

# Translocation reverses birth sex ratio bias depending on its timing during gestation: evidence for the action of two sex-allocation mechanisms

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**Abstract.** Many sex allocation mechanisms are proposed but rarely have researchers considered and tested more than one at a time. Four facultative birth sex ratio (BSR) adjustment mechanisms are considered: (1) hormone-induced conception bias; (2) sex-differential embryo death from excess glucose metabolism; (3) sex-differential embryo death from embryo–uterine developmental asynchrony; and (4) pregnancy hormone suppression and resource deprivation. All mechanisms could be switched on by the corticoadrenal stress response. A total of 104 female rhinoceroses (Rhinocerotidae), translocated from 1961 to 2004 at different stages of gestation or that conceived soon after arrival in captivity, were used to test for a reversal in BSR bias as evidence for the action of multiple sex-allocation mechanisms. Translocation induced a statistically significant BSR reversal between early gestation (86% male births from 0 to 0.19 gestation) and mid-gestation (38% male from 0.2 to 0.79 gestation). Captivity also induced a strongly male-biased (67% male) BSR for conceptions after arrival in captivity. The results indicate the action of at least two sex-allocation mechanisms operating in sequence, confirm the important role of sex-differential embryo death around implantation and of stress in sex allocation, and lend support to suggestions that sex-differential glucose metabolism by the preimplantation embryo likely plays a role in facultative BSR adjustment.

**Additional keywords:** conception, embryo, mammal, rhinoceros, stress, zoo studbook.

## Introduction

Sex allocation theories cannot be falsified when hypotheses lack a mechanism to test that causally links external influences with outcomes (i.e. the birth sex ratio (BSR)). Largely correlative testing for facultative BSR adjustment, without concomitant tests for its causative mechanisms, has resulted in considerable confusion (Sheldon and West 2004). However, an important article by Cameron (2004) has cut a swathe through this confusion by demonstrating the power of combining a hypothesis about the mechanism (i.e. causation and ontogeny, as defined by Tinbergen (1963)) with those about Darwinian function (i.e. adaptive value; see also Linklater 2004).

Hilborn and Stearns (1982) warned against assuming the action of single mechanisms because it leads researchers to rule out important causal influences. It is probable that more than one sex-allocation mechanism influences population BSRs (Clutton-Brock and Iason 1986) and each mechanism operates at different times, from before conception until birth (Grant 1996; Flint *et al.* 1997a; Mendl *et al.* 1998). It is also probable that each mechanism is switched on and off in response to some of the same, as well as different but variously correlated or independent, influences. Different mechanisms may interfere with

one another or have additive or synergistic effects in the same individual. Thus, there is a growing awareness that the action of multiple sex-allocation mechanisms may explain why numerous factors correlate with BSR variation in opposite and apparently contradictory directions in different studies. Multiple mechanisms are also likely to explain why related influences (e.g. dominance, resource conditions, body fat, density and social context) correlate with different BSRs depending on the type or timing of their measurement (Cameron 2004). The action of multiple mechanisms may also provide a more robust explanation for the evolution of sex allocation (Sheldon and West 2004).

No one has yet tested for multiple sex-allocation mechanisms operating in tandem. To make progress, we need to establish the timing and magnitude of the mechanisms involved and how they may interact to supersede, reverse or increase their individual effects. Many potential sex-allocation mechanisms are proposed. Sex-differential embryo death during mid- to late-gestation is well documented (Kruuk *et al.* 1999; Forsyth *et al.* 2004), although the exact mechanism(s) remains unclear. Nevertheless, on its own, embryo death during the latter stages of gestation remains theoretically problematic as a facultative sex-allocation mechanism because embryo loss is more costly to

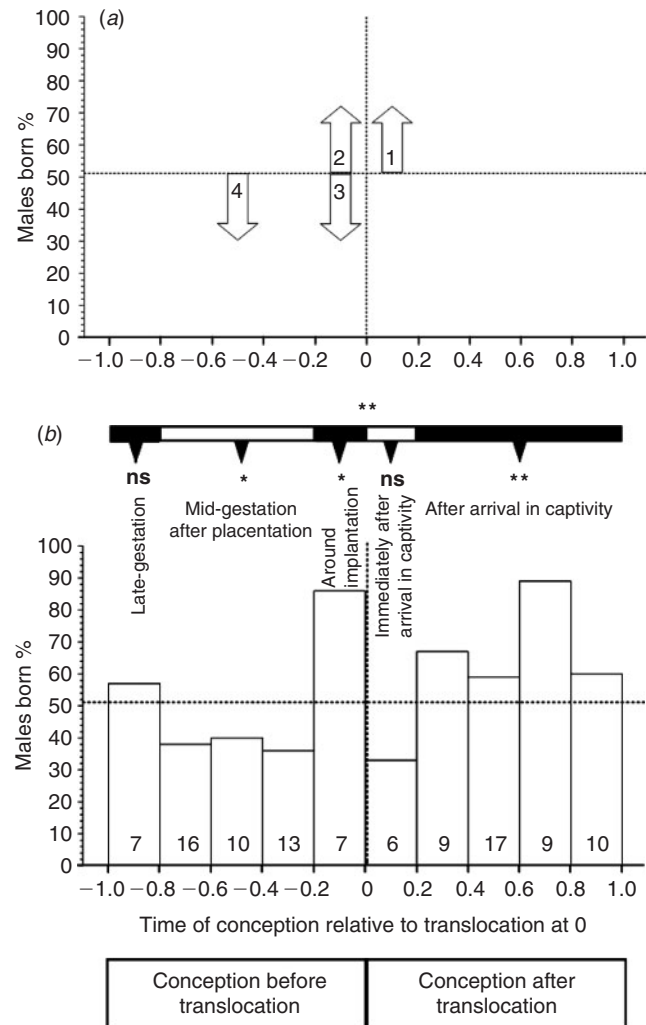
Darwinian fitness in late-gestation after the substantial maternal investment involved in placenta formation and embryo development. Consequently, some have advocated for steroid hormones (e.g. testosterone; Grant 1996) that predispose the ova to Y-sperm based partly on an apparent lack of evidence for early embryo death (e.g. in humans; James 1987a, 1987b). However, very early embryo death before or around implantation, although poorly documented, is not easily detected and has the potential to supersede any conception sex bias with a minimal, perhaps negligible, cost to fitness. Thus, although mechanisms for sex-biased conception and late-gestation embryo death may contribute, others have suggested that mechanisms influencing early embryo survivorship are more likely to adjust BSRs facultatively (Krackow 1995b).

There are currently four compelling mechanisms proposed for facultative birth sex ratio adjustment (Fig. 1a), as outlined below.

1. Exposure of the ova to extreme levels of testosterone before conception (i.e. during the follicular or luteal phases) changes the zona pellucida in a way that predisposes the ovum to insemination by Y-sperm (Grant 1996; James 2004).
2. A greater capacity for X-linked glucose metabolism, and the production of its toxic by-products, by female embryos before implantation mean that excess glucose in the oviduct and uterine fluids causes early female embryo death but facilitates early male embryo development and implantation (Peippo and Bredbacka 1995; Gutierrez-Adan *et al.* 2006).
3. Asynchrony between early embryo development and uterine readiness for implantation, which is mediated by progesterone, causes sex-differential embryo death (i.e. the 'developmental asynchrony' (DA) hypothesis; Krackow 1995a, 1997).
4. Environmental challenges and an animal's physiological response suppress the reproductive hormones necessary for the maintenance of pregnancy (e.g. progesterone) and deprive the embryo of resources, causing greater male than female death (Rivers and Crawford 1974; McMillen 1979).

Fortuitously, these mechanisms are sensitive to, and may be switched on by, the corticoadrenal stress response to external stimuli because stress (Teixeira *et al.* 2007) and distress (Brezile 1987) are known to: (1) induce a testosterone surge in the mother (for a review, see Grant 2007); (2) cause extreme increases in the concentration of circulating glucose (i.e. hyperglycaemia) in the mother (Brezile 1987); (3) suppress progesterone production and progesterone sensitivity in the mother (e.g. in the baboon; Albrecht *et al.* 1978); and (4) cause resource deprivation for the embryo where the mother's nutritional needs supersede those of her developing fetus (Coubrough 1985). Thus, the BSR from mothers that undergo an extreme stress event at different stages from oestrus through gestation to birth could be compared to test for the action of each of these mechanisms (Fig. 1a).

The translocation of wildlife into and between zoos involves their capture and transport, often international shipment and quarantine, and can occur at different times during gestation for pregnant females. The capture of a wild animal and its



**Fig. 1.** The birth sex ratio (BSR), expressed as percentage males born, from mothers subject to the stress of translocation at different times relative to conception. A negative value on the horizontal axis indicates that conception occurred before capture and translocation and a positive value indicates that conception occurred after arrival in captivity (e.g. a value of  $-0.4$  means that translocation occurred two-fifths into gestation, whereas  $+0.2$  means that conception occurred one-fifth of a gestation equivalent period (GEP) after arrival in captivity). The dashed horizontal line indicates the average rhinoceros BSR in the wild (Linklater 2006). (a) Predicted direction of BSR bias for each of the four hypothesised mechanisms if translocation stress activates them. Mechanism 1: stress before conception (i.e. 0.0–0.19 GEP) should induce a male-biased BSR if stress elevates the ovum's exposure to testosterone. Translocation soon after conception and around implantation (i.e.  $-0.19$  to 0.0 GEP) should cause a male-biased BSR if mechanism 2 operates, but a female-biased BSR if mechanism 3 operates. Mechanism 4: stress during mid- to late gestation should induce a female-biased BSR. (b) The BSR from mothers subject to the stress of translocation at different times relative to conception. Numbers at the base of each columns indicate sample size. \* $P < 0.1$ , \*\* $P < 0.05$ .

translocation into captivity is a major event in its life history (Teixeira *et al.* 2007). It is a period that includes repeated and prolonged stress-inducing events, beginning with the animal's capture. Translocation can occur over a period of weeks,

involving several novel stressors, including chemical immobilisation, capture, crating, transport, handling, release and acclimation to a new environment. Consequent stress and distress (Breazile 1987) repeatedly activate the hypothalamic–pituitary–adrenal (HPA) axis and a glucocorticoid response inducing changes in female body fluid chemistry that may return to precapture levels only after the rhinoceros, for example, has acclimated behaviourally, socially and physiologically to its new home (Teixeira *et al.* 2007). Importantly, the impact of translocation may not be unlike the impact of other stressors imposed by the physical or social environment, such as changes in social hierarchy or drought cycles causing resource deprivation, that are encountered by females in less-managed contexts. Translocation, therefore, is a potentially useful, albeit fortuitous, experimental manipulation to test for the role of stress in facultative BSR adjustment in mammals and to discriminate between the action of multiple sex-allocation mechanisms that may be turned on by the physiological perturbations of stress.

Zoos and zoo databases are fertile ground for studies of BSR adjustment (Hardy and Krackow 1995) because species studbooks quantify in detail the reproductive history of mothers transferred from the wild to captivity. Rhinoceros (Rhinocerotidae) studbooks are particularly useful in this regard because there have been many translocations of wild mothers into captivity. Moreover, rhinoceros, unlike many (particularly carnivorous or omnivorous) mammals have large offspring and rarely eat aborted, still-born fetuses or neonatal deaths. Thus, births (or lack thereof) are unlikely to be misreported. Moreover, the conservation status of the rhinoceros, their body size and the costs of acquiring and keeping them means that they are a considerable investment and so studbooks traditionally contain a high-quality record of individual life histories. The aim of the present paper was to use the rhinoceros international studbooks to detect extreme reversals in BSR in response to translocation stress at different times during a reproductive event as a fortuitous test for the action of each mechanism and its relative importance.

## Methods

The most recent international studbooks for black (*Diceros bicornis*: Rhinocerotidae; 1949–2004; Ochs 2005a), white (*Ceratotherium simum*; 1960–2004; Ochs 2005b) and Indian (*Rhinoceros unicornis*; 1947–2004; Hlavacek 2004) rhinoceros were used to identify all mothers captured from the wild and translocated into captivity. The sample population was confined to wild-caught mothers because captive rearing reduces the response to stress (e.g. domestic strain cf. wild-stock mice; Drickamer 1990) due to changes in reproductive and physiological development (i.e. physiological acclimation; Chapple *et al.* 1991; Zapata *et al.* 2004) and animals may habituate to similar and regular stressors (Dobson and Smith 1995). Moreover, rich captive diets are also suspected to drive population BSRs (Atkinson 1997) and therefore potentially confound a test that uses translocation stress as a stimulus of sex-allocation mechanisms. Thus, it is reasonable to expect that the transfer between institutions of captive-born and -raised females may not generate a physiological or reproductive response of the same type or magnitude.

The calving records of each wild-caught mother were examined to identify those that had calves born after arriving in captivity or after a transfer between captive institutions (northern white, *C. s. cottoni*,  $n = 1$ ; southern white, *C. s. simum*,  $n = 78$ ; Indian,  $n = 9$ ; east-central black, *D. b. michaeli*,  $n = 13$ ; south-eastern black, *D. b. minor*,  $n = 9$ ). The sex of each calf was recorded and back-dated from its birth date to its mother's arrival date to determine the time that each mother was translocated relative to the calf's conception. The sex of six calves was not reported and so they did not contribute to the analysis, leaving a total sample size of 104 mothers and their calves. The gestation time used to estimate conception from birth dates was an average calculated from measures of individual rhinoceros gestation times in the literature as 495 days for white (Owen-Smith 1988; Patton *et al.* 1999), 480 days for Indian (Schwarzenberger *et al.* 1993; Hlavacek 2004) and 460 days for black (Schwarzenberger *et al.* 1996; Berkeley *et al.* 1997; Garnier *et al.* 1998) rhinoceros.

For consistency and comparison, the time of conception relative to translocation was converted from days into a fraction of gestation length because each species has a slightly different gestation period (see above). Calves were grouped into 20% gestation equivalent periods (GEPs; as shown on the horizontal axis in Fig. 1b) based on the timing of their mother's translocation relative to conception and the BSR (percentage male) calculated for each GEP. The 20% GEP intervals were used for two reasons based on: (1) the estimated time of implantation and placentation during rhinoceros embryo development; and (2) available sample size. First, the timing of implantation in rhinoceros is not known, but may be estimated from the horse (*Equus caballus*: Equidae; a much better known Perissodactyl). In doing so, it was assumed that the timing of fetal development in rhinoceros species with an average gestation of 460–495 days can be scaled-up from that observed in the horse, with a gestation of 343 days. The equine blastocyst fixes its position around Day 17 and embryo cells begin migrating into the maternal endometrium around Day 37. Allantochoariaonic villi develop around Day 50 and full placentation is complete around Day 100 (Allen 2001). Thus, implantation likely occurs around 0.1 GEP ( $37/343 \text{ days} = 0.11$ ) and full placental function occurs around 0.3 GEP ( $100/343 = 0.29$ ). Second, the division of mothers into smaller GEPs is limited by the available sample size (i.e.  $n = 104$ ). Using equal intervals of 0.2 GEPs allows enough equally sized intervals to test for a switch in sex-biased embryo survival between implantation at 0.1 GEP and full placental function at 0.3 GEP while maintaining large enough numbers of mothers in each GEP to retain sufficient statistical power.

The time taken to complete translocation from capture to captivity varied greatly between mothers but capture dates were not always reported. However, arrival date in captivity was always recorded and is probably the most reliably recorded event. Thus, arrival date was used as the best available metric for the timing of translocation stress for most mothers. Capture dates were used only where the translocation period was so long that translocation stress occurred much earlier in gestation (i.e. an earlier GEP) than the arrival date indicated.

The six unsexed calves were conceived when they were at  $-0.83$ ,  $-0.38$ ,  $-0.22$ ,  $-0.08$ ,  $0.39$  and  $0.58$  GEP relative to translocation and so would have contributed data to GEPs

throughout the study period had their sex been known. Thus, it is unlikely that under-reporting of calf sex would markedly change the results.

Translocation techniques and procedures are likely to have changed over the period 1961–2004 that contributed translocated mothers in a way that may influence the results. For this reason, mothers were compared in each GEP for the years they were translocated to confirm that each GEP had a representative sample of mothers translocated throughout the period.

## Results

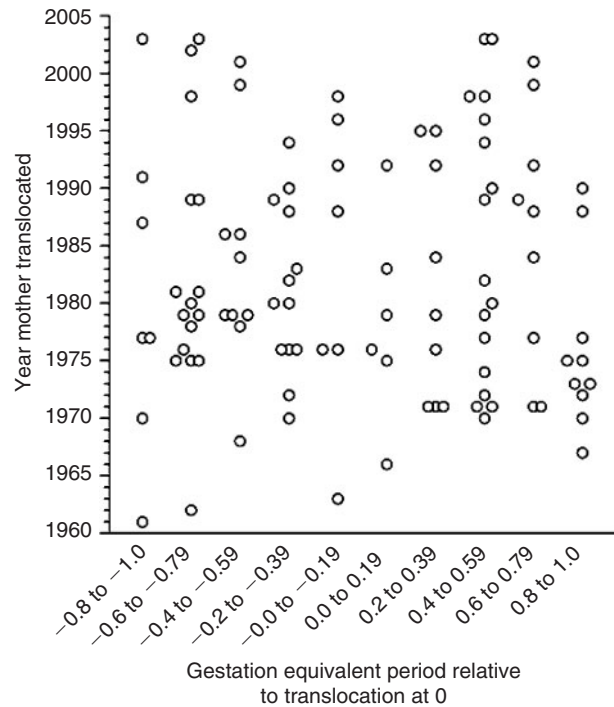
Translocation had a significant impact on the direction of the BSR bias depending on its timing during gestation (Fisher's exact test, five periods  $\times$  two sexes,  $P = 0.027$ ; Fig. 1b). In particular, there was a significant reversal from a male- to female-biased BSR in response to stress between early (0.0 to  $-0.19$  GEP) and mid- ( $-0.20$  to  $-0.79$  GEP) gestation (Fisher's exact test, two periods  $\times$  two sexes,  $P = 0.035$ ). The BSRs of mothers stressed in early gestation and mid-gestation were different from the expected wild BSR (i.e. 51.6% male; Linklater 2006), but the difference only approached statistical significance (Binomial test, mother translocated during early gestation,  $P = 0.074$ ; mother translocated during mid-gestation,  $P = 0.065$ ; Fig. 1b). These results indicate greater female embryo death during early gestation but greater male embryo death during mid-gestation in response to stress. Thus, at least two different mechanisms operating at different times and with opposite effects adjusted the BSR of translocated rhinoceroses.

Only two births to mothers stressed immediately before conception (i.e. they conceived soon after arrival in captivity; 0.0–0.19 GEP) were male, but the sample size is too small ( $n = 6$ ) to detect a stress-induced conception bias. However, mothers that conceived in captivity after this initial period (i.e. over 92–99 days after arrival) had male-biased BSRs that deviated significantly from the expected value (i.e.  $>51.6\%$  male; 0.2–1.0 GEP, Binomial test,  $P = 0.030$ ; Fig. 1b). The size of the male bias increased with additional time in captivity before conception. Births where 61.5% male for conceptions during the period 0.20–0.59 GEP ( $n = 26$ ), but 73.7% male for conceptions during the period 0.60–1.0 GEP ( $n = 19$ ) after arrival in captivity.

The mothers in each GEP were no more likely to be translocated during any particular sequence of years between 1961 and 2004 (Fig. 2). There was no significant correlation between BSR and average year for the different GEPs (Pearson correlation,  $r = 0.398$ ,  $n = 10$ ,  $P > 0.1$ ). Thus, the patterns in BSR described are unlikely to be caused by changes in capture and translocation techniques over the 43 years of records.

## Discussion

Female-biased BSRs caused by greater male embryo death due to stress during mid- to late-gestation are well documented (Labov *et al.* 1986; Pratt and Lisk 1989; Wauters *et al.* 1995; Kruuk *et al.* 1999; Forsyth *et al.* 2004) and supported by the results reported herein for translocated rhinoceroses. However, this is the first time that a significant reversal in BSR bias between early and mid-gestation has been demonstrated as a consequence of a male-biased BSR due to stress during early gestation. It indicates



**Fig. 2.** Distribution of translocated mothers through time for each gestation equivalent period (GEP) relative to translocation at 0. Birth sex ratio (BSR) data for each GEP consisted of mothers translocated in widely different years between 1961 and 2004, thus discounting the possibility that changes in translocation technique over the four decades may explain changes in BSR between GEPs.

that at least two different and potentially competing mechanisms operate to adjust BSR through sex-differential embryo death. The result explains the apparent contradiction between different studies showing both male- (e.g. Moorhouse and Macdonald 2005) and female-biased (e.g. Pratt and Lisk 1989) BSRs as a consequence of stress during gestation. Stress may produce both a male and female BSR bias depending on its timing due to the action of at least two post-conception sex-allocation mechanisms.

The switch from a female to male bias in embryo death between early and mid-gestation, respectively, lends support to the conclusions of others about the timing and importance of a sex-allocation mechanism early in gestation. Others have shown the critical period to be soon after conception (Cameron *et al.* 1999; Cameron 2004) and probably before implantation (Cameron and Linklater 2007). Data showing a BSR switch either side of the  $-0.20$  GEP for rhinoceros mothers translocated pregnant are consistent with a switch in BSR from male to female bias in response to stress after implantation at around  $-0.1$  GEP but before full placental function at  $-0.3$  GEP (Fig. 1b). Female embryos appear to be vulnerable to maternal stress before implantation, whereas male embryos are more vulnerable to maternal stress with placentation.

Unlike the mothers discussed above who were translocated while already pregnant, those that instead conceived

immediately after arrival in captivity (i.e. 0.0–0.19 GEP; Fig. 1b) when they were still subject to novel stressors (e.g. acclimating to a new physical and social environment in captivity) can be used to check for the action of stress-sensitive preconception sex-allocation mechanisms. Unfortunately, the sample size of mothers that conceived soon after arrival in captivity was too small for this test to be conclusive. Nevertheless, it should be noted that more females than males were conceived by mothers stressed immediately before conception, which is consistent with other studies showing that stress induces female- and not male-biased conceptions (e.g. Geiringer 1961; Lane and Hyde 1973) in contrast to the first mechanism. Stress before conception appears not to increase conception rates by Y-sperm. Either stress does not expose the ova to elevated testosterone in the rhinoceros or the mechanism proposed (Grant 1996; James 2004) is not supported. Work with rats (Geiringer 1961; Lane and Hyde 1973) supports the latter conclusion. Although preconception sex-allocation mechanisms may exist, they appear less influential on BSR, at least where the external driver is stress, than post-conception mechanisms.

Finally, although the body condition of females arriving in captivity will vary, they receive a richer and more abundant diet for less effort and, therefore inevitably, on average gain body condition. Those that do not, due to illness (or even death), are also unlikely to subsequently become mothers and so would not be part of this dataset. Others have found that changes in the maternal condition (i.e. whether the mother has a deficit or surplus energy balance) during the period before implantation is the strongest predictor of birth sex rather than condition *per se* (Roche *et al.* 2006; Cameron and Linklater 2007). My results showing a significant increase in male calves for conceptions after arriving in captivity support the role of changing body condition on BSR bias.

In summary, the three findings of male-biased BSRs for mothers stressed around implantation, the switch in BSR to a female-bias before placentation and greater male births for conceptions that occurred after arrival in captivity are consistent with the importance of facultative sex allocation before implantation via sex-differential embryo death. Elevated circulating glucose or an early progesterone surge after ovulation (Pratt and Lisk 1991; Flint *et al.* 1997a, 1997b; Cameron 2004; Gutierrez-Adan *et al.* 2006; i.e. hypotheses 2 and 3) are probable causes of sex-differential early embryo death and, so, each mechanism described in the Introduction is considered below as a potential explanation.

#### *Sex-differential glucose metabolism by preimplantation embryos*

Glucose concentrations of *in vitro* media ranging from 2.5 to almost 6 mM (Gutierrez-Adan *et al.* 2001; Larson *et al.* 2001; Kimura *et al.* 2005) have been shown to augment male embryo development towards the blastocyst stage but cause female embryo death (Peippo and Bredbacka 1995). Within this range of glucose concentrations, poorer female embryo development and survivorship have been attributed to the toxicity of by-products from glucose metabolism via the pentose-phosphate pathway (PPP; Larson *et al.* 2001; Kimura *et al.* 2005),

particularly reactive oxygen species like superoxide and hydrogen peroxide (Guérin *et al.* 2001). It is not that female embryos are more sensitive to such by-products, but that they produce more of them because key enzymes regulating the PPP (e.g. glucose-6-phosphate dehydrogenase) are X-linked and so toxic metabolite concentrations rise in a glucose-rich environment until they are fatal (Kimura *et al.* 2005).

Glucose concentrations of 1 mM in media *in vitro* do not have a sex-differential effect on embryo development (Kimura *et al.* 2005). Therefore, it is not surprising that ordinarily glucose concentrations in the oviduct and uterine fluids of large mammals during preimplantation embryo development are consistently less than 1 mM and maintained an order of magnitude lower than contemporary concentrations in circulating blood (e.g. bovid blood 1–3 mM cf. uterine fluid 0.05–0.2 mM; porcine blood 4.6 mM cf. oviduct fluid at 0.6 mM; Parrish *et al.* 1989; Nichol *et al.* 1992). Thus, baseline glucose concentrations in oviduct and uterine fluids are ordinarily lower than levels known to have a sex-differential effect on embryo survivorship.

Stress, however, is known to induce hyperglycaemia, which may also elevate glucose levels in the oviduct and uterus. Baseline circulatory glucose concentrations are estimated to be less than 3.2 and 4.6 mM in black and white rhinoceroses, respectively (concentrations measured immediately after chemical immobilisation; Seal *et al.* 1976; Kock 1992). However, during translocation, rhinoceroses show significant and prolonged increases in serum glucose ranging from 5.1 to 9.7 mM (Kock *et al.* 1990). The magnitude of this glucose response to stress above baseline is typical of that observed in many large mammals (e.g. big-horned sheep 6.6–12.4 mM, white-tailed deer 8.7 mM, impala 13.7 mM, roan antelope 12.0 mM, buffalo 10.3 mM and elephant 12.2 mM; Seal *et al.* 1972; McDonald *et al.* 1981; Kock *et al.* 1987a, 1987b; Hattingh 1988). We do not yet know how glucose levels in oviduct and uterine fluids respond to such a sudden or prolonged hyperglycaemia like that induced by stress. Nevertheless, it is reasonable to expect glucose concentrations to increase but be buffered by diffusion rates and metabolism from the extremes observed in the blood. Thus, a rise in blood glucose levels to almost 10 mM may also achieve increases in oviduct and uterine fluid glucose concentrations above 2.5 mM, like those observed to cause female embryo death *in vitro* (Gutierrez-Adan *et al.* 2001; Larson *et al.* 2001; Kimura *et al.* 2005).

Female-biased embryo mortality *in vitro* is observed even when the addition of  $\leq 6$  mM glucose is temporary (i.e. 6 h; Jimenez *et al.* 2003) and, therefore, greater female embryo mortality *in vivo* may be induced by acute, as well as chronic, stress. More extreme glucose levels (i.e.  $\geq 20$  mM) are known to reverse the sex-differential mortality *in vitro* towards a male bias (Jimenez *et al.* 2003). However, the highest blood glucose levels in large mammals induced by the stress of capture did not exceed 13.7 mM (see above). Thus, ordinarily, one would not expect a stress event to reverse female-biased embryo mortality towards a male bias. Taken together, the literature on baseline glucose concentrations in the circulation and the effect of stress on glucose levels, as well as the results presented herein, lend support to Cameron's (2004) expectation that a population's BSR may be driven by elevated levels of circulating glucose in mothers during a short critical period after conception, around

implantation and certainly before full placental function that are the consequence of a net energy surplus (i.e. excess nutrition).

Should a net energy (glucose) deficit occur, for example under-nutrition, the reverse (i.e. the death of males, the more vulnerable sexually selected sex) should be observed. Greater male embryo death may largely be the result of their greater demand for nutrient. Even in the early stages of embryo cleavage and the formation of the blastocyst, there can be large differences in the demand by males of resources owing to their faster cleavage rates (e.g. mice (Tsunoda *et al.* 1985) and bovids (Avery *et al.* 1989)); the higher nutritional needs of males are more often not met during times of maternal stress (Pratt and Lisk 1991). Therefore, male loss may also occur in early gestation (Pratt and Lisk 1989) and may be particularly common or marked in strongly sexually dimorphic species where male embryos are significantly more demanding of mothers' resources earlier in gestation. Thus, energy (glucose) balance that is driven by diet, body condition and stress in early gestation before implantation may generate extreme bias in BSRs in both directions, depending on environmental conditions.

#### *Progesterone and developmental asynchrony*

Although compelling on its own, sex-differential glucose metabolism by embryos may be only one of several related mechanisms that cause greater female embryo death around implantation. Krackow (1995a) has convincingly shown that asynchrony between uterine and embryo development may also cause sex-differential implantation failure (i.e. the DA hypothesis) and demonstrated the process in action (Krackow and Burgoyne 1998). Flint *et al.* (1997b) contributed further, showing that progesterone may determine sex-differential embryo survival through its influence on uterine sensitivity to the blastocyst's signal, interferon (IFN)- $\tau$ . Asynchrony in the timing of the blastocyst's signal relative to uterine sensitivity may cause pregnancy loss. The asynchrony can be caused by an early increase in circulating progesterone and kill the more slowly developing female blastocyst (Flint *et al.* 1997a). Importantly, an early progesterone surge after ovulation has been positively related to maternal dominance status and body condition in red deer (Flint *et al.* 1997b), thus proving the expected link between proxies for maternal Darwinian fitness and the DA mechanism.

However, a DA driven by progesterone is unlikely to explain the results presented herein for rhinoceros, where stress is the environmental driver, because stress probably has the opposite effect on progesterone levels and sensitivity to that of elevated status or nutrition. Increased adrenocorticotrophic hormone (ACTH) and corticosterone elevate prolactin (Lamming *et al.* 1974), suppress gonatrophin production (Dobson and Smith 1995) and, consequently, placenta-uterine progesterone (Albrecht *et al.* 1978). Moreover, cortisol has a greater affinity for uterine and placental receptors than progesterone, thus blocking progesterone recognition. Finally, cortisol inhibits uterine blood flow and capillary permeability that ordinarily facilitate implantation (Coubrough 1985; for a review, see Pratt and Lisk 1991). Thus, stress could interfere with or exacerbate a developing asynchrony between the embryo and uterus by delaying a progesterone surge or the readiness of the uterus. Pratt and Lisk

(1989) showed that stress around implantation caused early male, not female, embryo death in hamsters, probably as a consequence of progesterone suppression through the action of corticosterone and ACTH. Thus, in rhinoceros, translocation stress would have been expected to prevent differential female embryo death, and perhaps increase male embryo death, but it did not.

Nevertheless, the 'glucose metabolism' and DA hypotheses are not mutually exclusive. Glucose also controls embryo development and therefore the timing of the blastocyst's signal. Thus, regardless of embryo sex, small changes in glucose availability may speed up or slow down development, causing or preventing developmental asynchrony. Although sex-differential glucose metabolism is the more compelling mechanism to explain the effects of stress on embryo survival described herein, it does not exclude developmental asynchrony from also playing a role, particularly if energy balance and stress interact. A developmental asynchrony may be exacerbated or ameliorated by hyperglycaemia through stress depending on embryo sex and the timing of the stress event. The interaction of these two mechanisms could be extremely complex.

#### *Multiple mechanisms in sex allocation*

Sex allocation theories, for example the Trivers-Willard hypothesis (Trivers and Willard 1973; Brown 2001; Sheldon and West 2004) have been difficult to test because more than one, and probably several, sex-allocation mechanisms operate concurrently and sequentially. Consequently, other factors, such as stress (due to management, competition for resources or social context; Kruuk *et al.* 1999) may modify or obscure the relationship between parental energy balance (ability to invest) and the costs and benefits of investing in sons or daughters (Hardy 1997). The results of the present study demonstrate why measures of maternal quality (context, attributes or ability to invest) need to be carefully chosen and timed when testing sex allocation theories. The same condition or challenge measured at different times during gestation may generate a different relationship with BSR. For example, if rhinoceroses were used to test for the role of stress (or stress was used as a proxy for the influence of maternal fitness) on BSR, then authors that measured stress during early gestation would have received the opposite result to those who measured it in late gestation, but both would have been correct. The overall BSR from mothers translocated during gestation is near parity (24 males : 27 females, or 47.1% male; Fig. 1b) and so apparently not greatly modified by stress. However, closer analysis revealed a significant stress-induced BSR reversal sometime after implantation and before placentation. Indeed, translocation stress is likely to have reversed, obscured or reinforced expected relationships between maternal body condition and birth sex (as has been reported for horses (Cameron *et al.* 1999)) for captured rhinoceros depending on the interaction between the mother's condition and the timing of the stress during gestation.

The action of multiple mechanisms explains why Sheldon and West (2004) found that studies using measures of maternal dominance or condition before conception gave greater support for the Trivers-Willard hypothesis (Trivers and Willard 1973) than those where the measure was after conception. Sheldon and West (2004) attributed this to the confounding effect of two different

processes: (1) a positive effect of maternal condition on the sex ratio conceived (perhaps better expressed as early embryo survival before it is detectable); and (2) a negative effect of the sex ratio surviving to placentation on subsequent maternal condition, particularly for embryos in the latter stages of gestation, when they demand more from the mother. My results support this idea for two reasons, outlined below.

1. Preconception indices are likely to predict the effect during early gestation, whereas post-conception measures are more likely to straddle the period of implantation to full placental function. Thus, the latter would record across the reversal in female–male embryo vulnerability described herein for rhinoceros and, so, obscure both relationships.
2. If elevated or improving body condition induced hyperglycaemia and a male-bias in embryos surviving to full placental function, subsequent investment, or lack thereof, may reduce or even reverse the earlier bias, as was observed herein for rhinoceroses challenged at different times in gestation.

The present results also provide clues as to the evolutionary likelihood and relative importance of sex-allocation mechanisms at different times in the sequence from ovulation to birth and the difficulties of detecting them, particularly in field studies. The loss of an early embryo is hardly detectable because the opportunity exists for a second pregnancy soon after that does not extend the interoestrous period significantly. Thus, particularly in species that are only weakly seasonal breeders, like the horse (Linklater *et al.* 2004) and rhinoceros (Owen-Smith 1988), and in field studies of them, this differential mortality would be undetectable and have minimal consequences for maternal fitness. Therefore, mechanisms operating during early gestation before implantation should be highly susceptible to adaptation or exaptation (Gould and Vrba 1982) towards facultative sex allocation. In contrast, the fitness costs of embryo loss increase as gestation proceeds, so mechanisms operating after the period from implantation to full placental function should be more costly and less important in facultative sex ratio adjustment, or are only observed under extreme circumstances (e.g. extreme nutritional deprivation; Labov *et al.* 1986; Kucera 1991; Kruuk *et al.* 1999). Indeed, translocation immediately before parturition (−0.8 to −1.0 GEP; Fig. 1) in rhinoceros did not induce a BSR bias compared with earlier periods of gestation. This supports the idea that the costs of sex-allocation mechanisms operating in late-gestation in rhinoceros become too great to be facultative. This would be particularly true for a large, monotoxic, slow-reproducing species like rhinoceros. Once an embryo reaches the last one-fifth of gestation, the mother is evolutionarily committed. Finally, post-conception mechanisms will always be able to modify the outcomes of preconception mechanisms in sex allocation. Provided post-conception mechanisms happen soon enough after conception and do not cost the mother significant investment or future reproductive opportunity (i.e. the embryo is small, dies before placenta development and the opportunity to conceive again is not significantly delayed), they can operate to improve fitness and supersede any conception bias. Thus, preconception sex-allocation mechanisms should be secondary and less influential than those operating immediately after conception.

Although the present results support the importance of sex-differential early embryo death through glucose metabolism before the period from implantation to full placental function, its effects are countered by greater male embryo vulnerability in late gestation and may also be influenced by other mechanisms operating in concert or antagonistically, and concurrently or sequentially, such as DA (Krackow 1997) or hormonally induced conception bias (James 2004). Understanding adaptive sex allocation will depend on measuring the effects of these multiple mechanisms and modelling their combined outcomes and interaction in varying contexts where their influences wax and wane. The key to understanding what drives facultative BSR adjustment, being able to predict it and modify it, and to understand the historical confusion regarding the topic will depend on first appreciating that multiple mechanisms exist and interact.

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