relation between male fertility and the proportion of male offspring sired (squared correlation coefficient $r^2 = 0.41, P = 0.013$). More-fertile males sired a greater number of sons, and less-fertile males sired more daughters (Fig. 1A). There was also a significant relation between the percentage of morphologically normal spermatozoa and the proportion of male offspring sired per male ($r^2 = 0.37, P = 0.021$) (Fig. 1B). In contrast, no significant relation was found between sperm velocity parameters and the proportion of male offspring sired ($P > 0.05$).

Thus, of the two main determinants of male fertility—sperm swimming velocity and the proportion of normal spermatozoa—the latter was found to be associated with sex ratio. This may be the case because the proportion of normal spermatozoa is more likely to be inherited by sons (9) than sperm swimming velocity, which may be influenced to a greater extent by environmental factors (10). Thus, males with a higher proportion of normal spermatozoa may benefit from producing sons who will inherit the trait that will increase their fertility, and they will thus achieve high reproductive success. In contrast, low-fertility males will benefit from producing daughters who will not inherit their father’s poor ejaculate quality.

There are two possible mechanisms by which males may adjust sex ratio. First, although it is assumed that mammalian males produce equal numbers of X- and Y-bearing spermatozoa as a consequence of meiotic cell division, ejaculates may differ in the proportion of Y-bearing spermatozoa (11), resulting in biases in sex ratio at birth. Thus, high- and low-fertility males could differ in the proportion of Y-bearing spermatozoa in the ejaculate. Second, Y-bearing spermatozoa could be at an advantage in relation to X-bearing spermatozoa when produced by more-fertile males, whereas the opposite may occur among less-fertile males. Differences between males in the competitiveness of X- and Y-bearing spermatozoa could arise through differential expression of genes carried in the sex chromosomes (12). Such postmeiotic expression of germ line–specific X- or Y-linked genes has recently been demonstrated (13) and could influence sperm shape, size, and function. Furthermore, it has recently been shown that males with deletions in the Y chromosome produce Y-bearing spermatozoa with morphological abnormalities that are less efficient at fertilization, resulting in sex ratio biases toward females (14). Thus, red deer males with low fertility rates may have a lower proportion of morphologically normal spermatozoa as a consequence of genetic information on the Y chromosome, which would also impair the chances of fertilization of Y-bearing spermatozoa. On the contrary, males with high fertility rates may produce more-competitive Y-bearing spermatozoa. Alternatively, females could influence the fertilization success of X- and Y-bearing spermatozoa depending on the fertility of the male. This would require that females be able to assess ejaculate quality (and more specifically the proportion of normal spermatozoa) and bias sex ratio accordingly, given that in our experimental design females were prevented from evaluating male quality or copulatory behavior. This hypothesis assumes that differences in fertilization success between X- and Y-bearing spermatozoa are caused, not by differences in competitiveness between them (as proposed by the previous hypothesis), but by female selection in the reproductive tract.

Our experimental approach reveals unexpectedly large differences in fertility rates between males from natural populations when females are artificially inseminated. Are such differences in male fertility likely to occur in natural contexts? In the wild, low-fertility males could compensate by transferring more spermatozoa per ejaculation. This is unlikely to occur because in natural populations, low-fertility males have smaller testes that produce fewer spermatozoa (6), a trait that is known to have a major influence on fertility (15). Thus, the differences in fertility rates when all females are inseminated with equal sperm numbers are likely to be exacerbated when differences in sperm numbers come into play in natural contexts. Alternatively, low-fertility males could enhance their fertilization success by copulating more often with the same female, but the opportunities to do so may be limited. Because low-fertility males have smaller antlers (7), their ability to defend females for a long period of time may be constrained. Furthermore, in Mediterranean populations food is scarce during the mating season, and males either defend harems or establish territories where food resources are concentrated (16). Females move between territories and harems while searching for food; thus, repeated copulations with the same female may be rare. Finally, frequent copulations may lead to sperm depletion among low-fertility males given their limited sperm numbers; there is evidence that in natural populations, frequent copulation leads to sperm depletion and decreases male siring success (17). Thus, in natural populations, differences in fertility rates are likely to contribute substantially to differences between males in lifetime reproductive success.

Our findings suggest that mammalian males can manipulate the sex ratio of their offspring, thus creating an unforeseen evolutionary scenario that includes conflicts of interest between males and females. For instance, a fertile male may benefit from producing sons, but the costs of raising a male may be high for a female in poor physical condition (18). This level of conflict may improve our ability to explain biases in sex ratio at birth.

References and Notes
8. Materials and methods are available as supporting material on Science Online.
WNT and DKK Determine Hair Follicle Spacing Through a Reaction-Diffusion Mechanism

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Mathematical reaction-diffusion models have been suggested to describe formation of animal pigmentation patterns and distribution of epidermal appendages. However, the crucial signals and in vivo mechanisms are still elusive. Here we identify WNT and its inhibitor DKK as primary determinants of murine hair follicle spacing, using a combined experimental and computational approach. Transgenic DKK overexpression reduces overall appendage density. Moderate suppression of endogenous WNT signaling forces follicles to form clusters during an otherwise normal morphogenetic program. These results confirm predictions of a WNT/DKK-specific RD model (SOM text 3), providing in vivo corroboration of the reaction-diffusion mechanism for epidermal appendage formation.

The development of regularly arranged body parts has long fascinated experimental biologists and theoreticians alike. One area of long-standing debate has been the formation of epidermal appendages such as feathers and hairs. Theoretical models have provided seemingly simple solutions to complex developmental processes (1); in order to achieve regular patterns, the reaction-diffusion (RD) hypothesis of Alan Turing postulates a pair of activator and inhibitor with special characteristics (2) [supporting online material (SOM) text 1]. However, it remains largely unclear whether such predictions can be substantiated in molecular and mechanistic terms (3). Because canonical WNT signaling is essential for the induction of hair and feather follicles (4, 5) and forced stimulation of this pathway is sufficient to induce supernumerous appendages (6, 7), the pathway represents an appealing candidate for the primary signal that dictates follicle distribution. Here we set out to analyze its role in hair follicle arrangement by verifying predictions of a biologically adapted RD model.

The WNT pathway is active from the earliest stages of follicular development (5, 8) (Fig. 1A). Expression of the WNT inhibitor Dkk1 is directly controlled by secreted WNTs (9, 10). Further aspects of this pathway and the RD mechanism are discussed in SOM text 2 and fig. S1. In developing murine skin, mesenchymal Dkk1 expression is found adjacent to the early hair follicle bud (5) (Fig. 1B), whereas Dkk4, a further functional inhibitor of WNT signaling (11, 12), shows strong epithelial expression at discrete loci before hair placode formation (Fig. 1C). Weak expression in the early hair follicle bud indicates that Dkk4 expression marks the forming follicle (Fig. 1C). Five LEF/TCF consensus binding motifs are found within 700 base pairs (bp) upstream of the transcriptional start site of Dkk4, and regulation of the promoter by the canonical WNT signaling pathway was suggested by transfection studies (Fig. 1D). Hence, the available data support the role of WNT and DKK as primary determinants of hair follicle spacing patterns.

If WNTs and WNT inhibitor(s) represent the two components required by the RD hypothesis, it should be possible to derive, from a WNT/DKK-specific RD model (SOM text 3), predictions about the outcome of experimental alterations of activating and inhibitory functions.

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Fig. 1. WNT signaling and expression of Dkk genes are associated with hair follicle formation. (A) WNT signaling in mesenchymal cells is associated with developing hair follicles (arrowhead). BATgal mice harboring a WNT-responsive lacZ gene were used as a reporter. (B) Mesenchymal Dkk1 expression adjacent to epithelial placodes and buds (arrowheads). (C) Strong epithelial Dkk4 expression at discrete loci prior to hair placode formation. Expression rapidly declines after follicle budding (arrow). (A to C) Scale bars, 100 μm. (D) Reporter gene expression [relative light units (RLUs) ± SEM] after endogenous (white) and stimulated (black) canonical WNT signaling. *P < 0.0001 (f test) for stimulated WNT signaling (black columns).