Evidence for a priming effect on maternal resource allocation: implications for interbrood competition

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It is commonly assumed that there exists interbrood competition mediated by *in utero* growth. This could be manifested by a female reallocating saved resources to future broods. Here, we report results of a manipulation experiment designed to detect such reallocation. Two groups of female mice were allowed each to produce two broods. In the first brood, the test females were mated with phenotypically normal males heterozygous for an insulin-like growth factor 2 (*Igf2*) null allele, while the control females were mated to a wild-type male. The test sample females invested 20% less into their first brood than did the control sample. In both test and control groups the females were mated with a wild-type male in the second round of mating. Surprisingly, we found that females that invested little into their first brood also invested little (compared with other second broods) into their second brood. This 'priming' effect suggests that the assumptions of classical models of parent-offspring conflict are overly simplistic but cannot disprove the existence of interbrood competition.

Keywords: growth; parent-offspring conflict; imprinting

1. INTRODUCTION

When modelling resource allocation by parents to offspring (e.g. in models of parent-offspring conflict), it is typically assumed that siblings compete for resources (see Mock & Parker 1997). In mammals, for example, it is usually assumed that there is competition mediated by *in utero* demands of embryos. This competition may either be intra- or interbrood competition (Mock & Parker 1997). The existence of interbrood competition is required, for example, by the conflict model for the evolution of genomic imprinting (Moore & Haig 1991) to explain the presence of imprinting in species with singleton births or those (such as some species of *Peromyscus*; Foltz 1981) in which paternal unrelatedness occurs only between broods (Haig 1999).

Evidence for intrabrood competition is abundant: in numerous mammals there is an inverse correlation between mean neonate size and the number of progeny in a brood (McClaren 1965; Gregory *et al.* 1996; Taylor *et*

al. 1998). By contrast, the evidence for interbrood competition is more scant. Such competition could be manifested as changes in either the size or number of broods. A saving of resources in an early brood could permit more broods per lifetime either by increasing the mother's longevity and/or by reducing the time to the next brood. Alternatively, the saving could be manifested as an increased investment into subsequent broods. The latter is most probable as a female approaches what are to be her final broods.

While having a large first brood tends to make females delay reproduction (Drewett 1983) there is no experimental evidence, that we are aware of, suggesting that a female who invests heavily into a given brood invests less into the following brood than a female whose initial brood was relatively 'cheap'. It is this issue that we address here. We manipulate first-brood resource allocation by using mice lacking the insulin-like growth factor 2 (*Igf2*) gene. We then ask whether females who are made to invest little in the first brood invest more into the second brood than control females. Given that interbrood competition could be manifested in at least two other ways, our experiment is not designed to disprove the existence of interbrood competition. Our design could in principle, however, provide support for the model and is designed to make this as likely as possible. For example, we use the fact that mice will mate immediately after giving birth to control and minimize interbrood time.

2. MATERIAL AND METHODS

(a) Manipulation of first brood investment

Igf2 promotes *in utero* growth and is imprinted, being expressed only from the paternal allele in embryonic mice (DeChiara *et al.* 1991). Consequently, a mouse inheriting a null mutation (*Igf2Δ*) from the father is growth retarded, while one inheriting the null from the mother is phenotypically normal (DeChiara *et al.* 1990). To ask whether first-brood resource allocation affects second-brood allocation, we set up two series of matings of wild-type females. In the test series (group 1), wild-type females were mated with males heterozygous for the null allele (*Igf2Δ/+*). In the control series (group 2), females were mated with wild-type males. The date of coitus was recorded upon the appearance of a copulation plug, and the resulting brood was weighed on the day of birth, and sacrificed. Females for each group were randomly selected from an age-matched collection of females of the same strain.

The *Igf2Δ/+* fathers inherited the null allele from their mothers and were hence phenotypically normal. This ensures that any female investment into a given brood is unlikely to be skewed by a female's perception of a male's size and/or quality.

(b) Production of the second brood

The same females, from both groups, were then mated to wild-type males, and the weights of the second brood determined on the day of birth. Second broods are hence identical in all parameters but one: the investment into the first brood. We collected data from 31 females, 16 in the test group (first cross to *Igf2Δ* males) and 15 in the control group. Twenty-eight females mated with the second male within one oestrous cycle of the birth of her first litter (4 days), and all were mated by the second oestrous cycle (two in the test group, one in the control). Litters were excluded if there was evidence of cannibalism, or the number in the litter was less than seven (possibly owing to cannibalism that left no obvious traces). Excluding that latter restriction makes no qualitative difference to our results. For further details of methods see electronic Appendix A available on The Royal Society's Publications Web site.

3. RESULTS

(a) Inclusion of the *Igf2Δ* genotype into the first brood reduces the total brood weight

Mean total brood weight from *Igf2Δ* fathers was reduced by *ca.* 21% compared to the mean total brood weight from wild-type fathers (8.96 ± 0.27 g and

Figure 1. First brood weights of group 1 and group 2 mothers.

11.50 ± 0.34 g, respectively; figure 1; two-tailed *t*-test: $t = 5.93$, d.f. = 29, $p < 0.0001$). As *Igf2Δ* neonates are 60% wild-type weight (DeChiara *et al.* 1990) and the brood is composed of *ca.* 50% *Igf2Δ* neonates, such a brood would be predicted to weigh 20% less than a wild-type brood. The observed reduction compares, at first sight, well with this prediction.

To some extent, however, the difference between resource allocation in control and test crosses was not simply because of the reduced growth of *+Igf2Δ* progeny. Litters containing *Igf2Δ* neonates have significantly fewer neonates than those from wild-type crosses (mean number in the litter from *Igf2Δ* fathers: 7.69 ± 0.20 g, and in litters from wild-type fathers: 8.73 ± 0.32 g; two-tailed *t*-test: $t = 2.84$, d.f. = 29, $p < 0.01$). The low litter number is caused by pre-partum effects (see electronic Appendix A).

(b) Forced reduction in resource allocation to the first brood decreases allocation to the second brood

In contrast to our expectation, group 2 (control) broods were significantly heavier than group 1 (test) broods (figure 2a; mean brood weight of group 1: 12.85 ± 0.28 g and of group 2: 13.92 ± 0.33 g; two-tailed *t*-test: $t = 2.47$, d.f. = 29, $p = 0.019$; Mann-Whitney *U*-test: $N_1 = 16$, $N_2 = 15$, $p = 0.009$). (Note: we could replicate the usual finding of intrabrood competition; for details see electronic Appendix A.)

(c) Control females have more progeny in their second broods than do test animals

What is the mechanism of the unexpected difference between group 1 and group 2 females? The increased weight of group 2 broods over group 1 broods was not due to an increase in allocation per neonate, as the mean weights of second litter neonates in group 1 and group 2 did not significantly differ (figure 2b; 1.39 ± 0.02 g and 1.38 ± 0.02 g, respectively; two-tailed *t*-test: $t = 0.14$, d.f. = 29, $p = 0.887$). This would be consistent with the idea that there exists an evolutionarily stable strategy level of investment per neonate. Given the above result, we expect the increased weight of group 2 broods to be owing to their having more offspring. Indeed, group 2 females

had on average 10.13 ± 0.35 offspring per second litter, whereas group 1 females had 9.31 ± 0.28 offspring per second litter (figure 2c; one-tailed *t*-test: $t = 1.83$, d.f. = 29, $p = 0.04$). Higher resource allocation in second broods has previously been well described (Roberts 1961; Krakow & Gruber 1990).

4. DISCUSSION

While we find evidence for intrabrood competition, we did not find evidence that females made to invest lightly into the first brood invest more heavily in the second brood than a female who invested heavily into the first brood. Why might this be? For every 1 g increase in the weight of the first brood there was a 0.3 g increase in the weight of the second brood (Spearman's rank: $r = 0.488$, $n = 31$, $p < 0.01$; figure 3a). This appears to be because of there being more progeny in the second brood: there was a positive correlation between the number of neonates in the first brood and the number in the second brood (Spearman's rank: $r = 0.507$, $n = 31$, $p < 0.01$; figure 3b). These results suggest that females are somehow 'primed' by the experience in their first brood, such that the number in the second brood is determined in part by the number in the first.

This priming is not owing to pathology associated with carrying mutant mice. Excluding the test sample, the total brood weight is greater in the second brood than in the first brood (mean weight of first brood: 11.50 ± 0.34 g; mean weight of second brood: 13.92 ± 0.33 g, $p < 0.0001$; two-tailed paired *t*-test: d.f. = 14; figure 3c), and this was largely owing to increased number of progeny in the second brood (mean number in first litter: 8.73 ± 0.32 g; mean in second litter: 10.13 ± 0.35 ; paired *t*-test: $p < 0.005$, d.f. = 14; figure 3d). The same is found in the test group as well (mean weight in the first brood: 7.69 ± 0.20 ; mean number in the second brood: 9.31 ± 0.28 ; two-tailed paired *t*-test: $p < 0.0001$, d.f. = 14). What else might explain this priming? We propose a number of hypotheses.

If the differences in first brood sizes reflect early re-absorption rates, then possibly the uterine sites where embryos have implanted but have been re-absorbed, are not sites that are competent for implantation of other embryos within some critical time window. Under this model, implantation numbers will not be different in the females mated in the control group and those mated in the test group in their first broods, but will be different in the second broods. If the uterus recovers over time, a longer delay to second reproduction should diminish the correlation between first and second brood sizes.

Alternatively, if prior embryos change the maternal system, possibly by expanding the uterine vasculature, a female having many progeny may be more able to support many in subsequent pregnancies. This model predicts no differences in implantation rates in first and second broods, but differences in re-absorption rates.

The above considerations suggest that some constraint on second litter size might be placed by the number of progeny in the first litter. This may have masked an interbrood competition effect. We should then ask whether group 1 females have heavier second broods when controlling for first brood number. We find no such evidence: ANCOVA of second brood weight as a function of

Figure 2. (a) Comparison of second brood weights between females previously mated to *Igf2Δ* males (group 1; $n = 16$) or to wild-type males (group 2; $n = 15$). Group 1 broods weighed significantly less than group 2 broods. (b) Mean weights of offspring in the second brood do not differ between group 1 and group 2 females. (c) The number of offspring in second broods of group 2 females exceeds the number of offspring in group 1 second broods.

Figure 3. (a) The weight of the first brood positively correlates with the weight of the second brood, combining both test and control samples. The same comparisons on group 1 ($n = 16$) and group 2 ($n = 15$) samples are not significant; group 1: $p = 0.383$, Spearman's rank $r = 0.243$; group 2: $p = 0.405$, Spearman's rank $r = 0.232$. (b) The number of offspring in the first brood positively correlates with the number of offspring in the second brood, combining both groups. The same comparison on groups 1 and 2 are not significant; group 1: $p = 0.278$, Spearman's rank $r = 0.289$; group 2: $p = 0.083$, Spearman's rank $r = 0.462$. See also electronic Appendix A. (c) The mean total brood weight of group 2 is greater in the second brood than in the first brood. (d) The mean number of offspring in the litter of group 2 is greater in the second brood than in the first brood.

treatment with first brood number as the covariate: $F = 1.97$, d.f. = 1, $p = 0.172$; see figure 6 in electronic Appendix A. Indeed, from examination of residuals of regression of second brood weight on first brood number, it is again the control females that have the larger second broods (mean of residuals for group 1: -0.25 ± 0.28 ; group 2: 0.27 ± 0.30). For the corresponding ANCOVA

for mean weight and total number in the second brood see electronic Appendix A.

While the above supposes that the restriction on second brood litter size may be a constraint, the effect instead may be adaptive. We can imagine modifications to models of resource allocation that suppose that high prior investment improves the return from future investment. Should

such a model predict that increased investment will be owing to increased numbers rather than increased size of neonates, then the fact that we observe no difference in the mean size of neonates in groups 1 and 2 in the second broods would be supportive.

The above results, while suggesting that the situation is more complex than commonly assumed, do not disprove the existence of interbrood competition. If our pathological mechanisms are correct then the fact that we permitted females to reproduce immediately after giving birth may be important. We also chose to feed our mice *ad libitum* and it may be the case that in the wild resources are more limited and interbrood competition more evident. Alternatively, in the wild any putative cost may be experienced as differential mortality rates or reduced time to next reproductive event. Further, we deliberately avoided examining the consequences of lactation, which may be a more important drain on resources (Clutton-Brock *et al.* 1989). Hence, we cannot rule out the possibility that interbrood competition does exist. However, our results suggest that simple models of such competition, ignoring as they do the apparent priming effect, are overly simplistic.

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