biology letters

Early experience and parent-of-origin-specific effects influence female reproductive success in mice

Reinmar Hager and Rufus A Johnstone

Biol. Lett. 2006 **2**, 253-256
doi: 10.1098/rsbl.2006.0457

biology letters

Biol. Lett. (2006) **2**, 253–256
doi:10.1098/rsbl.2006.0457
Published online 7 March 2006

Early experience and parent-of-origin-specific effects influence female reproductive success in mice

Reinmar Hager* **and Rufus A. Johnstone**

Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK
Author and address for correspondence: Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, UK (reinmar.hager@cantab.net).

Recent studies on mammals investigating parent-of-origin-specific effects such as genomic imprinting and maternal effects have demonstrated their impact on short-term measures of fitness, for example offspring growth. However, the long-term fitness consequences of parent-of-origin-specific effects and their role outside the immediate mother–offspring interaction remain largely unexplored. Here, we show that female mice mated to males that inherited the same set of paternal and maternal genes as themselves have a higher reproductive success than females mated to males of reciprocal genotype. Furthermore, we demonstrate that the early social environment experienced by an individual influences its reproductive success. Females raised with unrelated siblings in a mixed litter had a subsequent lower reproductive success than those that were fostered together with all their biological siblings in unmixed litters. Our results highlight the important influence of parent-of-origin-specific effects and conditions in early development on long-term reproductive success in mammals and suggest that parent-of-origin-specific effects may provide the underlying mechanism for beneficial coadaptation between genotypes, for example, in mate choice.

Keywords: mice; reproductive success; genomic imprinting; early experience; parent-of-origin-specific effects

1. INTRODUCTION

Parent-of-origin-specific effects such as genomic imprinting have been shown to significantly affect measures of fitness in mammals (Li *et al.* 1999). Their evolution is thought to be the result of differential fitness effects in males and females and stems from a conflict between the sexes over the optimal level of maternal investment (Haig 2004). Recent studies in mice have demonstrated parent-of-origin-specific effects on maternal provisioning and revealed a positive interaction between the offspring's maternally inherited set of genes and genes expressed in their nursing foster mother (Hager & Johnstone 2003, 2006). Furthermore, other studies using gene targeting have shown pre- and post-natal effects such that young with a deactivated paternal copy of a paternally expressed gene (*Peg3*) were growth retarded (e.g. Li *et al.* 1999). These studies indicate that parent-of-origin-specific effects influence individual short-term fitness, yet, to date no work has been done to elucidate any long-term effects on reproductive success. Given theoretical studies (e.g. Haig 2000) and results of our own work, however, we predict such effects.

Moreover, the consequences of environmental conditions experienced early in life for an individual's fitness are increasingly recognized. For example, maternal condition and sibling competition are known to influence fitness and reproductive success in birds and mammals (Lindström 1999; Carere *et al.* 2005). It is, however, unclear how such effects might modify or interact with epigenetic and genetic effects influencing reproductive success, which may be crucial when designing experiments that investigate parent-of-origin-specific effects. More generally, the early social environment of an animal may prove to be a far more important predictor of its reproductive success than previously assumed.

In this study, we set out to investigate whether long-term female reproductive success is affected by a difference in the parent-of-origin of their mate's genotype. We assess this by mating females of two genotypes with males of either the same or reciprocal genotype with respect to the origin of their paternally and maternally inherited sets of genes and measure the rate at which these females produce offspring over a period of three litters. In a second step, we assess the impact of early social experience on reproductive success by raising focal females in litters composed half of their biological litter mates and half of unrelated individuals of reciprocal genotype. We discuss our results in the light of potential parent-of-origin-specific effects on female investment and long-term reproductive success, and how the social environment experienced early in life may affect these parameters.

2. MATERIAL AND METHODS

We created males and females of reciprocal genotype using mice of the B6 (C57Bl/6) and CBA (CBA/Ca) strains, which are an ideal model system to study fitness consequences of parent-of-origin-specific effects because they differ consistently in key life-history parameters such as litter size (Hager & Johnstone 2003) and inbred mice have been extensively used in research on genomic imprinting (Li *et al.* 1999). The general features of these strains and their breeding have been described elsewhere (Hager & Johnstone 2003, 2005).

In a first experiment, individuals were raised by their biological mothers in unmixed litters (i.e. together with all their biological siblings) until weaning and thereafter kept with same-sex litter mates until the onset of sexual maturity at the age of 5–7 weeks. Then, we paired 35 males and females (six of which failed to reproduce) from different litters (such that individuals were unfamiliar and unrelated) and kept both of them for a period of 120 days following the design described in table 1. Offspring were kept with their parents until weaning (day 21) unless the next litter was born earlier. We recorded the date of birth and the number and weight of young that females produced in three consecutive litters. Reproductive success is commonly defined as the number of young produced over a lifetime that survive to sexual maturity (Krebs & Davies 1993). In our study, 98.9% of young born survived to weaning such that the number of young at weaning can be safely taken as the number of young surviving to sexual maturity (mice become sexually mature at around five weeks of age). In addition, the time between litters is an important fitness component due to the limited breeding season in the wild, hence including a time component yields a better approximation of reproductive success (Fuchs 1982; Bartman & Gerlach 2001) and is here given

Received 9 January 2006
Accepted 30 January 2006

Table 1. Mating design of first experiment. (The first part of an individual's genotype denotes its maternal strain while the second refers to its paternal strain (e.g. a B6CBA individual had a B6 mother and CBA father).)

		male genotype	
		B6CBA	CBAB6
female genotype	B6CBA	B6CBA/ B6CBA	CBAB6/ B6CBA
	CBAB6	B6CBA/ CBAB6	CBAB6/ CBAB6

by the number of young produced over the number of days it took to produce them.

In a second experiment, we changed the early social environment of focal females by raising them in mixed litters. As in the previous experiment, we produced females and males of reciprocal genotype. Then, litter births were synchronized to allow partial cross-fostering (see Hager & Johnstone 2005 for details). Upon birth (i.e. day 1) we created mixed litters of unrelated and individually marked pups such that CBAB6 females were now fostered in litters consisting half of their biological siblings and half of individuals of the reciprocal genotype. Due to space limitations we were able to measure the reproductive success for only one female type, CBAB6. These females were mated to males of the reciprocal genotype (B6CBA). The reproductive success of these females was then compared to genetically equivalent females of the first experiment that were raised in a different social environment, i.e. with all their biological siblings.

We analysed our data in Minitab 13.1 using general linear models (GLM) with categorical and continuous predictor variables as outlined in the result tables. Data were checked for conformity with GLM requirements and error-structures for normality. The experiments described in this study do not require a project license and comply with UK Home Office regulations.

3. RESULTS

First, we investigated the long-term reproductive success of females that were mated to unfamiliar and unrelated males of equivalent genotype and either the same or different parent-of-origin of their paternally and maternally inherited sets of genes. As predicted, female reproductive success was significantly influenced by an interaction effect between female and male genotype but not a main effect of genotype (GLM; $F_{1,24}=9.12$; $p=0.006$; table 2). Figure 1 shows that females produced offspring at a higher rate when mated to males that had the same paternal and maternal genotype as themselves. Over and above the interaction effect, female bodyweight significantly influenced her reproductive success with larger females being at an advantage over smaller ones (GLM; $F_{1,24}=7.20$; $p=0.013$). The male's bodyweight showed no significant effect on this variable.

In the second experiment, we explored whether early experience of being fostered in mixed litters had an effect on female reproductive success. These individuals were raised in mixed litters consisting of pups of both reciprocal genotypes. As in the previous experiment, we analysed the factors that influence female reproductive success. CBAB6 females raised in unmixed litters that were later mated to B6CBA males had a significantly higher reproductive success than equivalent females raised in mixed litters that were mated to B6CBA males (t-test, $t=2.59$, $p=0.022$, d.f.$=14$).

Table 2. GLM model of factors influencing reproductive success of reciprocal females mated to males of either genotype. (Percentage variance accounted for=31.47, d.f.=28.)

full model	d.f.	F	p
female genotype×male genotype	1	9.12	0.006
female bodyweight	1	7.20	0.013
male genotype	1	1.31	0.264
female genotype	1	0.85	0.367
male bodyweight	1	0.02	0.888
minimal model	**coefficient**	**s.e. coefficient**	
constant	−0.08	0.02	
female genotype× male genotype	0.04	0.01	
female bodyweight	0.02	0.08	

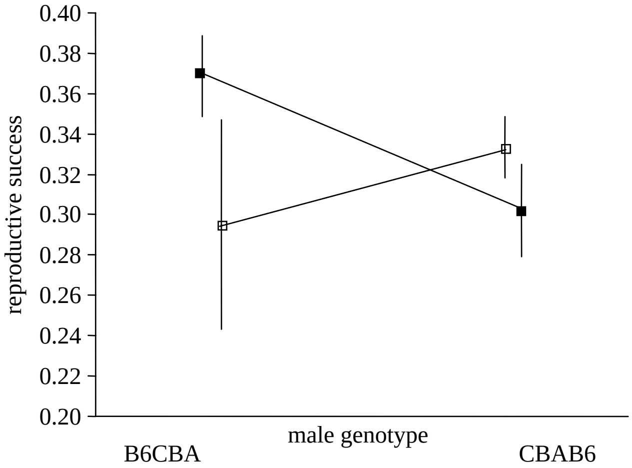

Figure 1. This interaction plot shows the reproductive success (number of pups produced in three consecutive litters over number of days required to produce three litters) of reciprocal females shown as a function of their own and their mate's genotype. The open boxes denote female genotype CBAB6 and the closed boxes represent the B6CBA genotype. The vertical lines connect the values for the same maternal genotype.

Looking at the two experiments in conjunction and analysing the reproductive success of CBAB6 females mated to males of both genotypes, we found that only the females' rearing condition had a significant effect (GLM; $F_{1,23}=9.12$; $p=0.006$). Controlling for the latter variable, male genotype did not significantly influence female reproductive success (table 3). In addition, neither male nor female bodyweight exerted any significant effect on our response variable.

4. DISCUSSION

Our study revealed two key results. First, females mated to males of the same paternal and maternal genotype had a higher reproductive success than those mated to genetically equivalent males of a reciprocal genotype. Second, changing the early experience of females was found to affect their reproductive success. Comparing females that were

Table 3. GLM model of factors influencing the reproductive success of CBAB6 females in the two experimental conditions. (Percentage variance accounted for = 23.22, d.f. = 27.)

full model	d.f.	F	p
rearing condition (mixed/unmixed)	1	7.72	0.010
male bodyweight	1	1.75	0.197
male genotype	1	1.14	0.296
female bodyweight	1	0.03	0.870

minimal model	coefficient	s.e. coefficient
constant	0.35	0.01
rearing condition (mixed/unmixed)	0.04	0.01

raised in litters consisting of their biological litter mates only (unmixed litters) to those that grew up in mixed litters containing 50% individuals of the reciprocal genotype, we found that females raised in unmixed litters had a higher reproductive success than those raised in mixed litters, irrespective of their mate's genotype.

(a) *Parent-of-origin-specific effect*

Results of the first experiment hint at the influence of parent-of-origin-specific effects such as genomic imprinting on long-term reproductive success; to our knowledge a novel finding. While our results suggest a role for parent-of-origin-specific effects in influencing reproductive success, at this stage we are unable to determine which set of parentally inherited genes causes this effect. Further experiments are required to disentangle the effects of paternally and maternally inherited sets of genes.

The evolution of genomic imprinting has been explained as a result of asymmetrical fitness consequences and hence a conflict between maternally and paternally derived genes (Haig 2000). Our results demonstrate an interaction effect of maternal and paternal genotype and point to the possibility of coadaptation between parent-of-origin-specific effects during evolution such that the expression of maternally and paternally inherited genes from the same parent-of-origin maximizes reproductive success, and will thus be selected for. Coadaptation could be achieved by female mate choice if females are able to detect their mate's epigenotype (e.g. via MHC gene products) and adjust their maternal investment accordingly (differential allocation, e.g. Sheldon 2000). Alternatively, offspring that inherit genes of the same parent-of-origin could be more efficient in their metabolism, grow faster *in utero* and are thus born at shorter intervals, hinting at the possibility of coadaptation between maternal and offspring genotype.

(b) *Early experience effect*

We also found an effect of the social environment experienced early in life on subsequent reproductive success. When females were raised in mixed litters such that they experienced males of the reverse genotype early in their development, the effect of the interaction with the male genotype became non-significant. Rather, females that were reared in mixed litters had a lower reproductive success than otherwise identical females reared in unmixed litters. This is a novel finding and synoptically viewed with recent results in birds (Carere *et al.* 2005) implies that early experience may be a more important predictor of reproductive success and other life-history parameters than previously assumed.

Moreover, our study suggests that early experience may have a greater influence on reproductive success than parent-of-origin-specific effects, underlining the significance of this factor in determining fitness. It should be noted, however, that our results are based on data from females of one genotype only (CBAB6). A possible explanation for these results could be that elevated levels of sibling competition affect an individual's condition in a way that is not immediately reflected in lower bodyweight or some other measurable variable. Alternatively, the presence of unfamiliar pups may influence the mother's generosity to the litter as a whole, which in turn affects the focal individual's condition and subsequent reproductive success. Young females may also experience males of reciprocal genotype as competitors. This may lower their attractiveness and as a consequence females reduce their level of maternal investment (Sheldon 2000; Drickamer *et al.* 2000). At present, the mechanism underlying this effect remains unclear and further studies are required to elucidate the evolutionary consequences of early experience on reproductive success. Moreover, future studies of parent-of-origin-specific effects on individual fitness should consider the effects of the social environment experienced early in life.

We would like to thank Ed Harris, Per Smiseth and two anonymous referees for helpful comments and Trinity College, Cambridge for funding.

Bartmann, S. & Gerlach, G. 2001 Multiple paternity and similar variance in reproductive success of male and female wood mice (*Apodemus sylvaticus*) housed in an enclosure. *Ethology* **107**, 889–899. (doi:10.1046/j.1439-0310.2001.00723.x)

Carere, C., Drent, P. J., Koolhaas, J. M. & Groothuis, T. G. G. 2005 Epigenetic effects on personality traits: early food provisioning and sibling competition. *Behaviour* **142**, 1329–1355. (doi:10.1163/156853905774539328)

Drickamer, L. C., Gowaty, P. A. & Holmes, C. M. 2000 Free female mate choice in house mice affects reproductive success and offspring viability and performance. *Anim. Behav.* **59**, 371–378. (doi:10.1006/anbe.1999.1316)

Fuchs, S. 1982 Optimality of parental investment: the influence of nursing on reproductive success of mother and female young house mice. *Behav. Ecol. Sociobiol.* **10**, 39–51. (doi:10.1007/BF00296394)

Hager, R. & Johnstone, R. A. 2003 The genetic basis of family conflict resolution in mice. *Nature* **421**, 533–535. (doi:10.1038/nature01239)

Hager, R. & Johnstone, R. A. 2005 Differential growth of own and alien young in mixed litters of mice: a role for genomic imprinting. *Ethology* **111**, 705–711. (doi:10.1111/j.1439-0310.2005.01097.x)

Hager, R. & Johnstone, R. A. 2006 The influence of phenotypic and genetic effects on maternal provisioning and offspring weight gain in mice. *Biol. Lett.* **2**, 81–84. (doi:10.1098/rsbl.2005.0403)

Haig, D. 2000 Genomic imprinting, sex-biased dispersal, and social behaviour. *Ann. NY Acad. Sci.* **907**, 149–163.

Haig, D. 2004 Genomic imprinting and kinship: how good is the evidence? *Annu. Rev. Genet.* **38**, 553–585. (doi:10.1146/annurev.genet.37.110801.142741)

Krebs, J. R. & Davies, N. B. 1993 *An introduction to behavioural ecology*. Oxford, UK: Blackwell Science.

Li, L. L., Keverne, E. B., Aparicio, S. A., Ishino, F., Barton, S. C. & Surani, M. A. 1999 Regulation of maternal behaviour and offspring growth by paternally expressed *Peg3*. *Science* **284**, 330–333. (doi:10.1126/science.284.5412.330)

Lindström, J. 1999 Early development and fitness in birds and mammals. *Trends Ecol. Evol.* **14**, 343–348.

Sheldon, B. C. 2000 Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397–402. (doi:10.1016/S0169-5347(00)01953-4)