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Experimental alteration of litter sex ratios in a mammal

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Adaptive theory predicts that mothers would be advantaged by adjusting the sex ratio of their offspring in relation to their offspring's future reproductive success. Studies investigating sex ratio variation in mammals, including humans, have obtained notoriously inconsistent results, except when maternal condition is measured around conception. Several mechanisms for sex ratio adjustment have been proposed. Here, we test the hypothesis that glucose concentrations around conception influence sex ratios. The change in glucose levels resulted in a change in sex ratios, with more daughters being born to females with experimentally lowered glucose, and with the change in glucose levels being more predictive than the glucose levels *per se*. We provide evidence for a mechanism, which, in tandem with other mechanisms, could explain observed sex ratio variation in mammals.

Keywords: Trivers–Willard hypothesis; sex allocation; glucose; maternal investment

1. INTRODUCTION

Variation in the production of sons and daughters is a key variable in life-history and evolutionary theory, with sex ratio at birth and hatching varying considerably (Clutton-Brock & Iason 1986; Cameron 2004; Rosenfeld & Roberts 2004; Sheldon & West 2004). Adaptive hypotheses predict systematic variation in the sex ratio when the fitness returns of producing sons and daughters vary between individual parents (Trivers & Willard 1973; Clark 1978). The Trivers–Willard hypothesis (TWH; Trivers & Willard 1973) is the most influential of these hypotheses. The TWH posits that, if one sex has more variable reproductive success (males in polygynous species), then (i) mothers in good condition with more resources to allocate would be advantaged by producing sons, as highly competitive sons would out–reproduce highly competitive daughters, who are constrained to a less variable reproductive rate, and (ii) mothers with less resources to allocate would be advantaged by producing a daughter, since even a moderately successful daughter would out-reproduce an unsuccessful son. This hypothesis depends on three key assumptions: the condition of young at the end of maternal investment tends to be correlated with maternal condition; these differences in condition endure into adulthood; and the slight condition advantages will have a greater influence on male reproductive success (Trivers & Willard 1973; Hewison & Gaillard 1999).

This hypothesis is logically appealing and has been extensively tested in a wide variety of mammalian taxa (Cameron 2004; Sheldon & West 2004). In mammals, few studies have produced conclusive results either confirming or refuting this hypothesis, and the inconsistent results have proved difficult to interpret (Festa-Bianchet 1996; Brown 2001; Cameron 2004). However, two recent reviews have suggested that the most consistent support for the TWH in mammals occurs when condition scores are taken around conception, whereas condition scores taken at other times during the reproductive cycle provide less consistent support (Cameron 2004; Sheldon & West 2004). This suggests that sex ratio adjustment is most likely to occur around conception (Cameron 2004). In addition, some of the variation arises owing to inconsistencies in the condition measures themselves (Cameron 2004). Recent studies have also shown consistent support for the TWH where change in condition around conception is used as a variable, rather than absolute condition (Roche *et al.* 2006; Cameron & Linklater 2007), suggesting that change in condition might be an overlooked variable.

The lack of a known mechanism for sex ratio adjustment hampers our understanding and interpretation of results both in mammals and other taxa (e.g. birds; Pike & Petrie 2003). Several hypotheses in mammals focus on the differences around conception or early development in relation to hormone fluctuations (e.g. Grant 1996, 1998; James 1996, 2004) and asynchrony in early embryo development (e.g. Krackow 1995; Krackow & Burgoyne 1998; Forchhamer 2000). It is probable that more than one process could influence sex ratios (Sheldon & West 2004), and another mechanism by which sex ratios might be adjusted was suggested recently (Cameron 2004). Briefly, male and female conceptuses are sexually dimorphic in their response to glucose (Gutiérrez-Adán *et al.* 2001) and in their ability to survive in mediums with different glucose concentrations (Larson *et al.* 2001). Added glucose enhances the development of the male conceptus, but inhibits female conceptus growth and development. This

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explains a frequently observed phenomenon: the embryos become increasingly male biased as they develop from differentiated cells to expanded blastocyst *in vitro* but not *in vivo* (Catt *et al.* 1997; Pegoraro *et al.* 1998; Hasler *et al.* 2002). The glucose added to *in vitro* cultures enhances male conceptus growth and development, resulting in a difference in sex ratios between *in vivo* and *in vitro* raised conceptuses. Other lines of evidence also support the hypothesis (Cameron 2004). For example, studies investigating diabetes that induce an increase in circulating glucose result in male-biased sex ratios (Machado *et al.* 2001). Furthermore, a recent study showed that a high-fat diet resulted in more sons even when the total caloric value was the same as a low-fat diet (Rosenfeld *et al.* 2003). The role of glucose levels in offspring sex ratio has yet to be tested *in vivo*.

Dexamethasone (DEX) is a steroid that inhibits glucose transport and reduces plasma glucose concentrations (Hahn *et al.* 1999; Buren *et al.* 2002) with little impact on follicular development (Maciel *et al.* 2001). Social stress causes male-biased sex ratios in many species (Krackow & Hoeck 1989; Perret 1990) and DEX has been successfully used to reduce these stress-related litter changes (Pratt & Lisk 1990). While the study by Pratt & Lisk (1990) attributed the differences to a reduction in stress levels caused by DEX, it could be also due to glucose concentrations since stress results in higher levels of circulating glucose (Battilana *et al.* 2001).

We aimed to test the hypothesis that a change in circulating glucose concentration during early cell division can result in biased sex ratios in mice using DEX to reduce circulating glucose concentrations in the blood during early cell division. Previous studies have argued that the assumptions of the TWH hold in mice (e.g. Meikle & Westberg 2001) and significant sex ratio variation in line with the TWH has been reported (e.g. Rosenfeld *et al.* 2003).

2. MATERIAL AND METHODS

We used 40 mice of the National Medical Research Institute (NMRI) strain from the UK, which were laboratory bred at South African Vaccine Producers, Edenvale, Johannesburg. They were kept under 12 L : 12 D photoperiod in a temperature-controlled room, and provided with food and water ad libitum.

Nulliparous females were housed in pairs from 42 days of age. At 56 days of age, females were weighed and had a small blood sample taken from the ventral tail vein for blood glucose analysis using an Accu-Chek Advantage blood glucose test with Advantage II test strips. Males were now introduced and left in the cage with the two females for 3 days and nights, during which time mating occurred. Only two females failed to conceive, so we had a sample size of 18 DEX-treated females and 20 control females. While the male was present, 1.0 $\mu g\ ml^{-1}$ of DEX was added to the drinking water of the treatment females for 3 days following the introduction of the male, thereby including conception and early conceptus development, but very little gestation period. Although this leads to a variable dosage of DEX, it was preferable to injection, as the stress of handling and injection could result in the elevation of cortisol (Tornello *et al.* 1982; Pratt & Lisk 1990), which in turn would elevate glucose concentrations, thereby potentially negating the treatment effects. After 3 days, females were once again weighed and blood samples taken for glucose analysis. Males were removed from the cages and all water was replaced with fresh tap water.

Females were maintained individually until parturition 19–20 days later. As soon as possible after birth, we counted the number of pups in case infanticide occurred. No pups were lost and there was no evidence of infanticide. At 21 days after birth, the pups were sexed by anogenital distance and the presence of small scrotal sacs. Pups were sexed blind as to whether they were born to a treatment or control female.

We treated litters as replicates for statistical analysis. Therefore, all results are reported on the litter sex ratio, unless stated otherwise. All tests were two tailed and means reported $\pm$ 1 s.e. Logistic regression was used to model the effects of G1 (pre-treatment glucose level), G2 (post-treatment glucose level), treatment (treatment group) and change from pre- to post-treatment of circulating blood glucose (ΔG). We used information theoretic approaches (Burnham & Anderson 2002) to assess *a priori* hypotheses explaining variation in sex ratio. Specifically, we used Akaike information criterion and normalized Akaike weights (Burnham & Anderson 2002) to assess the probability that a specific hypothesis was most likely among the considered. We also used the sum of Akaike weights across models containing a specific variable (e.g. G2) to assess the importance of specific variables in explaining variation in sex ratio. We also report estimates and standard errors of parameters linking explanatory variables to sex ratio in order to evaluate functional linkage between these variables and sex ratio.

3. RESULTS

The overall sex ratio in both groups combined was biased slightly towards females (52% females versus 48% males), but did not differ significantly from the expectation of a 50 : 50 sex ratio (two-tailed binomial test, $p=0.57$). DEX treatment had a significant effect on plasma glucose levels (control 5.24 ± 0.22, range 4.4–7.8; DEX 6.47 ± 0.19, range 4.9–8.4; $t_{36}=4.27$, $p<0.001$). A change in glucose levels was therefore recorded for DEX-treated females, but not for control females (control 0.01 ± 0.25; DEX -0.79 ± 0.16; $t_{36}=2.72$, $p=0.01$). There was a correlation between glucose levels and change in glucose levels, but the explanatory power was low (regression $F_{1,36}=5.86$, $r^2=0.14$). The sex ratio differed significantly between the treatment and control groups (rank-sum test: $Z=-2.18$, $p=0.03$), with DEX females giving birth to fewer sons (41.9%) than control females (53.5%). The sex ratio for control females did not differ significantly from the expectation of 50% (54%, two-tailed binomial test, $p=0.33$), but DEX-treated females gave birth to more females than would be expected (42%, two-tailed binomial test, $p=0.04$). As a consequence, females treated with a glucose blocker gave birth to fewer sons than control females, as well as less than those predicted from an expectation of parity. However, in our model selection analysis, treatment alone was not a strong predictor of sex ratio ($\Delta AIC=13.446$; table 1). Additionally, there was no significant difference in litter size between the two groups (two-tailed *t*-test, $p=0.13$), although DEX females tended to have slightly smaller litters (control 10.45 ± 0.60; DEX 9.17 ± 0.62; $t=1.53$, $p=0.13$).

Our model selection analysis revealed that change in circulating blood glucose (ΔG) was the most important

Table 1. Models used to estimate the effects of blood glucose on litter sex ratio of mice.

Model[a]	AIC[b]	ΔAIC	MW[c]	DEV[d]	NP[e]
ΔG	503.512	0.0	0.5446	499.512	2
ΔG+G2	505.237	1.725	0.2299	499.237	3
TRT+ΔG	505.371	1.859	0.2150	499.371	3
ΔG×G2	507.116	3.604	0.0824	499.116	4
G2	512.269	8.757	0.0063	08.084	2
TRT+G2	514.084	10.572	0.0028	509.822	3
TRT	516.958	13.446	0.0007	512.958	2
(·)	520.089	16.577	0.0001	518.089	1
G1	520.583	17.071	0.0001	516.583	2

[a] Notation for models follows where letters indicate that the model varies according to the given parameter. The following designation implies: ΔG, delta glucose; G1, glucose level before treatment; G2, glucose level after treatment; TRT, treatment; (·), constant model.
[b] Akaike information criterion.
[c] MW, model weight, probability that the given model is best among the suite of models considered.
[d] DEV, deviance (−2 log L).
[e] NP, number of parameters in the model.

predictor of sex ratio (table 1). Given that the top three models fall within 2 ΔAIC, we summed the Akaike weights of the top variables (i.e. treatment, G2, ΔG) in order to quantify the importance of each variable (Burnham & Anderson 2002). The change in circulating blood glucose (ΔG) had overwhelming support with an Akaike weight of 0.9911, while circulating blood glucose post-conception (Akaike weight = 0.2395) and treatment (Akaike weight = 0.2185) were not well supported (table 1). The change in circulating blood glucose (ΔG) also explained more than half the variation in sex ratio within our dataset ($R^2 = 0.5425$; figure 1), while circulating blood glucose post-conception ($R^2 = 0.2868$; figure 2) and treatment ($R^2 = 0.1498$) explained less of the variation. Consequently, the change in glucose levels was a more important predictor of sex ratio in litters than the concentrations of glucose *per se*.

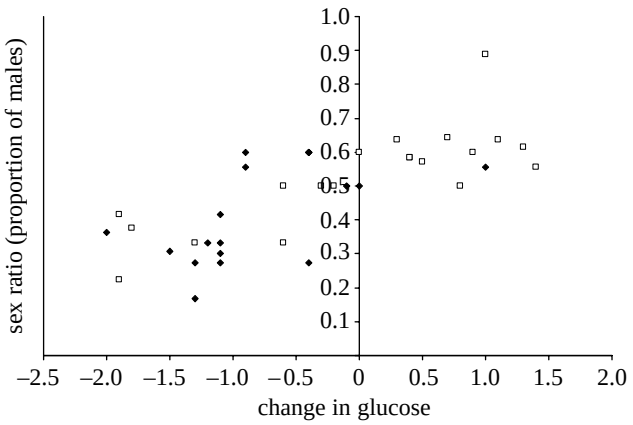

Figure 1. The relationship between the change in blood glucose concentrations from pre-treatment to post-treatment and the litter sex ratio of laboratory mice. Filled diamonds, litters from mothers treated with DEX to block glucose uptake; open squares, control mothers.

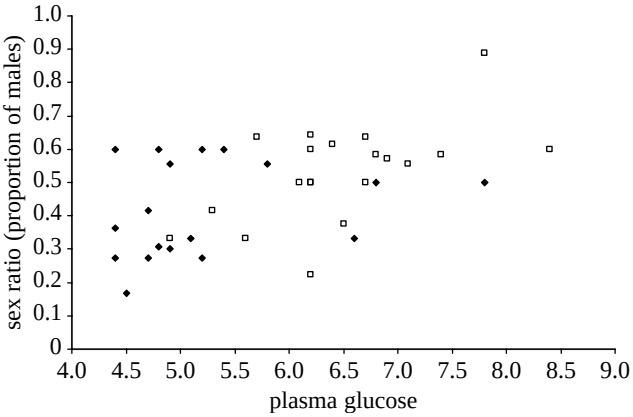

Figure 2. The relationship between blood glucose concentrations after conception and the litter sex ratio in laboratory mice. Filled diamonds, litters from mothers treated with DEX to block glucose uptake; open squares, control mothers.

4. DISCUSSION

These results demonstrate a mechanism to explain how sex ratio can be influenced *in utero* as a result of changing glucose concentration. The level of circulating glucose during early cell division influences the sex ratio of mice litters, consistent with studies *in vitro*, which show that added glucose differentially influences the survival of male and female conceptuses (e.g. Larson *et al.* 2001). Further research is needed to confirm if a similar effect is seen in monotocous species. Although it is possible that DEX administration had a direct effect on sex ratio, it is unlikely since the level of glucose was a significant predictor of sex ratio, regardless of treatment group (figure 1). The litter size did not vary significantly, and therefore the treatment seems to influence the ratio of surviving male and female blastocysts rather than the total number, although this may be due to a lack of statistical power owing to the small sample size. No difference in litter size would be intriguing, since the glucose hypothesis would predict differential survival of male and female blastocysts in relation to glucose levels. Although more corpora lutea are typically produced than being developed into blastocysts (Krackow & Burgoyne 1998), this result suggests that other factors are probably operating in tandem with any glucose effect. Nonetheless, variation in glucose levels provides a potential explanation for sex ratio adjustment in line with adaptive hypotheses proposed to take place in mammals. In addition, our results support recent studies that suggest that sex ratio adjustment occurs at or around the time of conception (Cameron 2004; Sheldon & West 2004) and provide a link between maternal condition and diet. Females only had their glucose levels altered during conception and early cell division, thus precluding any influences later in gestation. However, further investigations on the influence of glucose levels in the uterine fluid are required to ensure that variations in plasma glucose are reflected *in utero*.

If plasma glucose concentrations during the early development of the conceptus mediate sex ratios, either directly or through interaction with other factors (Cameron 2004), then several confusing results from previous studies investigating sex ratio variation could be explained. For example, social stress leads to the production of more sons than daughters (Krackow & Hoeck 1989; Perret 1990), which does not appear to have an adaptive explanation. An interaction between glucose levels and early development may also provide a link to other hypotheses (e.g. Krackow 1995; Forchhamer 2000) that focus on different developmental rates in males and females.

The change in plasma glucose concentration had a markedly greater influence on sex ratio than the level of glucose *per se*. In evolutionary terms, changing condition would be more likely to predict maternal condition once offspring were born, thereby influencing the mother's longer term ability to invest in offspring, providing a link with the assumptions of the TWH. Studies on sex ratio variation investigate indices of condition, but the rate of change of condition around conception has only rarely been considered as a variable that may be influencing sex ratios (see Roche *et al*. 2006; Cameron & Linklater 2007). We suggest that this may be a fruitful area for future research into sex ratio adjustment.

Variation in glucose levels might also provide a link with hypotheses related to maternal hormone levels (James 1996, 2004). Glucose levels are important for reproductive functioning through their interaction with luteinizing hormone (LH). Glucose enhances the LH secretion and release from the pituitary, whereas reduced glucose concentrations can inhibit the LH pulse (Murahashi *et al.* 1996; Nagatani *et al.* 1996). The LH in turn has an enhanced effect on glycolytic activity, enhancing glucose availability to the oocyte (Zuelke & Brackett 1992). Therefore, there is a positive feedback loop between the glucose levels and the LH; glucose enhances the LH secretion, which in turn enhances the glucose availability to the oocyte. The timing of insemination appears to be an important factor for skewing sex ratio, second only to manipulated food in terms of studies supporting the TWH (Cameron 2004). This could be explained by the close link between glucose and circulating LH. Furthermore, variation in human sex determination with time of ovulation can also be explained by the same relationship. Males are conceived either early or late in the fertile period (James 2000), coinciding with peaks in the LH production, and therefore availability of glucose to the conceptus. Variation in glucose levels, in tandem with other mechanisms, may mediate the sex of an offspring. Consequently, there may just be some truth in the traditional beliefs about certain foods dictating the sex of a conceptus.

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