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Coadaptation in mother and infant regulated by a paternally expressed imprinted gene

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This study investigates how a targeted mutation of a paternally expressed imprinted gene regulates multiple aspects of foetal and post-natal development including placental size, foetal growth, suckling and post-natal growth, weaning age and puberty onset. This same mutation in a mother impairs maternal reproductive success with reduced maternal care, reduced maternal food intake during pregnancy, and impaired milk let-down, which in turn reduces infant growth and delays weaning and onset of puberty. The significance of these coadaptive traits being synchronized in mother and offspring by the same paternally expressed imprinted gene ensures that offspring that have extracted 'good' maternal nurturing will themselves be both well provisioned and genetically predisposed towards 'good' mothering.

Keywords: genomic imprinting; *Peg3*; paternally expressed gene; mother–infant coadaptation; maternalism; conflict theory

1. INTRODUCTION

Among vertebrates, genomic imprinting appears to be unique to mammals, and recent evidence suggests that this evolved *ca*. 100 Myr ago at the time of divergence of placental from egg-laying mammals (Killian *et al.* 2000). Non-placental monotremes (platypus) express these genes but they appear not to be imprinted, while many imprinted genes are expressed in the placenta of all the mammalian species so far investigated (Killian *et al.* 2001). Interestingly, in marsupials and in extra embryonic tissue of some mammals, X-inactivation is determined according to parent of origin (Zeng & Yankowitz 2003); only the maternal X is expressed in the placenta (Kay *et al.* 1994), while in all other tissues the silencing of the X-chromosome is stochastic (Lyon 1999).

Epigenetic differences in chromatin structure influence gene expression in insects, and 'parent-of-origin' dependent gene expression found in *Drosophila* is indistinguishable from imprinting except for DNA methylation differences (Lloyd *et al.* 1999). This suggests that imprinting is an ancient and conserved mechanism for gene silencing based on differences in chromatin structure. Such differences in chromatin structure may have arisen by insertion of foreign DNA sequences such as retrotransposons, retroviruses and parasitic repetitive elements containing 5-methylcytosine (Yoder *et al.* 1997). The oocyte cytoplasm has the ability to discriminate between paternal genomes as seen in the selective elimination of paternal chromosomes in invertebrates (Werren & Hatcher 2000). Moreover, the mammalian fertilized egg undergoes a dramatic demethylation of the paternal but not the maternal genome, and the imprint for X-inactivation appears to involve a maternally inherited protein in the oocyte cytoplasm (Reik *et al.* 2001; Mak *et al.* 2002). Hence mechanisms for chromosomal recognition and selective gene silencing almost certainly preceded the establishment of mammalian gene imprinting, and natural selection may have acted upon epigenetic differences between parental alleles (Pardo-Manuel de Villena *et al.* 2000). Such selection pressures would operate where parental asymmetries prevail as in the context of 'parental conflict' (Haig 1997).

Around 70 Myr ago mammals underwent a significant diversification to form the present-day 109 families and *ca*. 4000 species (Springer *et al.* 2003). This rapid expansion, which colonized environmental extremes of land, sea and air, probably owed much to the hallmark features that mammals evolved, including viviparity, homeothermy, maternal care and milk provisioning. These traits have a common outcome, namely successful maternalism, and failure of any one of them would severely compromise lifetime reproductive success. In evolutionary terms there is something very special about the mother–infant relationship of eutherian mammals in that pre-partum maternal resources supplied by the placenta and post-partum lactation depend upon the foetal genotype (Wolf 2000). Growth and development of the placenta is important for transfer of nutrients (Constancia *et al.* 2002) while placental hormones increase maternal food intake (Wade 1986), prime the brain for maternal care (Bridges *et al.* 1997) and the mammary gland for milk production, and silence female sexual interest in males. The suckling stimulus from the post-partum infant sustains milk production, stimulates milk let-down and also suppresses maternal reproduction (McNeilly 1994; Woodside *et al.* 2000). Here, we show that targeted mutation of a paternally expressed gene (*Peg3*) in offspring reared with wild-type mothers experience 32% mortality and wild-type offspring reared with mutant mothers experience 28% mortality. The combined mutation in mother and offspring is 94% lethal (only 6% of pups survive to weaning age), which raises the question as to what the coadaptive effects of this gene are in mother and infant.

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2. METHODS

(a) *Subject animals, housing and care*

All subjects were laboratory mice (*Mus musculus*) and all procedures were undertaken with the relevant ethical approvals. The original *Peg3* mutation was developed on the 129sv inbred strain and the generation of the targeted mutation is described in detail in Li *et al.* (1999). Briefly, a 4.8 kb IRES-βgeo-SV40 polyadenylation selection cassette was inserted into the 5′ coding exon of the *Peg3* gene in embryonic stem cells of the 129sv inbred strain, using gene targeting. Heterozygous embryos inheriting the *Peg3*β*geo* mutation from the paternal germ line showed no detectable wild-type *Peg3* mRNA, but did show appropriate β-galactosidase (β-Gal) expression. Both heterozygous and homozygous subjects showed identical β-Gal expression, demonstrating the functional equivalence between heterozygotes and homozygotes.

Animals were housed at the sub-Department of Animal Behaviour (Madingley, Cambridge, UK) on a reverse 12 D : 12 L light cycle, under a constant temperature of 21 °C and 55% humidity, to facilitate observational studies. All animals were given *ad libitum* access to water and the RM3(E) chow diet supplied by Lillico. Fresh bedding, wood-shavings supplied by Lillico, was placed into cages weekly. Behavioural observations took place during the dark period of the light cycle, this being the period when the mice are most active. No behavioural trial took place on the day of cleaning, and all trials were carried out under dim red illumination.

Animals were identified as either wild-type or $Peg3^{+/-}$ by β-Gal staining. Cartilage tissue was removed from a 2 mm long piece of tail, cut from the offspring under a licensed procedure. Each tissue was incubated in a small 1 ml volume Eppendorf tube, in phosphate-buffered saline containing X-gal (1 mg ml^{-1}), 5 mM of $K_4Fe(CN)_6$-$3H_2O$, 5 mM of $K_3Fe(CN)_6$, 1 mM of $MgCl_2$ and 0.02% of NP-40 at room temperature, being placed on a Janke and Kunkel IKA-Vibrax-VXR orbital shaker overnight until colour developed. The presence of a blue–green colour in the tissue indicates the presence of β-Gal and therefore the $Peg3^{+/-}$ mutation.

(b) *Animal breeding*

Three separate groups of breeding pairs comprised the majority of this study (see figure 1): (i) wild-type female × wild-type male, offspring are all wild-type; (ii) wild-type female × homozygous $Peg3^{-/-}$ male, offspring are all heterozygous $Peg3^{+/-}$ but null for the *Peg3* allele because the gene is imprinted and paternally expressed; and (iii) $Peg3^{+/-}$ female × wild-type male, offspring are all wild-type (both +/+ and −/+ where the maternal allele is silent). All females were three months old and virgins. To examine intrauterine and post-natal effects on pup growth, a fourth group of breeding pairs was established. Multiparous wild-type females were mated with heterozygous $Peg3^{+/-}$ males giving mixed litters composed of both wild-type and $Peg3^{+/-}$ offspring. The growth of these offspring was compared with those born to multiparous mothers from conditions (i) and (ii) above.

(c) *Onset of oestrus*

One female from separate litters containing five to seven pups were removed from their mothers at day 28 post-natal (pn) and placed individually into small cages. From day 30 pn, a vaginal smear was taken daily and the body weight of females was recorded using an electronic balance. The vaginal smear was taken by swabbing the inside of the vagina with a small damp cotton bud and smearing the contents onto a clean glass Menzel-Glaser slide. Smearing occurred daily until the first oestrus smear was observed under a microscope as determined by the presence of cornified epithelial cells and an absence of leucocytes.

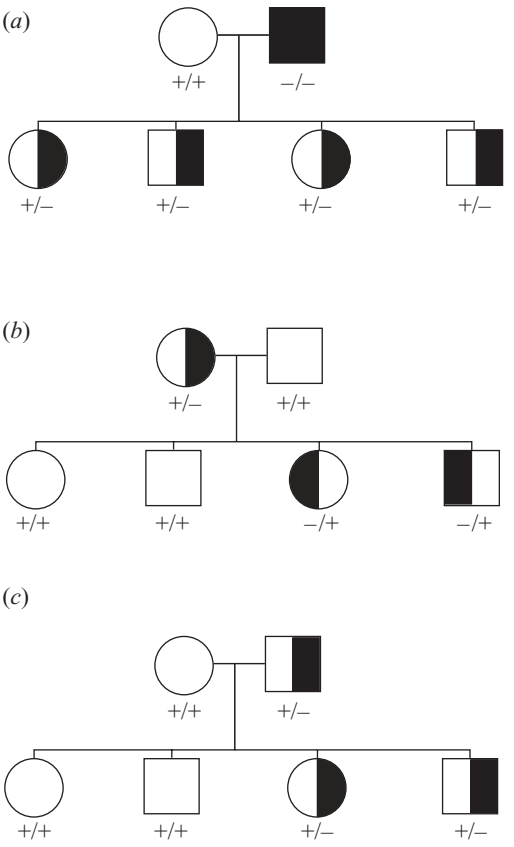

Figure 1. Animal breeding pairs used. (*a*) A wild-type female (open circle) mated with a homozygous mutant $Peg3^{-/-}$ male (filled square) produce litters composed of heterozygous mutant $Peg3^{+/-}$ sons (right-half filled squares) and daughters (right-half filled circles), who express the paternally active copy of the *Peg3* mutation. (*b*) A heterozygous mutant $Peg3^{+/-}$ female mated with a wild-type male (open square) produce litters composed of wild-type sons and daughters. Half of the litter are homozygous wild-types (+/+) and half are non-mutant carriers of the maternally silent *Peg3* mutation (−/+), but are still phenotypically wild-type (left-half filled circles and squares). (*c*) A wild-type female mated with a heterozygous mutant $Peg3^{+/-}$ male produce mixed litters. Half of the offspring are wild-type (+/+) and half are heterozygous mutant $Peg3^{+/-}$ who express the paternally active copy of the *Peg3* mutation. In addition, this study also used a group of wild-type males mated with wild-type females producing litters entirely composed of wild-type offspring (not shown).

(d) *Suckling by pups*

Behavioural testing took place on day 5 pn, 1 h after the start of the dark phase of the light cycle. Mutant $Peg3^{+/-}$ and wild-type litters were removed from their wild-type mothers and placed into a holding cage under a red light to keep them warm. The weight of litters and mothers was recorded using an electronic balance. After 2 h, litters and mothers were re-weighed and reunited. The weight of litters and females was subsequently recorded every hour for 4 h.

(e) *Milk let-down by mothers*

Behavioural testing took place on day 5 pn, 1 h after the start of the dark phase of the light cycle. Mutant *Peg3*$^{+/-}$ and wild-type mothers were removed from their wild-type litters and placed into a holding cage under a red light to keep them warm. The weight of litters and mothers was recorded using an electronic balance. After 2 h, litters and mothers were re-weighed and reunited. The weight of litters and females was recorded after 6 h of suckling by pups. Litters were standardized for size between wild-type and *Peg3*$^{+/-}$ mothers.

(f) *Body temperature of females*

The rectal temperature of three-month-old wild-type and *Peg3*$^{+/-}$ females was recorded using a Vet Tech Solutions Ltd miniature electronic rectal thermometer. Temperatures were taken 1 h after the start of the dark phase, once weekly, for 17 weeks.

(g) *Maternal behaviour*

Three-month-old primiparous wild-type and *Peg3*$^{+/-}$ females were tested on the day of birth of their litters. Three pups were randomly placed in the homecage of the female and the latency to retrieve a pup, nest-build and crouch over pups was recorded.

(h) *Thermoregulation by pups*

Individual wild-type and *Peg3*$^{+/-}$ pups from separate litters were removed from their mother and placed into a holding cage on day 12 pn. Their body temperature was measured by inserting an electronic thermistor underneath the skin fold at removal from their mother, and after 10, 15 and 30 min. Pups were replaced with their mothers after the 30 min testing period.

3. RESULTS

(a) *Effect of* Peg3 *mutation in offspring*

Offspring sired by homozygous mutant fathers mated with wild-type mothers express the mutated allele, while the wild-type allele that is inherited from the mother is silenced (see figure 1). There was no difference in the total number of pups in wild-type (+/+) or *Peg3*$^{+/-}$ mutant litters, although mutant litters had fewer pups that survived the first day of birth (4.96 ± 0.5 compared with 6.66 ± 0.48; *t*-test: $t = 2.31$, d.f. = 46, $p < 0.024$). Moreover, the mean pup weight was lower in *Peg3*$^{+/-}$ mutant litters than in wild-type (*t*-test: $t = 5.56$, d.f. = 49, $p < 0.001$), and placental size was 68% that of wild-type litters. Pup weight is a covariant of litter size and for both wild-type and *Peg3*$^{+/-}$ pups, weight decreased with litter size when genotype was a fixed factor (general linear model (GLM); $F_{1,47} = 45.9$, $p < 0.001$), with a similar relationship for each genotype ($F_{1,47} = 0.07$ n.s.). However, genotype also has a significant effect on pup weight controlling for litter size, with *Peg3*$^{+/-}$ pups weighing less at birth than wild-type pups ($F_{1,47} = 14.48$, $p < 0.01$; figure 2*a*).

The weight of wild-type females carrying wild-type or *Peg3*$^{+/-}$ mutant pups throughout their pregnancy was also recorded. In the last week of the three-week pregnancy, females carrying *Peg3*$^{+/-}$ litters failed to gain the same weight as females carrying wild-type litters (*t*-test: $t = 2.88$, d.f. = 33, $p < 0.007$). The body weight gain from before pregnancy compared with the immediate post-partum

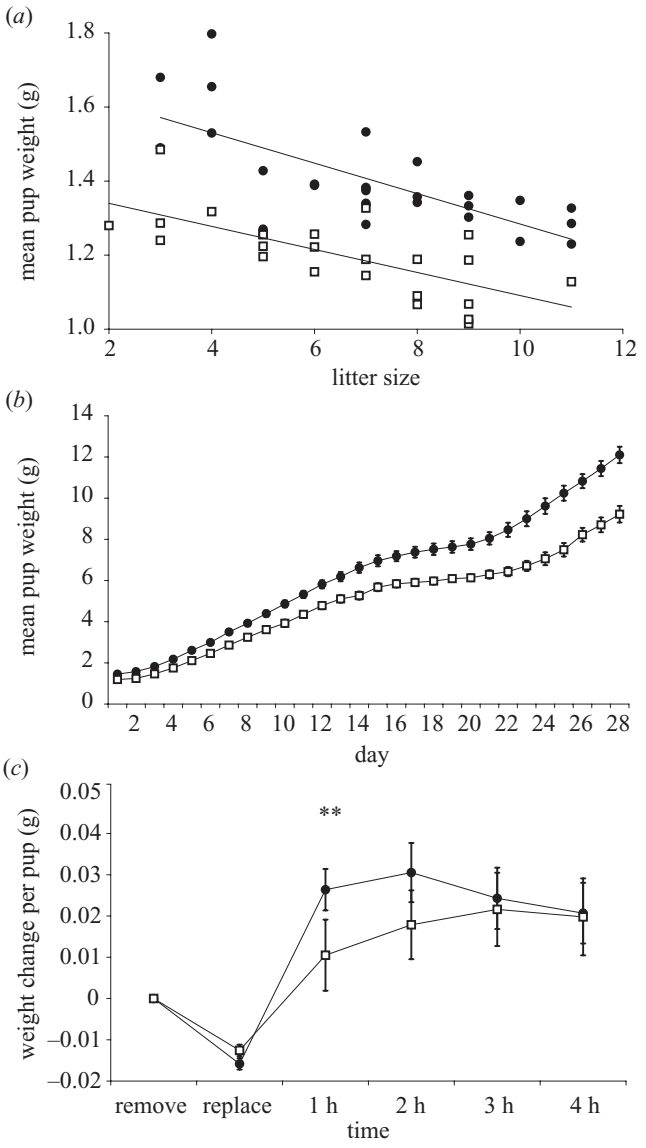

Figure 2. Development and behaviour of *Peg3*$^{+/-}$ pups (open squares) compared with wild-type pups (filled circles), all born to wild-type mothers. (*a*) *Peg3*$^{+/-}$ pups have lower birth weights over all litter sizes compared with wild-type pups (GLM: $F_{1,47} = 45.91$, $p < 0.001$). (*b*) *Peg3*$^{+/-}$ pups show further growth retardation over the first 28 days after birth (RMA: $F_{1,21} = 32.2$, $p < 0.001$). (*c*) Five-day-old *Peg3*$^{+/-}$ pups gain significantly less weight than wild-type pups after 1 h of suckling, having been replaced with wild-type mothers following 2 h of separation (*t*-test: $t = 3.01$, d.f. = 23, $p < 0.006$). Data are mean ± s.e.m.

period of wild-type females following the birth of their mutant pups was lower than that of controls, indicating that overall less reserves were gained during pregnancy (*t*-test: $t = 2.23$, d.f. = 35, $p < 0.032$; table 1).

Over the 28 days following birth, *Peg3*$^{+/-}$ mutant pups gained less weight than wild-type pups, although both groups were suckled by a wild-type mother (figure 2*b*). Moreover, this weight difference increased over time even into the post-weaning period after day 20 (GLM: $F_{1,63} = 30.5$, $p < 0.001$) and especially during the transition period to solid food (days 17–25: figure 2*b*).

The ability of *Peg3*$^{+/-}$ pups to suckle from wild-type mothers was directly assessed following a 2 h separation

from the mother. Both the mutant and wild-type pups lost the same weight during the period of separation and although both gained weight following reunion, $Peg3^{+/-}$ mutants gained significantly less weight over the first 2 h of returning to their mother (figure 2*c*). Mutant $Peg3^{+/-}$ mortality increased significantly over the suckling period, being higher both on the day of birth (Mann–Whitney $U = 384.5$, $p < 0.019$), during the first 5 days post-partum ($U = 299.5$, $p < 0.001$) and up to weaning at day 19 ($U = 325$, $p < 0.001$).

Following weaning at day 20, individually housed wild-type female mice enter puberty around day 40. In this study, mean time of puberty entry (first oestrus) for wild-type females was 42.2 ± 2.0 days while post-weaning $Peg3^{+/-}$ mutant females entered puberty significantly later (59.9 ± 2.1 days, *t*-test: $t = 5.32$, d.f. = 34, $p < 0.001$). Moreover, at the time of puberty they weighed significantly less than wild-type females (15.4 ± 0.2 compared with 16.9 ± 0.1 g; *t*-test: $t = 4.75$, d.f. = 34, $p < 0.001$).

Intrauterine and post-natal effects on infant growth were enhanced in litters of mixed genotype (produced from matings of multiparous wild-type females and heterozygous $Peg3^{+/-}$ males, thereby containing both wild-type and $Peg3^{+/-}$ pups). $Peg3^{+/-}$ mutant pups weighed less in such 'mixed' litters than $Peg3^{+/-}$ pups from litters that were exclusively mutant at day 28 pn (9.8 ± 0.2 compared with 10.9 ± 0.2; GLM: $F_{1,91} = 9.45$, $p = 0.003$). Conversely, wild-type pups weighed more in 'mixed' litters than wild-type pups in litters that were all wild-type at the same age (12.7 ± 0.2 compared with 12.3 ± 0.1; GLM: $F_{1,181} = 5.54$, $p = 0.02$).

(b) *Effect of* $Peg3^{+/-}$ *mutation in mother*

Considering the reverse condition of $Peg3^{+/-}$ mutation in the mother that has mated with a wild-type male and given birth to all wild-type pups (i.e. including the −/+ pups where the maternal allele is silent), a significant reduction in pup weight was recorded compared with pups born to wild-type mothers (figure 3*a*). The difference in pup weight was independent of litter size although with both maternal genotypes an increase of litter size produced pups of lower weight (figure 3*a*). There was no difference in litter size according to maternal genotype, nor in the number of pups born alive.

The change in female food intake during pregnancy measured in terms of the extra food females eat compared with pre-pregnancy showed significant differences between wild-type and mutant mothers (table 1). In the early stages of pregnancy the increase in food intake is stimulated by placental hormones and is primarily deployed for laying down energy stores ready for lactation. $Peg3^{+/-}$ mutant mothers failed to respond appropriately to this placental signal from wild-type pups and did not increase food intake as much as wild-type females (GLM: $F_{1,20} = 5.34$, $p < 0.03$). Throughout pregnancy (18 days), wild-type females consumed more food than $Peg3^{+/-}$ mutant females (*ca*. 10 g, 170 calories) with an overall day-by-day significantly greater food intake ($F_{1,24} = 8.93$, $p < 0.006$; table 1). One consequence of this failure to increase food intake sufficiently in $Peg3^{+/-}$ mutant mothers was seen following parturition when the extra body weight reserve carried over to the post-partum period was significantly reduced in the $Peg3^{+/-}$ mother (table 1).

$Peg3^{+/-}$ mutant females were impaired with milk let-down (figure 3*c*). Hence wild-type pups with a mutant $Peg3^{+/-}$ mother lost weight following the first day of birth while those with a wild-type mother gained weight (*t*-test: $t = 4.82$, d.f. = 26, $p < 0.001$). Moreover, wild-type pups born to $Peg3^{+/-}$ mutant mothers were significantly weight reduced throughout the period of weaning compared with those born to wild-type mothers (GLM: $F_{1,32} = 133.2$, $p < 0.001$; figure 3*b*). Following weaning when the offspring take to solid food, those wild-type pups born to the $Peg3^{+/-}$ mutant mother continued to be growth retarded compared with pups of the same genotype born to wild-type mothers (figure 3*b*) although they did catch up by weeks 8–10 post-partum. The difference in post-natal growth between litters born to wild-type mothers and $Peg3^{+/-}$ mutant mothers was observed over all litter sizes ($F_{1,21} = 16.32$, $p < 0.001$). Probably as a consequence of this delayed growth, wild-type pups born to $Peg3^{+/-}$ mutant mothers also commenced puberty late (42.2 ± 2.0 compared with 46.9 ± 1.5 days; *t*-test: $t = 5.32$, d.f. = 24, $p < 0.005$).

(c) *Coadaptation of pup thermoregulation and maternal behaviour*

Rodent pups are unable to maintain body temperature for the first 10 days post-partum. During this period the mother provides a nest and retrieves any pup that moves away from the nest until pup thermogenesis enables the pups to sustain their own body temperature. $Peg3^{+/-}$ mutant mothers were impaired relative to wild-type mothers in their maintenance of body temperature (figure 4*a*; repeated measures ANOVA (RMA): $F_{1,13} = 6.58$, $p < 0.025$) and in their ability to build nests and retrieve offspring (figure 4*b*; Mann–Whitney $U = 42.0$, $p < 0.005$). Moreover, on day 12 pn wild-type pups that were separated from their wild-type mother were able to maintain their body temperature, whereas $Peg3^{+/-}$ pups that were separated from a wild-type mother failed to accomplish this, and body temperature decreased by 2.3 °C over 30 min of separation (figure 4*c*; RMA: $F_{1,18} = 47.7$, $p < 0.001$).

4. DISCUSSION

The conflict theory of genomic imprinting predicts that paternal expression of imprinted genes in an embryo should demand more from a mother than maternally expressed genes (Haig & Graham 1991). Thus, paternally expressed imprinted genes should tend to increase offspring size, survival and ultimately reproductive success. As predicted, the offspring of a wild-type mother that have inherited the *Peg3* mutation from their father exhibited impaired infant suckling, weight gain and growth. $Peg3^{+/-}$ litters suffered high post-natal mortality leading to reduced litter size, and this trend continued until weaning. The placental size of mutant embryos was reduced by 25% and this had a major impact on birth weight. This was particularly notable in mixed mutant/wild-type litters where mutant pups are smaller than in litters that are all mutant, and wild-type offspring are larger than in litters that are all wild-type. Hence when there is intrauterine

Table 1. The relative effect of the *Peg3* mutation in the pup versus the mother.
(All data are mean ± s.e.m and significant results are represented with respect to the control situation as follows: $^{***}p < 0.005$, $^{**}p < 0.01$, $^{*}p < 0.05$. All statistical tests were undertaken using Student's *t*-tests. All groups have *N* values between 13 and 27.)

	control	$Peg3^{+/-}$ pups	$Peg3^{+/-}$ mother
mean litter size	6.86 ± 0.48	5.93 ± 0.51	6.46 ± 0.40
mean number of pups born alive	6.66 ± 0.48	4.96 ± 0.55*	6.31 ± 0.44
mean litter weight (g)	9.59 ± 0.51	6.35 ± 0.59***	8.02 ± 0.48*
mean pup weight (g)	1.40 ± 0.03	1.20 ± 0.02***	1.25 ± 0.06**
food consumed by mother during pregnancy (g)	93.2 ± 2.8	95.8 ± 3.6	83.6 ± 1.8**
maternal weight gain during pregnancy (g)	14.27 ± 0.69	11.86 ± 0.77*	11.91 ± 0.57*
maternal weight gain (g) (pre- versus post-pregnancy)	2.92 ± 0.19	2.32 ± 0.28*	1.88 ± 0.24**

and post-natal competition for maternal resources, the smaller placenta resulted in mutants being more severely impaired, and wild-type pups of the same litter were more advantaged. When the mutation was in the foetus, wild-type mothers ate less and failed to increase their food intake in the last week of pregnancy, suggesting an impairment of placental endocrine signals that are, in part, responsible for regulating maternal food intake. Moreover, mutant pups were also slower to regulate thermogenesis and maintain homeothermy, and later showed a delay in puberty onset. Although crucial to infant survival, these latter features do not fit comfortably with the conflict hypothesis.

$Peg3^{+/-}$ mutation in the mother produced a complementarity of dysfunctions, which was examined independently of the mutation in pups. Mutant females failed to increase their food intake in the early stages of pregnancy, ate less throughout pregnancy and carried over less bodyweight reserve into the post-partum period. Milk let-down was impaired in the mutant female during the post-partum period and their pups lost weight on day 1 following birth. Wild-type pups born to $Peg3^{+/-}$ mutant mothers remained significantly weight reduced throughout the post-partum period, which had a knock-on effect for puberty onset. Regardless of whether the *Peg3* mutation was present in the mother or in the pup the dysfunctional outcome was remarkably similar. The *Peg3* gene influenced suckling in the pup and milk let-down in the mother, placental nutrient transfer in the pup and food intake in the mother, post-natal growth in the pups and the time that the mother takes to achieve weaning weight of her pups. Finally, provision of warmth by maternal crouching and nest building, and the early onset of thermogenesis by pups, are also complementary aspects of maintaining body temperature, and each was impaired by mutation of the *Peg3* gene. While independently producing a complementarity of dysfunctions when mutations are either in mother or pups, the outcome was lethal, with only 6% of pups surviving beyond day 1 when the *Peg3* gene mutation was simultaneously inherited by mother and infant.

Although the consequences of this mutation in mother versus offspring are functionally complementary, the site of action whereby the effects are brought about are quite different. In the mother, *Peg3* is expressed strongly in the developing and adult hypothalamus and is known to affect size and structure of the paraventricular nucleus, especially involving oxytocinergic neurons (Li *et al.* 1999). The paraventricular nucleus of the hypothalamus regulates milk let-down, maternal care including nest building, food intake and body temperature (Russell *et al.* 2001), all of which are impaired in the adult mutant female. Hence these hypothalamic dysfunctions could account for all of the phenotypic impairments seen in the adult, while the delayed post-natal growth and late weaning of infants born to mutant mothers is probably secondary to the failure of the mother's hypothalamus to respond to placental hormone signals and post-natal suckling signals. In the foetus, pre-natal effects that reduce birth weight are probably secondary to *Peg3* action in the placenta. Impaired suckling by the infant also delays post-natal growth and weaning, and defects in the onset of infant thermogenesis are also probably consequences of hypothalamic dysfunction (Smith *et al.* 1998).

The cellular expression of *Peg3* encodes for a large zinc finger protein with 11 widely spaced C2H2 motifs (Kuroiwa *et al.* 1996) and two primary targets of expression are the hypothalamus and placenta. This chromosome region in the mouse is syntenic with human chromosome 19q13.4, which appears to have tumour suppressor properties in adults (Kohda *et al.* 2001). At the cellular level *Peg3* is a cell death mediator and cooperates with Siah1a in P53-mediated apoptosis. It is upregulated after exposure of primary cultured neuronal cells to hypoxic stress (Yamaguchi *et al.* 2002). *In vivo*, *Peg3* mRNA expression is induced abundantly in neurons by ischaemic damage and is thought to accelerate neuronal death through translocating Bax proteins from cytosol to mitochondria (Deng & Wu 2000; Johnson *et al.* 2002). The Peg3 protein also acts with Siah1, Siah2 and Traf2, which contain RING finger domains in their N-terminus. These proteins are involved in apoptosis and cell cycle arrest, indicating that Peg3 interacts with these RING finger domains to form multiprotein complexes during development (Deng & Wu 2000). One such protein complex involving Traf2 is thought to interact in the tumour necrosis factor signalling pathway to block apoptosis (Relaix *et al.* 1998). Hence the Peg3 protein, via regulation of apoptopic pathways, has the capacity to shape and remodel the development of those structures in which it is expressed.

The consequences of evolving imprinting for *Peg3* gene regulation are observed through its actions in the hypothalamic and placental tissues which, although of mother and infant genotype, interact with each other via neuroendocrine and placental hormonal factors in the same individual. The maternal consequences of the mutation show similarities with studies that have produced lesions of the hypothalamus (food intake, maternal behaviour,

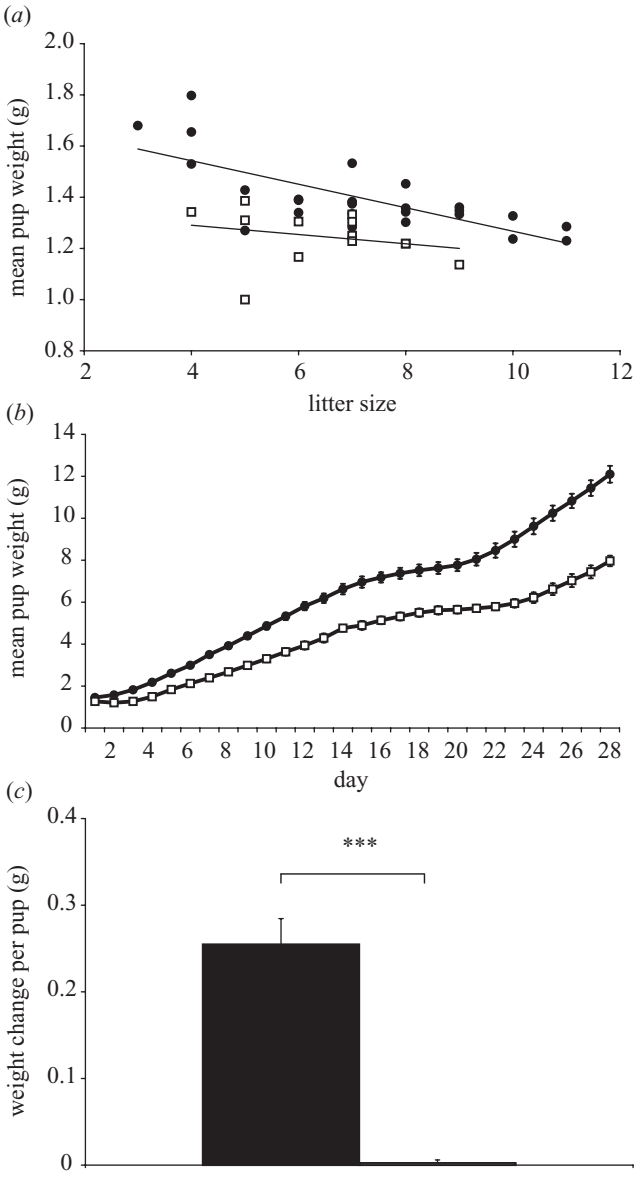

Figure 3. Development and behaviour of wild-type pups (+/+ and −/+; see text) born to *Peg3*$^{+/-}$ mothers (open squares) and wild-type mothers (filled circles). (*a*) Wild-type pups have lower birth weights over all litter sizes when born to *Peg3*$^{+/-}$ compared with wild-type mothers (GLM: $F_{1,13} = 11.07$, $p < 0.02$). (*b*) Wild-type pups born to *Peg3*$^{+/-}$ mothers show further growth retardation over the first 28 days post-birth (RMA: $F_{1,32} = 133.2$, $p < 0.001$). (*c*) Five-day-old wild-type pups with a *Peg3*$^{+/-}$ mother (open bar) do not gain as much weight following 6 h of suckling as those with wild-type mothers (filled bar) ($N^{+/+} = 16$, $N^{+/-} = 9$ litters per group, Mann–Whitney $U = 21$, $p < 0.005$). Data are mean ± s.e.m.

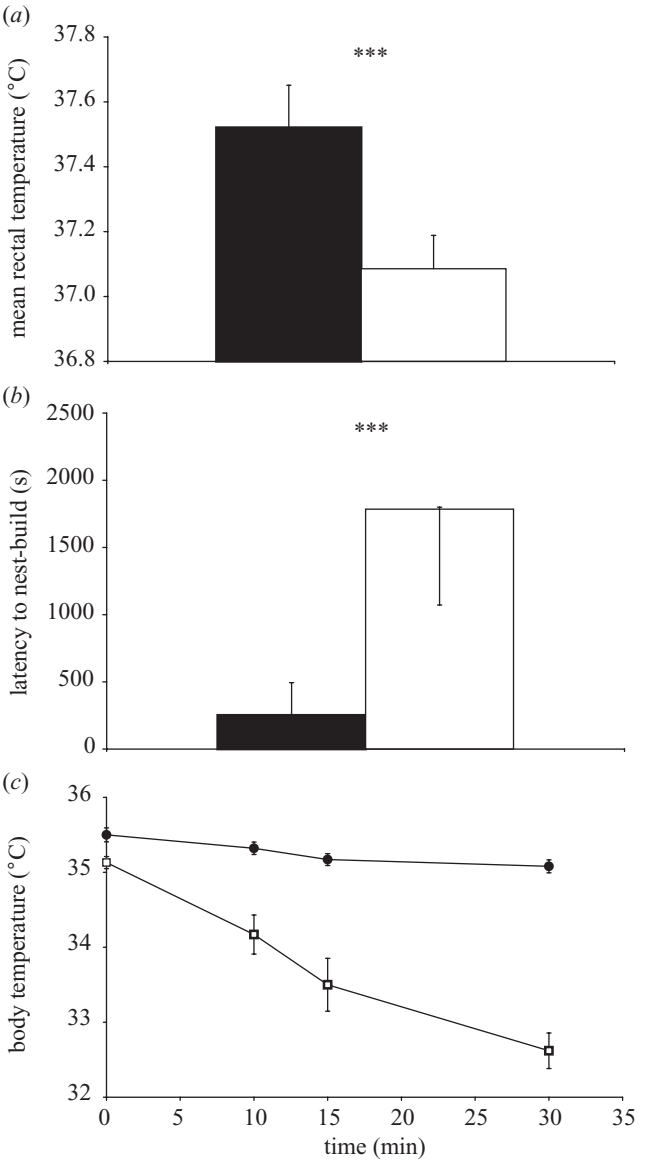

Figure 4. Mother and infant thermoregulation. (*a*) *Peg3*$^{+/-}$ mothers (open bar) have a lower core body temperature than wild-type mothers (filled bar) ($N^{+/+} = 8$, $N^{+/-} = 7$). Data are mean ± s.e.m. (*b*) Primiparous *Peg 3*$^{+/-}$ mothers (open bar) are slower to build nests than primiparous wild-type mothers (filled bar ($N^{+/+} = 8$, $N^{+/-} = 9$)). Data are medians and inter-quartile ranges. (*c*) *Peg3*$^{+/-}$ infants fail to maintain their own body temperature compared with wild-type infants when removed from their mothers on day 12 pn for 30 min ($N = 10$ per genotype). Data are mean ± s.e.m.

body temperature, milk let-down). Moreover, all of these hypothalamic phenotypes are also influenced by hormones produced by the placenta (food intake, maternal behaviour, milk production) or by post-natal pup development (pup thermogenesis and maternal body temperature) and post-partum behaviour of the pups (suckling: milk production and milk let-down). These interactions between mother and infant, involving the hypothalamus and placenta, provide the template for coadaptive selection pressures to operate on this paternally expressed gene. Moreover, early infant mortality accounts for the major part of the variance in fitness, providing a substantial opportunity for selection of traits that are expressed early in life (Mousseau & Fox 1998). The potential evolutionary outcome of this linked coadaptation is that offspring that have extracted 'good' maternal nurturing would themselves be both well provisioned and genetically predisposed towards good mothering when adult, thereby enhancing the spread of this gene across generations.

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