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Milk ejection in mice LG/J x SM/J

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Abstract In mammals, milk provision is crucial to offspring survival and growth from birth to weaning. Milk deficiency early in life may cause death or changes in the progeny metabolism that later may lead to obesity and metabolic disorders. This study investigates milk ejection (ME) the first day after birth (D1) in F_2 females from the intercross of LG/J and SM/J inbred mice strains. The absence of milk in F_3 pups' stomach at D1 is directly associated with their survival ($p < 0.001$) and growth pattern ($p < 0.001$) in the early stages of life. Furthermore, late growth pattern is also affected by this lack of nutrients at D1 because pups that survive this absence, mostly males, are heavier at weaning ($p < 0.001$) which, after necropsy, is shown to be due to significant higher total fat deposition ($p < 0.01$). We performed QTL analysis for ME at D1 in these F_2 females. Maternal performance of ME revealed a complex genetic architecture which even though it contains only a single QTL (accounting for 8 % of the variation in ME), it is totally context-dependent on the genetic background. We discovered many regions involved in epistatic interactions that together with the single QTL explain 19 % of the genetic variation for this trait. Milk ejection is an important component of maternal care, and understanding the mechanisms modulating its variation, along with other maternal features, may help to disentangle the complexity that is the mother/offspring relationship.

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Introduction

In mammals, maternal care is essential to survival and growth of the offspring, especially in the early stages. Rodents, in general, display a variety of maternal behaviors that may be nonpup-directed (nest-building, placentophagia, and defense of the young) and pup-directed (nursing, retrieval, grouping, anogenital/body licking, and tactile stimulations) (Krasnegor and Bridges 1989; Kuroda et al. 2011). Milk provision completes the set of actions displayed by females to ensure the success of their offspring and helps establish the postpartum maternal care period, since most maternal behaviors decline as weaning approaches (McLean and Speakman 1997).

As milk has components that maintain the pups' nourishment and allow their growth and development, it must be provided immediately following delivery to guarantee offspring survival (Silver 1995). Normal lactation requires typical development of the mammary gland. This involves a change from the quiescent stage to extensive cell proliferation at puberty, and culminating in massive proliferation and differentiation during pregnancy (Gjorevski and Nelson 2011). This development is controlled by metabolic hormones, growth factors, prolactin, and sex steroid hormones (Lamote et al. 2004) and is dependent on estrogen and progesterone during gestation (Svennersten-Sjaunja and Olsson 2005).

After birth, the secretion of milk depends not only on physiological changes that prepare the mammary gland for milk production, but also on milk availability in the mammary ducts and consequently its ejection. Milk production is controlled by lactogenic hormones like prolactin and growth hormones (GHs) (Neville et al. 2002). Prolactin activates STAT (signal transducers and activators of transcription) proteins that promote the expression of specific

genes involved in epithelial cell differentiation and milk protein's gene expression (Liu et al. 1997). GHs act via local growth factors that have been shown to be involved in mammary function, for instance, insulin-like growth factor (IGFs), epidermal growth factors (EGFs), and members of the transforming growth factor family (TGFβ) (Forsyth 1996; Plath et al. 1997). On the other hand, milk ejection is initiated by stimulation of neural receptors in the nipple done by pups through sucking behavior. This sucking, together with audible and visual stimuli from the pups, induces a rapid increase of plasma oxytocin (OT) in the mother, which promotes milk ejection in the mammary alveoli by contracting mammary myoepithelial cells (Dyball and Leng 1986; Cunningham and Sawchenko 1991; Yamamuro and Sensui 1998).

Whether mothers have issues with milk production and ejection or not, a very important aspect is the timing of milk provision, because nutritional deficiencies early in life compromise survival or may result in problems in development, which may be reflected by problems later in life (Noguera et al. 2011). Nutritional restriction in fetuses and neonates may set them to adapt to an undernourished environment through physiological and metabolic changes. Consequently, if those animals adapt to food scarcity, new access to a normal diet may trigger metabolic disorders such as obesity and type II diabetes, an effect also known as the "thrifty phenotype" (Hales and Barker 1992, 2001). It is suggested that the mechanisms behind these changes in developmental programming are due to permanent structural changes, epigenetic modifications, and/or to mitochondrial dysfunction (Warner and Ozanne 2010).

Genetic factors involved with milk provision in rodents have been investigated, mostly by knockout technology (Brown et al. 1996; Ormandy et al. 1997; Li et al. 1999; Palmer et al. 2006). However, as a complex trait, we expect a complex genetic architecture to be involved with milk provision. However, quantitative trait locus (QTL) analyses are more adequate in this case since QTLs are able to elucidate the total number of genetic regions involved, their direct effects (additivity), interallelic interactions at the same locus (dominance), interactions between different loci (epistasis), and the extension of effects across other phenotypes (pleiotropy) (Falconer and Mackay 1996; Erickson 2005).

Our group has been studying the genetic architecture of maternal care features in LG/J × SM/J female mice (Peripato and Cheverud 2002; Peripato et al. 2002, 2004; Sauce et al. 2012). In another study we found an important milk ejection variation between LG/J and SM/J mothers in which the former usually delay by 1 day the provision of milk (Chiavegatto et al. 2012). Here we investigate the genetic architecture of milk ejection in females from a F_2 intercross as well as the consequences of this 1-day delay on growth and fat deposition in their offspring.

Materials and methods

Animals

We used inbred mice strains LG/J and SM/J acquired from the Jackson Laboratory (Bar Harbor, ME, USA). These strains have been selected for large and small body size, respectively, at 60 days (Goodale 1938; MacArthur 1944). The details on the establishment of these strains may be found in Hrbek et al. (2006).

Animals born in our facility were individually identified within the first week of life. Three weeks after birth they were weaned and placed in single-sex cages with at most four other animals. At 7 weeks of age, each female was randomly mated with a male from a different family. When the female was pregnant, we removed the male from the breeding cage and then registered maternal attributes. We designed an F_2 intercross progeny by crossing ten LG/J females with ten SM/J males and ten SM/J females with ten LG/J males which produced a total of 68 heterozygous offspring. These F_1 mice were crossed among themselves with all combinations in order to get the Y chromosome and mitochondrial DNA from both SM/J and LG/J represented in F_2. At 10 weeks of age, 258 F_2 females were randomly mated with F_2 males to produce an F_3 generation. Seven days after birth, each F_3 animal had weight and tail measured weekly until weaning. After data collection, animals were sacrificed and necropsied. During necropsy, we recorded the tail length and total weight; weight of internal organs such as heart, liver, spleen, and kidney; and the weight of reproductive, mesenteric, inguinal organs, and kidney fat pads. It is noteworthy that there are several traits with known genetic variation in generations from the intercross of LG/J and SM/J strains besides behavioral ones like maternal care (Peripato et al. 2002) and prepulse inhibition (Samocha et al. 2010); these include growth (Cheverud et al. 1996; Kramer et al. 1998; Vaughn et al. 1999), bone length (Norgard et al. 2008), obesity (Cheverud et al. 2001; Ehrich et al. 2005), litter size (Peripato et al. 2004), and maternal effect on offspring growth (Wolf et al. 2002, 2011).

Animals were fed ad libitum with Nuvilab CR1/Nuvital (Colombo, PR, Brazil) and their facility was maintained at a constant temperature of 21 °C with a 12-h light/dark cycle at Federal University of Sao Carlos, São Paulo, Brazil. Experiments were carried out in accordance with the Ethics Committee of the Federal University of Sao Carlos (Brazil).

Scoring maternal performance

Females were monitored as soon as pregnancy was detected and were maintained until 7 days after delivery. Several maternal features were evaluated in this cross, including nest building before and after delivery and pup retrieval (see Chiavegatto et al. 2012; Sauce et al. 2012). The capacity for milk provision (i.e., the normal functioning of mammary glands and the recognition of offspring) was confirmed by the presence of milk in the stomach of the pups for 7 days (Fig. 1), which we have shown not to be due to the pups' sucking impairment (Chiavegatto et al. 2012). We assessed the trait of milk ejection in the same way, but using only the first day after delivery (D1). Generally, females with milk ejection at D1 maintained this behavior during the 7 days evaluated. Lack of milk ejection by the female was considered the cause when all littermates had empty stomachs. We scored litter size information at the date of birth and monitored survival daily during the first week. We monitored a total of 234 F_2 primiparous females and conducted all procedures between 8 a.m. and 12 p.m.

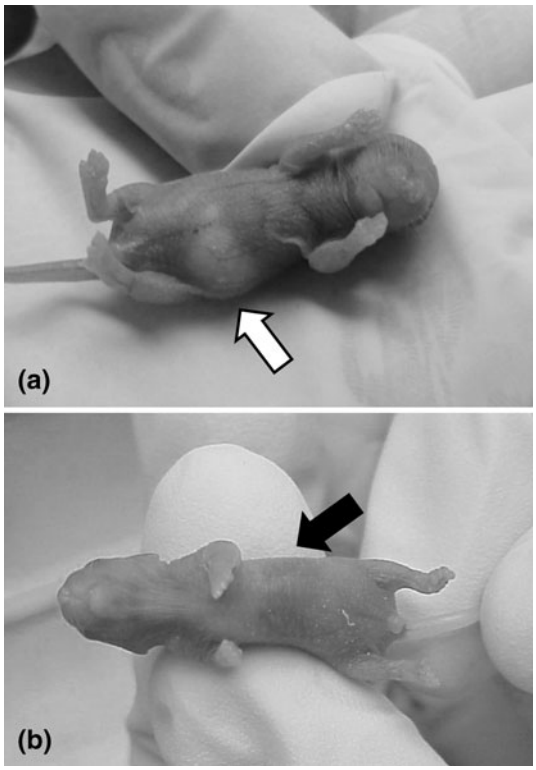

Fig. 1 Milk provision observation. **a** Presence of milk in the stomach of the pup, indicated by a *white arrow*. **b** Absence of milk in the stomach of the pup, indicated by a *black arrow*

Traits in F_3 animals versus milk ejection in F_2 females

We studied the effect of the lack of milk in D1 on F_3 pups compared to pups with milk. We assessed viability by the survival rate of pups ($n = 209$) and measured growth using weight and tail length at D1 ($n = 1{,}094$). We also used weight gain during the first week (daily) and at the day of weaning (D21). We measured weight and tail length only up to 3 weeks after birth because we were studying the mother–pup relationship that occurs until weaning. We compared the weight of organs and fat pad between animals lacking milk and animals with milk at D1 in F_3 males kept for reproduction (since most of the mice that survived the lack of milk were males). The organs and fat pads weighed were heart, spleen, liver, and kidney and reproductive, mesenteric, inguinal, and kidney fat pads ($n = 109$, age $\cong$ 9.4 weeks).

Statistical procedures for phenotypes

The association among nominal variables was tested by cross tabulation using Pearson's χ^2 test and ϕ coefficient in SYSTAT v10.0 (Systat, Chicago, IL, USA). We tested for heterosis in milk ejection (measured as the deviation from the middle of the parent value) using a *t*-test. We also tested the effect of the presence or absence of milk at D1 on distinct variables by using a GLM procedure in SYSTAT v10.0. The family effect was considered for each analysis. Differences were considered statistically significant when $p < 0.05$.

Molecular genotyping and QTL analysis

Total cellular DNA was extracted from the liver by using a guanidine thiocyanate protocol (Nelson and Krawetz 1992). PCR amplification of microsatellite loci was performed according to the protocol described by Dietrich et al. (1992) and modified by Routman and Cheverud (1994). We used 101 polymorphic loci covering the 20 chromosomes as completely as possible (Sauce et al. 2012). PCR products were visualized using 1–6 % agarose gel and ethidium bromide staining.

Single QTL analysis

Linkage map distances were calculated from F_2 animals of this intercross using MapManager QTX (Manly et al. 2001). Interval mapping of single QTLs (Lander and Botstein 1989) was undertaken by regressing milk ejection (ME) at D1 onto genotype scores every 2 cM along each chromosome, as described by Haley and Knott (1992).

The probabilities of a gene affecting ME at specific chromosome positions were obtained using the MIXED

procedure SAS v9.0 software (SAS Institute, Cary, NC, USA), and statistical significance of one-QTL models was evaluated using LOD scores.

Significance thresholds were calculated for each chromosome (chromosome-wise 5 % level) as well as an overall 5 % genome-wise threshold level by the effective marker number approach of Cheverud (2001) as modified by Li and Ji (2005). Confidence intervals for each QTL were determined by the one-LOD drop rule (Lynch and Walsh 1998).

Epistasis analysis

We used an interchromosomal two-way genome-wide scan performed every 2 cM along the mouse chromosomes to test for epistatic interactions for ME across the whole genome by using the F_2 model (Cockerham 1954) extended to two loci following orthogonal contrast scales using Cockerham's model (Cockerham and Zeng 1996; Kao and Zeng 2002).

Bonferroni thresholds for genome-by-genome interactions were estimated using the Li and Ji method (Li and Ji 2005). In our case, we considered interactions significant at $p < 1.51 \times 10^{-5}$ (Bonferroni threshold level of 0.05). We also considered it significant if one of the four modes of epistasis (additive-by-additive, additive-by-dominance, dominance-by-additive, and dominance-by-dominance) had $p < 3.77 \times 10^{-6}$ (1.51×10^{-5} divided by 4), even if the overall epistasis model was not significant. Epistatic QTLs located within 10 cM of one another were considered the same.

Results

Maternal performance

Milk ejection at D1 varies across parental strains and the F_1 and F_2 generations (Table 1). We found significant differences between SM/J vs. LG/J females ($\phi = 0.57$, $p < 0.001$), SM/J vs. F_1 females ($\phi = -0.31$, $p < 0.01$), and SM/J vs. F_2 females ($\phi = -0.17$, $p < 0.01$). LG/J females differed significantly between F_1 ($\phi = -0.79$, $p < 0.001$) and F_2 ($\phi = -0.61$, $p < 0.001$) for milk ejection. For all comparisons, LG/J females showed the poorer maternal performance of milk ejection at D1. The middle parent value for milk ejection deviates significantly from F_1 females for this trait ($p < 0.001$), suggesting, by definition, heterosis.

Table 1 Litter size and milk ejection at first day after deliver across generations

Generation	*N*	Litter size	Litter size viable	Presence (%)	Absence (%)
SM/J	30	4.05	2.95	25 (83.3)[a]	5 (16.6)
LG/J	23	6.59	2.33	9 (39.1)[b]	14 (60.9)
F_1	59	10.84	9.66	57 (96.6)	2 (3.4)
F_2	207	8.731	7.79	193 (93.3)	14 (6.7)

Litter size viable is the number of litter that survived after first week

[a] Significant differences among SM/J versus LG/J ($\phi = 0.57$, $p < 0.001$), SM/J versus F_1 ($\phi = -0.31$, $p < 0.01$), and SM/J versus F_2 ($\phi = -0.17$, $p < 0.01$)

[b] Differences among LG/J versus F_1 ($\phi = -0.79$, $p < 0.001$) and LG/J versus F_2 ($\phi = -0.61$, $p < 0.001$)

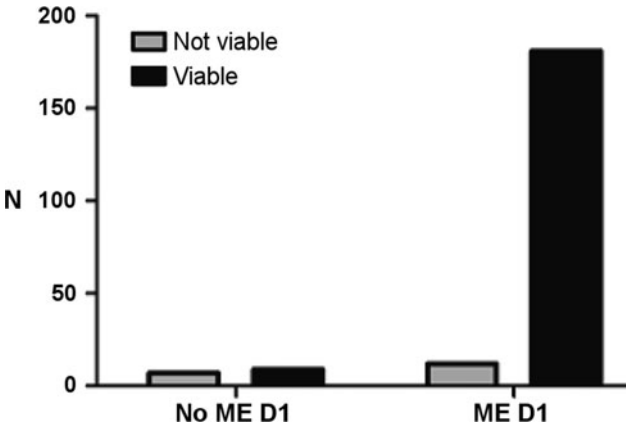

Fig. 2 Relationship between the presence or absence of milk at D1 and survival in F_3 animals. *ME* milk ejection

Litter sizes for LG/J, SM/J, F_1, and F_2 females are given in Table 1. LG/J females have a larger number of offspring, but 65 % of them do not survive through the first week compared to 28 % of SM/J females' offspring. F_1 and F_2 females have a survival rate of 89 % for both generations through the first week.

Traits versus milk ejection

The first F_3 trait investigated was viability indicated by survival rate. Figure 2 shows the relationship between receiving milk at D1 and the survival in F_3 animals. About half of the litter that was not fed right after birth was not viable. When we contrast the presence/absence of milk ejection at D1 with offspring survival, we find a significant association ($p < 0.01$), indicating that a pup's viability is directly dependent on having milk in its stomach as soon as it is born.

Milk is important not only for survival but also for offspring growth, as shown on Table 2 for weight at D1 and weaning, for weight gain during the first week and from the first week to weaning, and for tail length from the first week to weaning. The absence of milk the first day after birth is associated with animals having a reduced birth weight but being heavier at weaning ($p < 0.001$ and $p < 0.01$, respectively). The weight gain in the first week of life does not seem associated with the presence/absence

Table 2 F_3 weight and growth gain of animals with presence or absence of milk in their stomach the first day after birth

Trait	Milk presence D1	Milk absence D1
Birth weight (g)	1.44 ± 0.13**	1.32 ± 0.10
Weaning (g)	8.59 ± 1.94**	9.92 ± 2.12
Weight gain first week (g)	2.11 ± 0.66 ns	2.05 ± 0.08
Weight gain from first week to weaning (g)	4.67 ± 1.65**	4.85 ± 1.9
Tail length from first week to weaning (cm)	3.27 ± 0.71 ns	3.4 ± 0.24

Data are average ± standard deviation, ** Significant differences ($p < 0.01$) between both categories in that row

ns no significant differences for both categories in that row

Table 3 Necropsy data of F_3 males

Necropsy data	Presence D1 (males)	Absence D1 (males)
Weight (g)	26.882 ± 2.88 bs	29.546 ± 4.73
Tail (cm)	9.332 ± 0.49 ns	9.32 ± 0.28
Heart (g)	0.099 ± 0.02 ns	0.096 ± 0.02
Left kidney (g)	0.190 ± 0.04 ns	0.2 ± 0.04
Right kidney (g)	0.194 ± 0.04 ns	0.206 ± 0.04
Spleen (g)	0.058 ± 0.02 ns	0.054 ± 0.01
Liver (g)	1.267 ± 0.25 ns	1.174 ± 0.31
Reproductive fat pad (g)	0.352 ± 0.16*	0.534 ± 0.24
Kidney fat pad (g)	0.117 ± 0.07**	0.248 ± 0.10
Mesenteric fat pad (g)	0.423 ± 0.15*	0.588 ± 0.19
Inguinal fat pad (g)	0.454 ± 0.23**	0.746 ± 0.32
Total fat pad (g)	1.347 ± 0.52**	2.116 ± 0.85

Data are average ± standard deviation, * and ** significant differences ($p < 0.05$ and $p < 0.01$, respectively) between both categories in that row

bs borderline significance ($p = 0.06$), *ns* no significant differences for both categories in that row

of milk at D1 ($p = 0.58$). However, the weight gain from the first week to weaning demonstrates that animals lacking milk right after birth have a higher weight gain through weaning ($p < 0.001$). Though weight gain of the offspring through weaning appears to be associated with the absence of milk on the first day at birth, it is not accompanied by an overall growth. We found no significant differences in tail growth or internal organs at weaning between the two offspring categories ($p = 0.44$).

Comparing necropsy data of males that were not fed right after birth with that of males that had milk in their stomachs at the first day of birth, we found a borderline significant effect ($p = 0.06$) for weight at necropsy: a tendency of heavier males for the first category followed by a significant association with fat pad deposition (reproductive, kidney, mesenteric, inguinal, and total fat pad, $p < 0.05$, $p < 0.001$, $p < 0.05$, $p < 0.01$, and $p < 0.01$, respectively). The organs evaluated showed no significant difference between the two categories. Male necropsy data are given on Table 3.

QTL analysis

The regression of the data of milk ejection at D1 of 234 F_2 females on their interval mapping genotype scores allowed the investigation of QTLs for this trait. Analyses located one QTL affecting milk provision at the first day after birth (Fig. 3). This QTL, named *ME3 (Milk Ejection locus on chromosome 3)*, is significant at the genome-wide level (3.27). It is centered 14 cM downstream of the marker *D3Mit14*, 61.3 cM away from the most proximal marker of the telomere on chromosome 3, and has a confidence region of 11 cM (57–68 cM).

The additive (0.11) and dominance (0.08) genotype values show a predominantly additive effect of *ME3*, in which females with the LG/J allele (L) have better milk ejection first day after delivery. *ME3* explains 8 % of the total phenotypic variance in milk ejection at D1.

We found 24 chromosomal regions interacting epistatically in 18 different chromosomes. These pairs of loci are summarized in Table 4. All four forms of epistasis are represented in the results (additive-by-additive, dominance-by-dominance, additive-by- dominance, and dominance-by-additive).

The single QTL here identified (*ME3*) also interacts significantly with other regions across the genome (*ME1*, *ME11.a*, and *ME12*). Chromosomes *5*, *6*, and *10* were the only ones that did not have significant interactions for epistatic QTLs for ME at D1. We found a major network involving 11 loci in ten connections and five small ones in which *ME3* plays a central role (Fig. 4). With epistasis, all QTLs for milk ejection at D1 account for 19 % of the phenotypic variation (adjusted multiple r^2).

Discussion

LG/J and SM/J females display distinct maternal performance (Peripato et al. 2002; Chiavegatto et al. 2012), and milk ejection at D1 seems to be a major difference between them. In mammals, milk is a fundamental key to offspring survival and growth in the early stages of life and it must be provided as soon as possible. In mice, if the pups have no milk in their stomachs at most 6 h after birth, their survival may be compromised (Silver 1995). We found that LG/J females have impaired milk ejection at D1 when compared to SM/J females. On the other hand, F_1 and F_2 females showed superior milk ejection at D1 over their homozygous parental inbred lines. These results reveal a

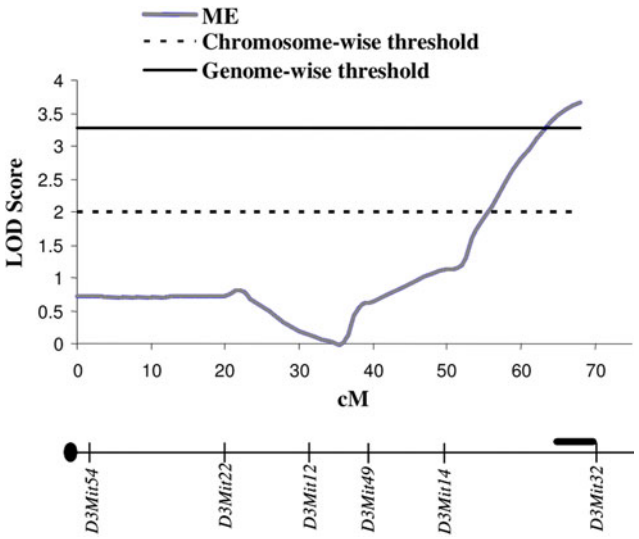

Fig. 3 LOD plot of chromosome 3. Significant LOD score (3.27) at genome-wise threshold level 14 cM downstream of the marker *D3Mit14* indicates highly significant evidence of QTL in this position. The chromosome-wise significance threshold for this chromosome is 1.99. Confidence region is 11 cM

heterosis effect for milk ejection at D1. Three genetic models have been hypothesized to explain heterosis: dominance, overdominance, and epistasis models (Falconer and Mackay 1996). The heterosis for milk ejection in LG/J × SM/J mice suggests a role of a nonadditive genetic pattern in modulating this trait.

Litter size is a reproductive trait directly related to fitness (Falconer and Mackay 1996), and maternal environment may have significant impact on viable litter sizes (Krasnegor and Bridges 1989; Peripato et al. 2004). LG/J females have lower survival rates compared to SM/J, F_1, and F_2 females. As they also had milk ejection deficiency at D1, certainly this milk impairment affected survival in newborns. We have shown that offspring with empty stomachs were capable of sucking (Chiavegatto et al. 2012.), which indicates that the lack of milk is related to the dam. This is exactly what we found when we contrasted milk ejection in F_2 females and survival rate in F_3 offspring. The progeny viability was significantly associated with being fed on D1. Furthermore, not only is survival dependent on maternal care features (a postnatal effect), in this case milk ejection, but the offspring's growth trajectory in early and late stages of life is also affected (Lee et al. 1991; Nogueira et al. 2011). It is also noteworthy that prenatal maternal effects may also influence offspring growth in mice as well as interact with other direct-effect genes (Wolf et al. 2011). In our study, growth patterns are different in animals that survive the lack of milk on D1. At birth, the lower weight of pups without milk may reflect impaired fetal growth (Warner and Ozanne 2010) or that they were not fed, naturally losing weight immediately after birth (Wright and Parkinson 2004). As soon as they get milk this scenario reverts, and the average weight gain in the first week of life is similar to animals fed since the first day. Interestingly, this pattern changes when we contrast weaning weight and weight gain from the first to the third week (weaning) in animals with and without milk in their stomach at D1. The unnourished mice at D1 show a significant increase in weight gain at weaning. However, despite being heavier, these animals do not differ in size when compared to mice continually fed since birth, suggesting a tendency toward fat storage. These results resemble thrifty phenotypes in which scarce nutrient supplies early in life drive physiological and metabolic adaptations that trigger disorders when the offer of nutrients increases (Hales and Barker 1992, 2001).

The adaptations during gestation and the perinatal period may permanently change essential cell structures that impact offspring during development, especially in the brain (Davidowa et al. 2003), kidney (Nwagwu et al. 2000), and endocrine pancreas (Garofano et al. 1999). The decreased birth weight, as we found here, has been associated with the risk of developing an aspect of metabolic syndrome such as type 2 diabetes (Poulsen et al. 1997; Bo et al. 2000) and hypertension (Bergvall et al. 2007). The low birth weight is followed by a rapid gain in weight which can be fat deposition perhaps irregularly concentrated on viscera (Modi et al. 2006), which is exactly what we found in the necropsy of the F_3 animals. Heavier males tended to be those that lacked milk at D1 and have a significant gain in total fat deposition or individual fat storage areas (reproductive, renal, mesenteric, and inguinal). Although we have not quantified the glucose levels or monitored for high blood pressure, the growth pattern and fat accumulation of animals with food deprivation at birth agrees with the thrifty phenotype profile. The mechanism for poor nutrition adaptations behind thrifty phenotypes implicates changes in the expression of specific genes due to cell structure changes and oxidative stress (Meaney et al. 2007; Warner and Ozanne 2010; Noguera et al. 2011). Gene expression is also altered by epigenetic markers that may reprogram the expression of specific genes that impact the way cells store energy, improving the chance of survival in a poor nutrient environment. These markers can be established in critical periods, such as gestation and the perinatal stage, and are maintained even after an increase in the availability of nutrients (Warner and Ozanne 2010; Barnes and Ozanne 2011), leading to greater storage of calories, i.e., fat deposition, and all consequences related to this. In brief, milk ejection is an important trait in mammals and the synchronism with birth seems to be critical for growth pattern, especially in the late stages on life, as we saw in our data.

Investigating the genetic architecture of milk ejection in F_2 females, we found one QTL and 24 epistatic regions modulating this trait. This significant QTL at genome-wide

Table 4 Epistatic interactions between QTLs affecting milk ejection at D1

QTL	Marker 1	Position 1 (cM)	Position 2 (cM)	QTL	Marker 2	Position 1 (cM)	Position 2 (cM)	Prob. epistasis	Epistasis type	Genotypic value	Prob. genotypic value
ME1	*D1Mit541*	16	112	*ME3*	*D3Mit14*	14	70	$6{\cdot}65 \times 10^{-6}$	AA	0.159	8.90×10^{-5}
									AD	0.112	4.15×10^{-4}
ME1	*D1Mit541*	10	106	*ME15*	*D15Mit143*	12	30	5.27×10^{-6}	AD	0.210	2.17×10^{-5}
									DA	0.114	9.50×10^{-3}
ME1	*D1Mit14*	14	94	*ME17.b*	*D17Mit10*	34	78	1.32×10^{-6}	AA	0.219	6.71×10^{-6}
									DD	−0.139	2.47×10^{-4}
ME2	*D2Mit370*	0	32	*ME11.a*	*D11Mit14*	0	68	1.51×10^{-5}	AD	−0.102	1.35×10^{-3}
									DA	0.0924	3.54×10^{-3}
ME3	*D3Mit14*	14	70	*ME12*	*D12Mit6*	14	66	7.62×10^{-7}	AA	0.167	7.13×10^{-5}
									DA	0.116	5.44×10^{-4}
ME11	*D11Mit15*	6	56	*ME3*	*D3Mit14*	12	68	1.17×10^{-5}	AA	0.124	9.12×10^{-4}
									DA	0.0868	5.04×10^{-3}
ME17.a	*D17Mit46*	0	2	*ME3.b*	*D3Mit12*	2	42	1.44×10^{-6}	AA	−0.213	8.88×10^{-4}
									DA	0.203	2.45×10^{-4}
ME4	*D4Mit235*	0	2	*ME12*	*D12Mit6*	0	52	2.23×10^{-7}	AD	−0.115	3.10×10^{-4}
									DA	−0.107	7.42×10^{-4}
ME7	*D7Nds1*	0	52	*ME8.a*	*D8Mit58*	28	30	2.12×10^{-6}	DD	-0.184	5.62×10^{-7}
									AA	0.104	1.60×10^{-2}
ME7	*D7Mit227*	12	36	*ME9.b*	*D9Mit8*	8	64	8.25×10^{-6}	DA	−0.200	9.18×10^{-5}
									DD	0.172	2.49×10^{-4}
ME13.b	*D13Mit147*	0	74	*ME8.b*	*D8Mit343*	28	68	1.70×10^{-10}	DA	0.176	8.77×10^{-7}
									AD	-0.135	4.82×10^{-5}
ME9.a	*D9Mit4*	0	38	*M13.a*	*D13Mit115*	20	34	2.04×10^{-6}	AD	-0.205	1.17×10^{-4}
									DA	0.154	4.21×10^{-4}
M13.b	*D13Mit9*	6	68	*ME11.b*	*D11Mit14*	14	82	3.40×10^{-7}	DD	−0.124	5.12×10^{-4}
									AD	0.133	1.10×10^{-3}
ME13.a	*D13Mit115*	0	14	*ME16*	*D16Mit2*	0	2	2.42×10^{-12}	AD	5.89	2.29×10^{-5}
									DA	−5.89	2.41×10^{-5}
ME14	*D14Mit5*	0	30	*ME15*	*D15Mit143*	0	18	1.49×10^{-5}	DA	−0.157	1.96×10^{-5}
									AA	0.0975	9.45×10^{-3}
ME15	*D15Mit143*	12	30	*ME18*	*D18Mit17*	0	22	2.35×10^{-7}	DA	0.195	3.16×10^{-5}
									AD	0.143	6.50×10^{-4}
ME17.b	*D17Mit10*	12	56	*ME19.b*	*D19Mit35*	6	66	7.56×10^{-6}	AA	0.302	2.92×10^{-5}
									DD	−0.192	3.01×10^{-3}
ME19.a	*D19Mit39*	2	32	*MEX*	*DXMit64*	0	38	1.11×10^{-16}	AA	0.314	8.77×10^{-8}
									AD	−0.238	6.91×10^{-8}

QTL are named as trait affect, chromosome number, and a and b indicate different regions at the same chromosome. Position 1 is the QTL's distance from the nearest proximal marker on the chromosome and Position 2 is the telomeric distance from the most proximal marker on the chromosome, in Haldane's cM

Epistasis types: *AA* additive-by-additive, *DD* dominance-by-dominance, *AD* additive-by- dominance, *DA* dominance-by-additive

threshold, *ME3*, is additive for the LG/J allele and explains 8 % of the total genetic variation for milk ejection at D1. Having LG/J alleles associated with a better maternal performance of milk ejection does not agree with milk ejection phenotyping data from parental lines because LG/J females have impaired milk ejection at D1. Besides the additive inheritance pattern for this locus, a more complex genetic mechanism modulates milk ejection, and even this single locus is affected by others. This context dependence of a locus modulates phenotype variation (Wolf et al. 2000) and is easily seen in Fig. 4 because *ME3* also has epistatic effects and is pivotal in the major network of interactions. Of the 18 interactions among the 24 epistatic QTLs, three are involved with *ME3*. In all cases, we found additive-by-additive and additive-by-dominance/dominance-by-additive epistatic interactions. The single-locus additive value for locus *ME3* changes depending on loci *ME1*, *ME11*, and *ME12*. In additive-by-additive epistasis, the *ME3* locus with homozygous LG/J alleles will have the same effect as that of the single QTL only in a homozygous LG/J allele's context, with opposite effect in the presence of a single or double SM/J allele. This sort of data reveals the importance

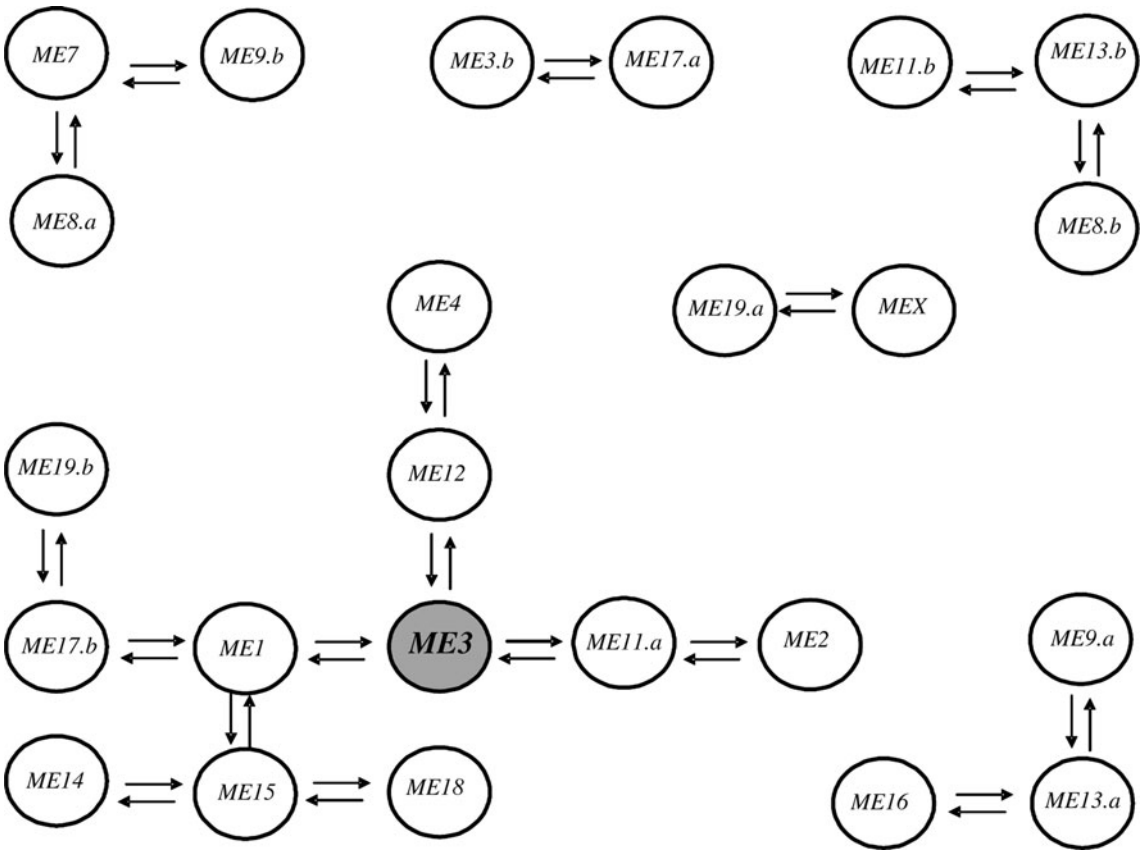

Fig. 4 Epistatic interaction patterns for milk ejection at D1. For details, see Table 4. Note that *ME1* was also detected as a single QTL

of considering epistasis in a QTL analysis, especially when one is interested in markers as a tool to select for economic traits, i.e., marker-assisted selection. In this situation, overlooking epistasis may direct to biased effects for a single QTLs (Carlborg and Haley 2004), consequently leading to negative selection response.

We found six networks among epistatic QTLs: a major one with 11 epistatic QTLs, three involving three regions, and two minor ones having two QTLs interacting epistatically. Together with the single QTL, these regions account for 19 % of milk ejection variation in F_2 animals from the LG/J × SM/J intercross. It is surprising that we found only one QTL that has an additive effect in contrast with numerous pairwise epistatic interactions. The relative absence of individual loci affecting litter size is compared with studies of maternal performance for offspring survival (Peripato et al. 2002), litter size (Peripato et al. 2004), and nest building (Sauce et al. 2012) in this same intercross. This lack of data could have been the result of small sample sizes, but this does not seem to be the case since there were several more epistatic QTLs detected, suggesting that there might be sufficient degrees of freedom to find more direct-effect QTLs. On the other hand, these data may point to the relative importance of epistatic QTLs when compared to the direct effect on these fitness-related traits (Merilä and Sheldon 1999; Sauce et al. 2012). When we contrast these results with phenotypic analyses, we reinforce the contribution of a nonadditive genetic pattern modulating milk ejection, as suggested by heterosis. Furthermore, we suggest the participation of epistasis in this heterosis effect, though we cannot rule out dominance/overdominance as well.

Maternal care is an intriguing trait due to its complexity and role as an environmental influence on the phenotypes of the offspring. It is a result of a combination of factors that together reflect the success of progeny through survival and growth in the early stages of life. Here we investigated a specific maternal care component, milk ejection after birth. This trait revealed a complex genetic architecture that even though it contains only a single QTL, it is totally context-dependent with other loci. Additionally, we have many regions interacting epistatically which explains genetic variation for this trait. Milk ejection is only a piece of this puzzle and much still remains to be understood as to how the mother/offspring relationship guarantees the progeny success.

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