Sexual growth dimorphism affects birth sex ratio in house mice

S. Krackow^{1,2,3*}, T. A. Schmidt^{4,5} and A. Elepfandt²

¹*Verhaltensbiologie, Zoologisches Institut, Universität Zürich-Irchel, Winterthurerstrasse 190, 8057 Zürich, Switzerland*

²*Abteilung Sinnesbiologie, Institut für Biologie, Humboldt-Universität, Invalidenstrasse 43, 10115 Berlin, Germany*

³*Forschungsbereich Populationsbiologie und Züchtungsforschung, Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf-Rostock, Germany*

⁴*Forschungsbereich Züchtung und Genetische Ressourcen, Bundesforschungsanstalt für Landwirtschaft, Institut für Tierzucht, Hoeltystrasse 10, 31535 Neustadt, Germany*

⁵*Institut für Tierzucht und Vererbungsforchung, Tierärztliche Hochschule Hannover, Bünteweg 17p, 30559 Hannover, Germany*

We present the first empirical evidence that mammalian sex-ratio deviations result from variation in adult-weight sexual dimorphism via correlated effects on blastocyst development. Two selection lines of mice exhibiting high and low sexual dimorphism in adult weight showed correlated sexual weight differences at birth and at weaning, caused by relatively decelerated growth of males in the low line from before birth. The sex ratio at birth was significantly female-biased in the low line, and significantly lower than in the highly dimorphic line. Concomitantly, blastomere numbers were at significantly higher variance in the low than in the highly dimorphic line, owing to an increased frequency of slowly growing blastocysts. Since low-dimorphism mice produced more corpora lutea and more female pups than the high-dimorphism mice, but not more males, birth sex-ratio bias most parsimoniously resulted from the loss of slowly growing male blastocysts. This is in agreement with the observation that sex-ratio skews in mammals arise when timing of uterine responsiveness (i.e. its temporally limited capacity for implantation) varies in relation to sex-specific embryonic growth rates. Hence, natural mammalian sex-ratio variation that stems from developmental asynchrony might be a by-product of natural selection for sexual dimorphism in adult weight.

Keywords: developmental asynchrony; blastomere counts; selection lines

1. INTRODUCTION

The question of adaptive sex-ratio variation in mammals (*sensu* Williams 1979) is still controversial (Hardy 1997; West *et al.* 2002). Perspectives range from the optimistic view that the ultimate causations of deviations can be disentangled by increasing empirical and theoretical research efforts (Cockburn *et al.* 2002) to meta-analysis revealing that a statistical artefact could, potentially, account for the significant results (Brown & Silk 2002) or suggesting that deviations are caused mostly by non-adaptive by-product mechanisms (Krackow 2002).

Much of the agony of argument stems from the lack of understanding of the physiological mechanisms causing the deviations (Krackow 2002; West *et al.* 2002). We know of only one experimentally validated mechanism (Krackow & Burgoyne 1998) that could allow for birth sex-ratio adjustment in mammals: the timing of uterine implantation capability in relation to sex-specific blastocyst growth rates (Krackow 1995*a,b*). First, this mechanism is suggested by the observation of higher developmental rates of male pre-implantation embryos now reported in a number of mammals (cf. Krackow & Burgoyne 1998), including laboratory mice (Burgoyne 1993; Burgoyne *et al.* 1995). Second, it relies on the fact that the uterus will respond to embryonic implantation

signals for only a certain time-period (see Lessey (1994) and references therein). Hence, exceptionally delayed embryos have been shown to fail at implantation in laboratory mice (e.g. McLaren & Mitchie 1956; Pope 1988). Exceptionally accelerated ones have also been suggested to face implantation failure, but no direct experimental evidence is available (Krackow 1995*a*). Consequently, when male and female pre-implantation developmental rates differ, sex ratios at birth can potentially vary if the time-frame of relative uterine responsiveness changes (Krackow 1997*a*). Likewise, when sex dimorphism in embryonic developmental pace changes, birth sex-ratios may be affected (Krackow & Burgoyne 1998).

In mammals, males generally differ from females in body weight (Ralls 1976), this dimorphism often being present from birth. Adult sexual dimorphism would be expected to affect birth sex-ratios if it implied sexually dimorphic embryo development that led to sex-differential prenatal mortality (*sensu* Forchhammer 2000; Krackow 1995*a*). Hence, selection for sex dimorphism in adult body weight might ultimately provide the basis for sex-ratio variations in mammals, owing to its correlated effects on early development. In that case, artificial selection for sexual dimorphism in weight is expected to result in diverging developmental asynchrony of male and female embryos and, thereby, potentially to lead to birth sex-ratio deviations between selection lines.

In mice, it is well known that sexual dimorphism in adult weight can be selected for (Eisen & Legates 1966;

* Author for correspondence (skrackow@zool.unizh.ch).

Table 1. Number of males and females per litter, sex ratio (proportion of males) and weight dimorphism (g) at birth, weaning (21 days) and maturity (42 days) for GD+ and GD− mice (*n* = number of litters).

	GD+			GD−		
	<i>n</i>	mean	s.e.	<i>n</i>	mean	s.e.
males	105	4.25	0.20	114	3.98	0.17
females ^a	105	4.64	0.22	114	5.39	0.19
sex ratio ^a	105	0.473	0.019	114	0.424 ^b	0.016
weight dimorphism (male weight−female weight) at:						
birth ^a	96	0.053 ^c	0.010	110	0.013	0.009
21 days ^a	83	0.325 ^c	0.093	74	−0.790 ^c	0.103
42 days ^a	84	5.647 ^c	0.149	75	1.192 ^c	0.211

^a Significantly different between strains.
^b Significantly different from even sex ratio.
^c Weight differs highly significantly between the sexes ($F_{1, n-1} > 12.3$, $p < 0.001$, in all cases).

Korkman 1957). Furthermore, subadult growth has been found to show correlated responses to selection for adult body weight and post-weaning weight gain, although not always positive ones (see Urrutia & Hayes (1988) and references therein). Also, sexually dimorphic blastocyst developmental rates have been shown to cause sex-ratio shifts (Krackow 1997a; Krackow & Burgoyne 1998). Therefore, we investigated the effects of selection for differences in adult sexual dimorphism on weight differences at birth and weaning, blastocyst development and litter sex ratio in two lines of laboratory mice divergently selected for high and low sexual dimorphism in adult body weight.

2. MATERIAL AND METHODS

We used laboratory mice from two lines that contrasted starkly in adult weight dimorphism. Selection lines were initiated from several strains of outbred and wild mice. Lines were successfully divergently selected for sexual dimorphism in adult weight, with increased dimorphism in line GD+ and decreased dimorphism in line GD−, as described by Schmidt (1993). In the current study, data were collected from mice of generations 15–17, kept under standard laboratory conditions. Weights of offspring were measured at birth, weaning (21 days) and as adults (42 days). For each litter, mean weights for males and females were calculated for data analysis. At birth, pups were counted and sexed by means of anogenital distance by animal breeding staff unaware of the experimental prediction, and sex was confirmed at weaning.

(a) *Corpora lutea counts and blastomere numbers*

Females of generation 16 were paired with randomly selected non-brother males of the same line and generation when found in oestrus by standard inspection of vaginal smears. Mating then took place overnight and animals were separated at 0700. Three days later, at 1200–1500, females were sacrificed, their ovaries were recovered, and their uteri were flushed. Corpora lutea were counted using a stereomicroscope. Immediately after flushing, blastocysts were transferred for 30 min into a 1% natrium citrate solution, using Pasteur pipettes. They were fixed on slides using standard methanol–acetic acid fixation, i.e. individual blastocysts were placed on a slide and covered by a drop of 1 : 1 methanol : acetic acid, which caused spreading of blastomeres,

i.e. dispersion of individual cells. The slides were then dried in 3 : 1 methanol : acetic acid for 4 h and then stored at −20 °C. For blastomere counting, individual blastocyst spreads were stained with DAPI (4',6-diamidino-2-phenylindoldihydrochloride, 0.05 g l^{−1} in 50 µl of mountant) and counted using a fluorescent microscope (Olympus Vanox AHB T 3).

(b) *Statistical analysis*

Strain differences in weight dimorphism (difference between male and female mean weight per litter) were tested using one-way analysis of variance (ANOVA). Generalized linear model analysis of covariance (ANCOVA) was used to test for strain differences in either male or female mean litter weights, correcting for concomitant effects of litter size. Least-squares mean weights for each strain, removing the effects of litter-size differences, could then be calculated. Male and female mean weights within litters were compared by repeated-measures ANOVA, and correlations between weight dimorphisms at birth, weaning and maturity were tested. Strain differences in litter sex ratios (percentage males) and percentage blastocysts recovered and fixated were tested using generalized linear models that tested for effects on the binomially distributed numbers of males (or blastocysts) given the respective litter size (or number of corpora lutea) using logits as a link function and a likelihood ratio test for significance, and a Pearson's scaling factor for overdispersion (cf. Krackow & Tkadlec 2001). The numbers of corpora lutea and male and female pups were compared between strains with a generalized linear model taking the data as Poisson distributed and using a log-link function. A mixed model was applied to test for differences in blastocyst development, using the base 2 logarithm of blastomere numbers as the response variable and strain as the independent fixed effect, adding maternal identity as a random effect (Littell *et al.* 1999). *F*-tests were applied to compare variance components of log₂-blastomere numbers between strains. Deviations from even (Mendelian) sex ratios were tested by comparing litter sex ratios with an expected value of 0.5 using Wilcoxon's signed-rank test statistics (*S*). Results were regarded as significant at $p < 0.05$ (two-tailed). All data handlings and analyses were carried out using SAS statistical facilities (SAS 1989).

3. RESULTS

Sex ratios differed significantly between strains ($F_{1,217} = 6.28$, $p < 0.02$; table 1). There were significantly

Table 2. Mean litter weights (g) of males and females at birth, weaning (21 days) and maturity (42 days) (n = number of litters).

	GD+			GD−			$F_{1,n-2}^b$	p
	n	mean ^a	s.e.	n	mean ^a	s.e.		
birth								
males	99	1.59	0.016	111	1.51	0.015	12.77	< 0.001
females	101	1.55	0.014	112	1.51	0.013	6.11	< 0.02
21 days								
males	84	10.99	0.177	76	9.81	0.186	20.82	< 0.0001
females	83	10.73	0.165	80	10.67	0.168	0.06	n.s.
42 days								
males	84	27.17	0.276	75	22.80	0.293	114.80	< 0.0001
females	84	21.50	0.200	77	21.61	0.210	0.13	n.s.

^a Least-squares means corrected for litter-size effects.^b Test statistics for strain difference from ANCOVA, correcting for litter-size effects.Table 3. Numbers of corpora lutea and blastocysts recovered and fixated on slides in GD+ and GD− mice (n = number of litters), and $\log_2$ of the number of blastomeres (n = number of blastocysts).

	GD+			GD−		
	n	mean	s.e.	n	mean	s.e.
corpora lutea ^a	13	11.85	2.15	9	14.44	1.24
recovered	13	9.46	2.03	9	12.89	1.76
fixated	13	8.54	2.07	9	11.89	1.83
$\log_2$ (blastomeres)	104	5.500	0.061	88 ^b	5.264	0.085

^a Significantly different between strains.^b One litter was accidentally lost before counting.

more female pups in litters of line GD− ($F_{1,217} = 6.37$, d.f. = 1, $p < 0.02$), while there was no difference in the numbers of males in the two lines ($F_{1,217} = 1.04$, d.f. = 1, n.s.; table 1). The sex ratio was significantly skewed in favour of females in GD− mice ($|S| = 1344$, $p < 0.0001$), while in GD+ mice the sex ratio did not significantly deviate from 0.5 ($|S| = 362$, n.s.; table 1).

Weight dimorphism was smaller in GD− mice than in GD+ mice at birth ($F_{1,204} = 8.45$, $p < 0.01$), weaning ($F_{1,155} = 64.91$, $p < 0.001$) and maturity ($F_{1,157} = 308.39$, $p < 0.001$; table 1). Males were always significantly heavier than females in GD+ mice (table 2). In GD− mice, male weights did not significantly differ from female weights at birth, were significantly lower at weaning and significantly higher at maturity (table 2). Males of line GD+ were significantly heavier than males of line GD− at all ages, while females were significantly heavier in GD+ than in GD− at birth, but insignificantly different at later ages (table 2). Within strains, weight dimorphisms of all age groups correlated significantly with one another (figure 1).

Numbers of corpora lutea and blastocysts recovered from uteri and successfully fixated on slides are given in table 3. GD− mice had significantly more corpora lutea than GD+ mice ($F_{1,20} = 9.76$, $p < 0.01$). Recovery and fixation rates did not differ significantly between lines ($F_{1,20} < 1.7$, ns, in both cases). Blastocysts of GD+ contained 49.89 ± 23.64 s.d. blastomeres, while those of GD− exhibited 42.99 ± 16.64 s.d. blastomeres ($F_{1,19} = 2.31$, n.s.; table 3). The within-litter variance in GD−

(0.486) was significantly larger than in GD+ (0.125) ($F_{80,91} = 3.89$, $p < 0.0001$). This difference disappeared if blastocysts of fewer than eight blastomeres, which occurred only in GD−, were excluded from analysis ($F_{76,91} = 1.12$, n.s.). The between-litter variance in blastomere number was not significantly different between lines ($F_{12,7} = 1.71$, n.s.).

4. DISCUSSION

Our results show that selection for sexual dimorphism in adult weight resulted in congruent differences in weight at birth and weaning, and, concomitantly, in a significant difference in litter sex-ratios at birth. Males from the line exhibiting reduced adult dimorphism (GD−) had decelerated growth from before birth in comparison with males from the line with increased dimorphism (GD+), as evidenced by their significantly lower weights from birth onwards. While birth weights appeared not to differ between the sexes in line GD−, GD+ males were significantly heavier than GD+ females at birth. The number of ova ovulated (corpora lutea) and the blastocyst developmental variance were significantly higher in GD− mice, the latter caused by some exceptionally slow-growing blastocysts in this line. Given that the number of females was higher at birth in GD− mice, but the number of males was not, these results are consistent with the assertion that in GD− mice, male blastocysts suffered from reduced implantation success caused by slower pre-implantation growth. This assertion is based on the fact that, in mice,

Figure 1. Regressions of (a) adult and (b) weaning weight dimorphism on dimorphism at birth, and (c) of adult on weaning weight dimorphism, in GD+ (dots) and GD- (circles) mice. All coefficients are significant.

after initiation of implantation by the blastocysts, the uterine environment changes (decidual reaction) so as to reduce the likelihood of implantation by exceptionally retarded blastocysts (cf. McLaren 1965; Pope 1988).

If this scenario of lower male implantation success owing to reduced developmental pace in GD- mice holds, it implies that there might have been an implicit selection for higher ovulation numbers in GD- mice, as otherwise the average litter size would have declined. The increase in the number of corpora lutea found in GD- mice might therefore be seen as a correlated selection response. Furthermore, the absence of a biased sex ratio in GD+ mice may indicate that the natural time-frame of uterine responsiveness allows for accelerated blastocyst growth, at least within the range encountered in that line, without the need to infer increased losses at implantation.

Hence, our study strongly suggests that sexual dimorphism in adult weight exerts correlated effects on blastocyst development, which can cause sex-ratio shifts as a result of interactions with the timing of uterine responsiveness. Developmental asynchrony between sex-specific embryonic growth and uterine mortality has been argued potentially to cause a wide range of sex-ratio distortions reported in mammalian populations (cf. Krackow 1995a,b, 1997b; Forchhammer 2000). Clearly, natural selection for adult sexually dimorphic body weight could provide the ultimate basis for many sex-ratio findings, because litter sex ratios would be unaffected by any variation in uterine survival in the absence of developmental sex differences.

Of course, correlated effects of adult dimorphism are not the only potential reason for sex differences in embryonic growth. Genes on the Y chromosome that cause male embryos to accrue relatively more maternal resources, even at the expense of female embryos, are known to spread as a consequence of genetic conflict (Hurst 1994). Hence, such 'neo-imprinted' genes might cause accelerated growth and earlier implantation of male blastocysts in spite of the fact that this might reduce female survival at implantation (Hurst 1994). The relative importance of these two ultimate mechanisms remains to be resolved. Clearly, understanding the ultimate reason for embryonic developmental sex differences does not lead to conclusions about the adaptive significance of any sex-ratio variation encountered in empirical studies. Nevertheless, it gives us a reasonable framework for interpretation of empirical results that include potentially non-adaptive or even parentally maladaptive effects. At least, according to the scenario outlined here, binomial variation of an even ratio is a rather fragile null hypothesis for the mammalian sex ratio at birth.

Invaluable technical help from A. R. Thornhill, Benita Lucht, Undine Schneeweiss and E. 'Garby' Garbely is kindly acknowledged.

REFERENCES

- Brown, G. R. & Silk, J. B. 2002 Reconsidering the null hypothesis: is maternal rank associated with birth sex ratios in primate groups? *Proc. Natl Acad. Sci. USA* **99**, 11 252–11 255.
- Burgoyne, P. S. 1993 A Y-chromosomal effect on blastocyst cell number in mice. *Development* **117**, 341–345.
- Burgoyne, P. S., Thornhill, A. R., Boudreau, S. K., Darling, S. M., Bishop, C. E. & Evans, E. P. 1995 The genetic basis of XX–XY differences present before gonadal sex differentiation in the mouse. *Phil. Trans. R. Soc. Lond. B* **350**, 253–260.

- Cockburn, A., Legge, S. & Double, M. C. 2002 Sex ratios in birds and mammals: can the hypotheses be disentangled? In *Sex ratios: concepts and research methods* (ed. I. C. W. Hardy), pp. 266–286. Cambridge University Press.
- Eisen, E. J. & Legates, J. E. 1966 Genotype–sex interaction and the genetic correlation between the sexes for body weight in *Mus musculus*. *Genetics* **54**, 611–623.
- Forchhammer, M. C. 2000 Timing of foetal growth spurts can explain sex ratio variation in polygynous mammals. *Ecol. Lett.* **3**, 1–4.
- Hardy, I. C. W. 1997 Possible factors influencing vertebrate sex ratios: an introductory overview. *Appl. Anim. Behav. Sci.* **51**, 217–241.
- Hurst, L. D. 1994 Embryonic growth and the evolution of the mammalian Y chromosome. I. The Y as an attractor for selfish growth factors. *Heredity* **73**, 223–232.
- Korkman, N. 1957 Selection with regard to the sex difference of body weight in mice. *Hereditas* **43**, 665–678.
- Krackow, S. 1995a The developmental asynchrony hypothesis for sex ratio manipulation. *J. Theor. Biol.* **176**, 273–280.
- Krackow, S. 1995b Potential mechanisms for sex ratio adjustment in mammals and birds. *Biol. Rev.* **72**, 225–241.
- Krackow, S. 1997a Effect of mating dynamics and crowding on sex ratio variance in mice. *J. Reprod. Fertil.* **110**, 87–90.
- Krackow, S. 1997b Further evaluation of the developmental asynchrony hypothesis of sex ratio variation. *Appl. Anim. Behav. Sci.* **51**, 243–250.
- Krackow, S. 2002 Why parental sex ratio manipulation is rare in higher vertebrates (invited article). *Ethology* **108**, 1041–1056.
- Krackow, S. & Burgoyne, P. S. 1998 Timing of mating, developmental asynchrony and the sex ratio in mice. *Physiol. Behav.* **63**, 81–84.
- Krackow, S. & Tkadlec, E. 2001 Analysis of brood sex ratios. *Behav. Ecol. Sociobiol.* **50**, 293–301.
- Lessey, B. A. 1994 The use of integrins for the assessment of uterine receptivity. *Fertil. Steril.* **61**, 812–814.
- Littell, R. C., Milliken, G. A., Stroup, W. W. & Wolfinger, R. D. 1999 *SAS system for mixed models*. Cary, NC: SAS Institute Inc.
- McLaren, A. 1965 Maternal factors in nidation. In *The early conceptus, normal–abnormal* (ed. W. W. Park), pp. 27–33. University of St Andrews.
- McLaren, A. & Mitchie, D. 1956 Studies on the transfer of fertilized mouse eggs to uterine foster-mothers. I. Factors affecting the implantation and survival of native and transferred eggs. *J. Exp. Biol.* **33**, 394–416.
- Pope, W. 1988 Uterine asynchrony: a cause of embryonic loss. *Biol. Reprod.* **39**, 999–1003.
- Ralls, K. 1976 Mammals in which females are larger than males. *Q. Rev. Biol.* **51**, 245–276.
- SAS 1989 *SAS/STAT user's guide: version 6*. Cary, NC: SAS Institute Inc.
- Schmidt, T. A. 1993 Divergent selection for sexual dimorphism in mice—6 generations of selection. In *44th Ann. Mtng EAAP*, vol. 1, pp. 268–271. Aarhus, Denmark: European Association for Animal Production.
- Urrutia, M. S. & Hayes, J. F. 1988 Selection for weight gain in mice at 2 ages and under *ad libitum* and restricted feeding. 1. Direct and correlated responses in weight gain and body weight. *Theor. Appl. Genet.* **75**, 415–423.
- West, S. A., Reece, S. E. & Sheldon, B. C. 2002 Sex ratios. *Heredity* **88**, 117–124.
- Williams, G. C. 1979 The question of adaptive sex ratio in outcrossed vertebrates. *Proc. R. Soc. Lond. B* **205**, 567–580.