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1 **Genotype dependent responses to levels of sibling**
2 **competition over maternal resources in mice**

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23

24 **Abstract:** Research on phenotypic plasticity has often focused on how a given
25 genotype responds to changing physical environments such as temperature or diet.
26 However, for many species the social environment plays an equally important role
27 due to competition for resources. During early development, the level of competition
28 for limited (maternally provided) resources will often depend critically on the number
29 of siblings. Therefore, competition among siblings should drive the evolution of genes
30 that allow flexible responses to realized levels of competition and maternal resource
31 availability. However, it is unknown whether genetically based differences between
32 individuals exist in their response to the social environment that affects their future
33 development. Using a quantitative trait locus approach in an experimental population
34 of mice we demonstrate that effects of sibling number on body weight depend on
35 individual genotype at seven loci, over and above the general negative litter size
36 effect. Overall, these litter size-by-genotype interactions considerably modified the
37 degree to which increasing litter size caused reduced weight. For example at one locus
38 this effect leads to a 7% difference in body weight at week 7 between individuals
39 experiencing the extremes of the normal range of litter sizes in our population (five to
40 nine litter mates). The observed interaction between genotype and the competitive
41 environment can produce differences in body weight that are similar in magnitude to
42 the main effect of litter size on weight. Our results show that different genotypes
43 respond to the social environment differentially and that interaction effects of
44 genotype with litter size can be as important as genotype-independent effects of litter
45 size.

46

47 **Introduction**

48 Phenotypic plasticity describes the ability of organisms to respond to changes
49 in the environment (West-Eberhard 2003) and is generally referred to as flexibility
50 when phenotypic change is reversible and as developmental plasticity when it is not
51 (Stearns 1989; Piersma and Drent 2003). Plasticity may be adaptive if the range of
52 phenotypes shown by a given genotype across environments, or the reaction norm,
53 increases fitness when compared to a single phenotype in these environments (Via &
54 Lande 1985; DeWitt *et al.*, 1998). Given the obvious fitness advantages of plasticity,
55 studies sought to elucidate associated costs and constraints as plasticity has not been
56 as universally observed as one might have expected (Snell-Rood *et al.*, 2010). A large
57 part of this research focused on how a given genotype responds to changes in the
58 physical environment such as diet or temperature. Yet, for many species the social
59 environment is certainly of equal importance in determining individual fitness and
60 explaining trait variation. How do given genotypes respond to changing social
61 environments? Further, research into costs and constraints of plasticity requires an
62 understanding of the underlying genetics as pleiotropy is an important source of
63 evolutionary constraint, and because genetic variation is the prerequisite for evolution
64 (e.g. Scheiner 1993; DeWitt *et al.*, 1998; Auld *et al.*, 2010). In this study, we tackle
65 these issues and investigate whether genetic variants can respond differentially to a
66 changing social environment in an experimental population of mice by focusing on
67 effects of the competitive environment on body weight.

68 An adaptive response to changing levels of competition may be favoured by
69 natural selection as the associated cost / benefit trade-off will change as well (e.g.
70 Stockley and Parker 2002; Wright and Leonhard 2002). This seems particularly
71 relevant to early development because levels of competition may be indicative of

72 available resources during this crucial developmental period (Gyekis *et al.*, 2011).
73 Sibling competition over access to resources is common in species that provide
74 significant parental care and have multiple offspring in a litter or brood (Mock and
75 Parker 1997). In mammals with multiple offspring per litter, sibling competition is
76 manifested largely in scramble competition, rather than contest competition, and is
77 thus crucially dependent on the number of competitors (MacNair and Parker 1979;
78 Mock and Parker 1997; Hager and Johnstone 2005). In mice the number of litter
79 mates is rarely greater than the number of nipples (10), but access is often limited to
80 one side of the female and offspring remain staunchly attached until all milk supply is
81 depleted (Gilbert 1995). Moreover, teats differ in their productivity (Barnard *et al.*,
82 1998), exacerbating sibling competition over the most productive nipples (usually the
83 anterior ones). Females have been shown to increase milk supply as litter size
84 increases (Knight *et al.*, 1986) which may mitigate competition on average but, at the
85 same time, per capita milk supply may decrease and thus competition will increase.

86 The number of siblings at birth may serve as an indicator of expected postnatal
87 sibling competition for maternal resources, and the number of litter mates at weaning
88 may be indicative of post-weaning competition for resources. In rodents, the number
89 of competitors may decrease or increase before weaning because pups may die or fall
90 victim to prey, or another female may produce a litter in the same nest (several
91 females often litter together in mice, König 1997) and pups are nursed by either
92 female (König 1994). Consequently, levels of competition and associated costs
93 (Trivers 1974; Clutton-Brock 1991; Godfray 1991) can increase or decrease
94 postnatally. The key question we address here is whether any response to this change
95 in the competitive environment has a genetic basis that differs between individuals or
96 populations.

97 Our aim for this study was to investigate whether bodyweight and growth
98 during the first 10 weeks of life is affected by the number of litter mates at birth or at
99 weaning and whether these effects depend on individual genotype. Body weight is a
100 key indicator of resource utilization during development and often a good predictor of
101 fitness under natural conditions where weight is associated with higher reproductive
102 potential, advantages in intra-sexual competition and female mate choice. Using a
103 quantitative trait locus (QTL) design, we first investigate main effects of litter size,
104 independent of genotype, and then establish whether individual genotype interacts
105 with the number of litter mates at birth or at weaning. This enables us to investigate
106 genetic variation in the response to the competitive environment, manifested in
107 differential responses to the number of competitors for different genotypes. We
108 predict that genotypes show different responses to the postnatal competitive
109 environment.

110

111 **Methods**

112 Our study population is an intercross of two inbred mouse strains that were selected
113 for divergent bodyweight at day 60, the Large (LG/J) and Small (SM/J) strains
114 (Goodale 1938; MacArthur 1944). The two strains differ in litter size: while *Large* has
115 an average litter size of 6.1, *Small* has an average litter size of 5.0, a difference of
116 18%; Ehrich *et al.*, 2003). For our analysis we used the F₂ and F₃ generation that
117 originated from the matings of ten Large males to ten Small females resulting in 52 F₁
118 individuals. These F₁ individuals were randomly mated to generate 510 F₂ mice,
119 which, after random mating, produced 1632 individuals of the F₃ generation in 200
120 full-sib families. At birth half litters were cross-fostered in 158 families (Kramer *et*
121 *al.*, 1998). Thus, the size of cross-fostered litters may be larger or smaller compared

122 with litter size at birth. It is important to bear in mind that such differences in litter
123 size can cause effects from as early as week 1 body weight and not just after weaning.
124 In this analysis we focus on body weights taken once weekly from week 1 to week 10
125 as well as pre-weaning (week 1-3) and postweaning growth (week 3-10) as
126 phenotypes. We have previously analyzed these traits for main effects considering the
127 autocorrelation between the weights, splitting the growth period in two, early and late,
128 (Hager *et al.*, 2009b). A genome scan using such a multivariate approach will pick up
129 QTL that affect growth in this particular period. However, a disadvantage of this
130 approach is that loci that affect only few traits will be missed and given the pleiotropic
131 nature of most QTL does not yield a picture of when during development QTL begin
132 to show their effect, when they decrease and stop. We have thus analyzed the above
133 traits separately, following Wolf *et al.*, (2008).

134 All F₂ and F₃ mice were genotyped at 353 polymorphic single nucleotide
135 polymorphic markers (SNPs) that were evenly spaced (4-5 cM apart) across the
136 genome, except where the two strains are monomorphic, using the Illumina Golden
137 Gate assay (Wolf *et al.*, 2008). Haplotypes were reconstructed in Pedphase using the
138 Integer Linear Programming (ILP) algorithm (Li and Jiang 2005) to produce a set of
139 unordered haplotypes for the F₂ generation and a set of ordered (by allelic parent-of-
140 origin) haplotypes for the F₃. We distinguish four ordered genotypes denoted *LL*, *LS*,
141 *SL*, *SS*, (paternal / maternal allele) with the *L* allele originating from the LG/J strain
142 and the *S* allele from the SM/J strain.

143 We first analysed the effects of two litter size parameters on growth and
144 development: litter size at birth (*LSB*; i.e. size of the litter born by the dam) and litter
145 size at weaning (*LSW*). Using the Mixed Procedure in SAS (SAS version 9.1.3; SAS
146 Institute, Cary, NC, USA) we fitted a mixed model using maximum likelihood to

147 model the trait as a function of two litter size parameters with biological and foster
 148 family (*dam, nurse*) as random effect class variables to control for shared
 149 environmental effects. Our aim was to establish which litter size parameter, if any,
 150 affected weekly weights and growth.

151 We then used the marker loci to scan the genome for quantitative trait loci
 152 (QTL) that interacted with either litter size at birth or with litter size at weaning to
 153 affect weekly weights and growth. In a first step we assigned the four ordered
 154 genotypes at the marker loci additive (*a*), dominance (*d*), and parent-of-origin (*i*)
 155 genotypic index scores following Wolf *et al.*, (2008). These index scores are arrayed
 156 in a genetic design matrix to relate variation in the mean phenotypes (i.e. genotypic
 157 values) of each of the ordered genotypes ($\overline{LL}, \overline{LS}, \overline{SL}, \overline{SS}$) to a vector of genetic
 158 effects:

159

$$160 \begin{bmatrix} \overline{LL} \\ \overline{LS} \\ \overline{SL} \\ \overline{SS} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 \\ 1 & 0 & 1 & -1 \\ 1 & -1 & 0 & 0 \end{bmatrix} \begin{bmatrix} r \\ a \\ d \\ i \end{bmatrix}$$

161

162 This linear equation can be used to solve for the genetic effects by inverting the
 163 design matrix and multiplying it by the vector of genotypic values to yield a definition
 164 of the genetic effects (in terms of genotypic values):

165

$$166 \begin{bmatrix} r \\ a \\ d \\ i \end{bmatrix} = \begin{bmatrix} \frac{\overline{LL}}{2} + \frac{\overline{SS}}{2} \\ \frac{\overline{LL}}{2} - \frac{\overline{SS}}{2} \\ \frac{\overline{LS}}{2} + \frac{\overline{SL}}{2} - \frac{\overline{LL}}{2} - \frac{\overline{SS}}{2} \\ \frac{\overline{LS}}{2} - \frac{\overline{SL}}{2} \end{bmatrix}$$

167

168 where r is the reference point for the model (the mid-point between homozygotes), the
169 additive effect is defined as half the difference in the mean phenotype of the two
170 homozygotes, the dominance effect is the difference of the mean heterozygote
171 phenotype from the mean of the homozygotes, and the genomic imprinting effect is
172 half the difference in mean phenotype between the two reciprocal heterozygotes
173 (Wolf *et al.*, 2008).

174 The genotypic index scores for a locus were used in a linear mixed model
175 fitted by maximum likelihood using the Mixed Procedure in SAS. In the first of these
176 models (Model 1) we included the three genetic effects (a , d , i) and the two litter size
177 parameters (LSB and LSW) as main effects, and the six pair-wise interactions between
178 the genetic and litter size parameters. The biological and foster family (dam , $nurse$)
179 were included as random effect class variables to control for the background
180 influences of other loci and shared environmental effects that can inflate significance
181 values. Cross-fostering was not included in this model as we have previously shown
182 that there is no main effect of cross-fostering in this data set (Hager *et al.*, 2009a). The
183 fixed effects in Model (1) can be expressed as a linear model where Y_j is the trait
184 value (ten weekly weights or growth) of individual j and $X_{a(j)}$, $X_{d(j)}$, $X_{i(j)}$ are the
185 genotypic index scores for the direct genetic effects (additive, dominance and
186 imprinting) of individual j :

187

$$\begin{aligned} 188 \quad Y_{(j)} = & LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + LSW_{(j)} * aX_{a(j)} + LSW_{(j)} * dX_{d(j)} + \\ 189 \quad & LSW_{(j)} * iX_{i(j)} + LSB_{(j)} * aX_{a(j)} + LSB_{(j)} * dX_{d(j)} + LSB_{(j)} * iX_{i(j)} + e_{(j)} \end{aligned} \quad (1)$$

190

191 To generate a test for the overall effect of a locus we generated a likelihood ratio test
192 by subtracting the $-2 \log$ likelihood from Model 1 generated by the Mixed Procedure

193 from the -2 log likelihood from a reduced model that included only LSB , LSW , and
 194 the same random effects as in Model (1). This difference in the -2 log likelihoods of
 195 the two models (reduced model minus full model, which always gives a positive
 196 value) is approximately χ^2 distributed with nine degrees of freedom (i.e. the two
 197 models differ by a total of nine model terms). The probability values calculated from
 198 the χ^2 distribution (with 9 d.f.) were then transformed into a log probability ratio
 199 (LPR) in order to make them comparable to LOD scores (LPR = $-\log_{10}[\text{probability}]$).

200 To distinguish between interactions of genotype with litter size at birth or at
 201 weaning we fitted two further models with the same random effects as in Model (1),
 202 the three main genetic effects, the two litter size parameters and either the interactions
 203 of genotype and litter size at birth (2) or genotype and litter size at weaning (3):

204

$$205 \quad Y_{(j)} = LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + LSB_{(j)} * aX_{a(j)} + LSB_{(j)} * dX_{d(j)} + LSB_{(j)} \\ 206 \quad * iX_{i(j)} + e_{(j)} \quad (2)$$

207

$$208 \quad Y_{(j)} = LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + LSW_{(j)} * aX_{a(j)} + LSW_{(j)} * dX_{d(j)} + \\ 209 \quad LSW_{(j)} * iX_{i(j)} + e_{(j)} \quad (3)$$

210

211 Models (2) and (3) were then individually compared (using the -2 log likelihood
 212 values as described above) to a further reduced model (Model 4) that contained only
 213 the three genetic effects and the two litter size parameters but not the interactions:

214

$$215 \quad Y_j = LSW_{(j)} + LSB_{(j)} + aX_{a(j)} + dX_{d(j)} + iX_{i(j)} + e_{(j)} \quad (4)$$

216

217 Thus, the only difference between Models (2) and (3) is the type of interaction effect
218 included. Using Models (2), (3) and (4) we generated two tests of interaction effects.
219 The comparison of Model (2) to Model (4) ($-2 \log$ likelihood of Model 4 minus that
220 of Model 2) provides a chi-square test (with 3 d.f.) of the interaction of the three
221 genetic effects with litter size at birth. The comparison of Model (3) to Model (4) (-2
222 \log likelihood of Model 4 minus that of Model 3) provides a chi-square test (with 3
223 d.f.) of the interaction of the three genetic effects with litter size at weaning.
224 Depending on which of the interaction effects (with litter size at weaning or at birth)
225 was significant, we identified whether litter size at birth or at weaning is causal to the
226 interaction effect.

227 To generate significance thresholds we used the effective number of markers
228 method, which is based on the Eigenvalues of the marker correlation matrix (Li and Ji
229 2005). This approach calculates the number of independent tests in a genome scan and
230 adjusts significance using a Bonferroni correction. Briefly, one first calculates the
231 correlation matrix for the marker loci and then estimates the Eigenvalues of the
232 correlation matrix. The integer parts of the Eigenvalues are replaced by 1 when the
233 value is ≥ 1 and 0 when the value is < 1 . This integer part is then added to the original
234 decimal part to yield the effective number of markers contained in that Eigenvalue.
235 For example, an Eigenvalue of 3.75 yields 1.75 effective markers, while an
236 Eigenvalue of 0.75 yields 0.75 effective markers. The sum of these converted values
237 represents the effective number of markers, which we used in the Sidak equation to
238 generate the threshold for genome-wide tests (i.e. we used the effective number of
239 markers on the whole genome to generate thresholds). We have previously
240 demonstrated that the thresholds obtained are very similar to those obtained through
241 computationally intensive simulation (Hager *et al.*, 2008a). We thus determined the

242 thresholds for all traits and identified significant loci when the overall locus LPR
243 value or the interaction effect LPR value exceeded the genome-wide threshold. To
244 investigate pleiotropic effects we included QTL effects whenever the effect of a given
245 locus is significant at the pointwise threshold ($p < 0.05$; $LPR > 1.3$) assuming the
246 QTL has exceeded the genome-wide significance threshold for a different trait (Wolf
247 *et al.*, 2008).

248 We have previously established that parent-of-origin dependent effects on
249 offspring phenotypes may be caused by either maternal genetic effects or genomic
250 imprinting (Hager *et al.*, 2008b). In essence, differences in maternal genotype can
251 cause differences between phenotypes of heterozygous offspring and thus cause the
252 same parent-of-origin effect patterns as those caused by genomic imprinting effects.
253 This also applies to the appearance of additive effects due to the genetic correlation of
254 offspring with their parents at a locus (where, at a particular locus, the correlation is
255 $\frac{1}{2}$). Thus, a locus expressed in the mother may affect her offspring's phenotype, but
256 since offspring inherit one allele from their mother it appears as if that locus directly
257 affects offspring phenotype. This scenario applies to non-cross-fostered animals only
258 as the autocorrelation between maternal and offspring genotype is broken in cross-
259 fostered animals. We therefore tested all loci with a significant interaction to
260 determine whether the interaction effect could be explained by a maternal genetic
261 effect or was associated with a change in the direct effect of a locus. This was
262 achieved by using a mixed model to test whether the parent-of-origin-dependent effect
263 or additive effect differed significantly between individuals reared by homozygous
264 versus heterozygous mothers (Hager *et al.*, 2009a).

265

266 **Results**

267 We first analyzed main effects of litter size, independent of any genotype effects. The
268 average litter size at birth in our experimental population was 8.54 with a range from
269 4 to 13 pups per litter. All traits were highly significantly affected by litter size at
270 weaning (i.e. post natal litter size), including week 1 body weight, whereas litter size
271 at birth only affected weeks 1 to 3. Invariably, litter size effects were negative such
272 that average individual weight decreased as litter size increased. Litter size at birth
273 had a standardized effect of -0.15 for week 1 weight, decreasing to -0.07 for week 3.
274 To illustrate the magnitude of these effects we compare litter sizes of five and nine
275 individuals at week 1. Pups born into the larger litter would then be 14.38% or 0.61 g
276 smaller compared to those born with five litter mates (average weight at week 1 is
277 4.23g). Unsurprisingly, the effects of litter size at weaning are greatest for week 2 and
278 week 3 (NB. cross-fostering took place at birth) with standardized estimates of -0.55
279 and -0.43 . However, these effects extend all the way to week 10 (standardized
280 estimate -0.19), at which time pups born into litters of nine are still 2.6% smaller on
281 average compared to those born into litters of five.

282 After having established the main effects of litter size, we performed a genome
283 scan across all 19 autosomes for loci that showed a significant interaction between
284 genetic (additive or dominance) or epigenetic (genomic imprinting) effects and litter
285 size at birth or weaning on weight and growth. We denote loci that show interaction
286 effects with litter size at birth $LSBy.z$ and loci interacting with litter size at weaning
287 $LSWy.z$. LSB refers to litter size at birth, LSW to litter size at weaning, y identifies the
288 chromosome and z the individual QTL on that chromosome in case several QTL are
289 found on one chromosome. For all loci we confirmed that any imprinting or additive
290 interaction effect is not caused by a maternal genetic effect.

291 Five loci on separate chromosomes (chromosomes 1, 4, 6, 11 and 16) showed
292 an interaction with litter size at birth (Table 1). Two loci showed interactions with
293 additive effects, two loci interacted with dominance and one with imprinting effects.
294 One might have expected that the interaction of genotype with litter size at birth
295 predominantly affects early weights, however, late weights are equally affected and
296 *LSB16.1* only affected body weight from week 5 onwards. Turning to loci that
297 interacted with litter size at weaning, we identified two QTL located on chromosomes
298 10 and 15 (Table 1). With the exception of *LSB11.1* all loci affected several traits
299 showing clearly when during development their effects become manifested, when
300 they are greatest and when they cease to show detectable effects (see LPR values in
301 Table 1). A locus on chromosome 10 showed an unusual pattern in that additive
302 interactions with litter size at birth influenced pre-weaning growth whereas
303 dominance interactions affected week 4 – 10 (at the same locus).

304

305 *Effects of litter size interactions*

306 The nature of the interaction can be examined by looking at the litter size effect in
307 individuals with specific alleles at a locus, which can be inferred from the sign of the
308 interaction given in Table 1. Overall, average body weight decreases with increasing
309 litter size regardless of genotype at all loci. However, the degree to which increasing
310 litter size leads to a reduction in weight across genotype varies and is manifested in
311 the interaction. A positive interaction of additive effects and litter size ($+a \times LS$) means
312 that *LL* homozygotes show a smaller reduction in weight compared to *SS*
313 homozygotes with increasing values of the litter size parameter (either litter size at
314 birth, *LSB*, or litter size at weaning, *LSW*, respectively). The reverse applies to a
315 negative interaction effect ($-a \times LS$). Positive dominance interactions indicate that

316 heterozygotes showed a smaller reduction in weight compared to homozygotes with
317 increasing litter size values. Again, the reverse applies to negative dominance
318 interaction effects. Finally, for positive imprinting interactions, *SL* heterozygotes
319 showed a stronger decrease in their weight compared to *LS* heterozygotes with
320 increasing litter size.

321 Overall, the loci show both positive and negative interactions with litter size
322 for additive and dominance effects. Thus, no general pattern can be established across
323 all traits for the direction of effect for a given genotype such that, for example, *LL*
324 homozygotes always increased in weight with increasing litter size. However, we can
325 illustrate the effects of the interactions for body weight, for example comparing
326 homozygotes and heterozygotes at *LSB16.1* where we assume a difference in litter
327 size of four, e.g. comparing homozygous and heterozygous pups born into litters of
328 five versus those born in litters of nine litter mates (about the average litter size in our
329 population). Figure 1 shows the average phenotype of both homo- and heterozygotes
330 for week 7 bodyweight when litter size at birth was nine next to their average weights
331 when litter size was five. We see that while homozygotes showed a reduction in
332 weight of 11.3%, from their average weight with litter size of nine to their average
333 weight with litter size of five, the corresponding value for heterozygotes was only 4%.
334 The differences between genotypes in their weight increase when comparing litters of
335 greater size difference. For example, focusing on differences between the two
336 heterozygotes (at *LSB6.1*) and comparing litters of five and 11 pups, we find that *SL*
337 heterozygotes would be over 11% smaller while *LS* heterozygotes would be only ~5%
338 smaller in litters of 11 compared to litters of five litter mates.

339

340 **Discussion**

341 Although the effects of litter size on growth and levels of competition have been
342 demonstrated in different systems (Mock and Parker 1997; Stockley and Parker
343 2002), we show that such effects can depend on the genotype of individuals at specific
344 loci. This advances on previous studies that generally demonstrated a genetic basis for
345 such interactions (e.g. Merilä and Fry 1998). In addition, our study confirms the
346 existence of a substantial main effect of litter size on weight such that average weight
347 was inversely proportional to litter size (e.g. Reading 1966; Epstein 1978). The effects
348 of litter size on weight decrease over time but even at week 10 are still significant:
349 body weight at week 10 is affected by litter size at weaning with a standardized effect
350 of -0.19, which would cause a reduction of 2.6% of weight comparing pups with nine
351 litter mates at weaning to those that had five litter mates. This suggests that, although
352 compensation for lower body weight caused by being born in larger litters is possible,
353 such compensatory growth is only partial and, consequently, a negative effect in terms
354 of smaller body size remains even weeks later. Our previous work analyzed main
355 genetic and genomic imprinting effects on weight and growth in this population
356 (Hager et al. 2009b) and we can thus compare these loci with those found in the
357 present study. We had discovered 18 main effect loci on 13 chromosomes that show
358 additive, dominance or imprinting effects (Hager *et al.*, 2009b, Table 1 therein).
359 While none are identical to the interaction loci we found in this present study, three of
360 the interaction loci (*LSB4.1*, *LSW10.1* and *LSW15.1*) are located directly adjacent to
361 main effect loci (*adi4.1*, *adi10.1* and *adi15.1*). The locus on chromosome 4 shows an
362 additive main effect on weights and growth as well as an interaction of the additive
363 effect with litter size at birth. Loci on the other chromosomes, however, do not show
364 the same main and interaction effects and it seems thus less likely that they could be
365 the same. In conclusion, with one exception on chromosome 4 (*LSB4.1*), the main

366 effect QTL are different from those showing the interaction with litter size suggesting
367 that the litter size interaction QTL described here are more specifically responding to
368 the social environment, unlike the main effect QTL,

369 Overall, we detected five loci with effects on weight and growth that were
370 dependent on (i.e. showed an interaction with) the number of siblings at birth, and two
371 that were dependent on litter size at weaning. Such genotype by (social) environment
372 interactions may enable genotypes to respond differentially to changes in
373 environmental conditions in relation to sibling competition and resource availability.
374 The differential effects of the prenatal (uterine) competitive environment (i.e. pre-
375 natal litter size) on postnatal growth could arise from differential genetic priming of
376 offspring to expectations of future resource availability and sibling competition. For
377 example, an individual born into a small litter might expect lower competition levels
378 due to adequate milk supply for all litter mates, whereas individuals born into large
379 litters may expect high levels of competition. Only a direct investigation of
380 competitive behaviour of pups cross-fostered into litters that differ in their size at birth
381 and at weaning would allow a confirmation of this hypothesis.

382 We were able to clearly demonstrate that genetic variants modulate individual
383 responses to changes in the competitive environment, in essence phenotypic plasticity
384 in social environments. However, whether or not the observed effects are adaptive is
385 more difficult to ascertain. If the effects were adaptive and selection favoured larger
386 body size, then we might expect genotypes adapted to larger litter size to show better
387 performance (i.e. larger body weight) as litter size increases compared to genotypes
388 adapted to smaller litter size. However, we do not find a consistent allele effect in
389 that, for example, individuals being homozygous for the *L* allele show a smaller
390 reduction in weight (or increased fitness) compared to *SS* homozygotes. Instead, as

391 indicated by the sign of the interaction for additive effects in Table 1, being
392 homozygous for the *L* allele results in a negative effect at one locus (*LSB1.1*), but a
393 positive effect at another (*LSB4.1*). For example, one might have expected that in the
394 strain that produces larger litters (*Large* has an average litter size of 6.1 compared to
395 an average litter size of *Small* of 5.0, a difference of 18%; Ehrich *et al.*, 2003), levels
396 of competition are higher than in strains with smaller litters and thus *Large* pups were
397 to some degree selected to develop in a more competitive environment. The *L* alleles
398 might thus confer a competitive advantage compared to *S*, reflected in increased
399 fitness of *LL* homozygotes when litters increase in size. This is not the case. Similarly
400 inconsistent is the pattern of interaction for dominance interactions, which overall
401 suggests non-adaptive reasons for the observed plasticity.

402 One explanation for the absence of clear adaptive benefits of plasticity is that
403 the loci may have pleiotropic effects on other traits that are not plastic (DeWitt *et al.*,
404 1998). While this remains an untested possibility, it seems more likely that the
405 condition of litter size change experienced in our experimental population is outside
406 the range of conditions in the original populations (Ghalambor *et al.*, 2007). Given
407 that the average litter size and range in our population exceeds that in both pure
408 strains by about three individuals, levels of competition may be different as may be
409 the costs (Chappel *et al.*, 2002).

410 The key result emerging from our study is that a given genotype results in
411 different phenotypes depending on the number of siblings. This genotype by social
412 environment interaction can be similar in magnitude as the strong main effects of litter
413 size (which results in considerable reduction in weight with increasing litter size) and
414 thus should thus be ascribed biological importance equivalent to the main effect. In
415 contrast to litter size main effects, the genotype by litter size interactions do not show

416 a consistent effect across genotypes. Therefore, although genetic variation to respond
417 to changes in the competitive environment exists, the observed phenotypic plasticity
418 in our study may be regarded as non-adaptive at present.

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420

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427

428 **Conflict of interest**

429 The authors declare no conflict of interest.

430

431

432 **Data archiving**

433 Data identifiers to be added

434

435 **References**

- 436 Auld JR, Agrawal AA, Releya RA (2010). Re-evaluating the costs and limits of
437 adaptive phenotypic plasticity. *Proc R Soc Lond B* **277**: 503-511.
- 438 Barnard CJ, Behnke JM, Gage AR, Brown H, Smithurst PR (1998). Maternal effects
439 on the development of social rank and immunity trade-offs in male mice (*Mus*
440 *musculus*). *Proc R Soc Lond B* **265**: 2087-2093.
- 441 Chappel MA, Bachman GC (2002). Energetic costs of begging. In: Wright J, Leonard
442 ML (eds) *The evolution of begging*, Kluwer Academic Press: Doordrecht. pp
443 143-162.
- 444 Clutton-Brock TH (1991). *The Evolution of Parental Care*. Princeton University
445 Press: Princeton.
- 446 DeWitt TJ, Sih A, Wilson DS (1998). Costs and limits of phenotypic plasticity.
447 *Trends Evol Ecol* **13**: 77-81.
- 448 Ehrich TH, Kenney JP, Vaughn TT, Pletscher S, Cheverud JM (2003). Diet, obesity
449 and hyperglycemia in LG/J and SM/J mice. *Obesity* **11**: 1400-1410.
- 450 Epstein HT (1978). The effect of litter size on weight gain in mice. *J Nutr* **108**: 120-
451 123.
- 452 Gilbert AN (1995). Tenacious nipple attachment in rodents: the sibling competition
453 hypothesis. *Anim Behav* **50**: 881-891.
- 454 Godfray HCJ (1991). Signalling of need by offspring to their parents. *Nature* **352**:
455 328-330.
- 456 Goodale HD (1938). A study of the inheritance of body weight in the albino mouse by
457 selection. *J Heredity* **29**: 101-112.

458 Gyekis J, Blizard DA, Stout JT, Vandenbergh DJ, McClearn GE, Hager R (2011).
459 Genetic and maternal effects on offspring mortality in mice. *Evolutionary*
460 *Biology* DOI 10.1007/s11692-011-9131-x

461 Hager R, Johnstone RA (2005). Differential growth of own and alien young in mixed
462 litters of mice: a role for genomic imprinting. *Ethology* **111**: 705-711.

463 Hager R, Cheverud JM, Leamy LJ, Wolf JB. (2008a). Sex dependent imprinting
464 effects on complex traits in mice. *BMC Evolutionary Biology*, **8**, e303.

465 Hager R, Cheverud JM, Wolf JB (2008b). Maternal effects as the cause of parent-of-
466 origin-dependent effects that mimic genomic imprinting. *Genetics* **178**: 1755–
467 1762.

468 Hager R, Cheverud JM, Wolf JB (2009a). Change in maternal environment induced
469 by cross-fostering alters genetic and epigenetic effects on complex traits in
470 mice. *Proc R Soc Lond B* **276**: 2949-2954.

471 Hager R, Cheverud JM, Wolf JB. (2009b). Relative contribution of additive,
472 dominance and imprinting effects to phenotypic variation in body size and
473 growth between divergent selection lines of mice. *Evolution* **65**:1118-1128.

474 Knight CH, Maltz E, Docherty AH (1986). Milk yield and composition in mice:
475 effects of litter size and lactation number. *Comp Biochem Physiol* **84A**: 127-
476 133.

477 König B (1997). Cooperative care of young in mammals. *Naturwissenschaften* **84**: 95-
478 104.

479 König B (1994). Components of lifetime reproductive success in communally and
480 solitarily nursing house mice - a laboratory study. *Behav Ecol Sociobiol* **34**:
481 275-283.

482 Kramer MG, Vaughn TT, Pletscher LS, King-Ellison K, Adams E, Erikson C *et al.*
483 (1998). Genetic variation in body weight gain and composition in the
484 intercross of Large (LG/J) and Small (SM/J) inbred strains of mice. *Genet Mol*
485 *Biol* **21**: 211-218.

486 Lander ES, Botstein D (1989). Mapping Mendelian factors underlying quantitative
487 traits using RFLP linkage maps. *Genetics* **121**: 185–199.

488 Li J, Ji L (2005). Adjusting multiple testing in multilocus analyses using the
489 eigenvalues of a correlation matrix. *Heredity* **95**: 221-227.

490 Li J, Jiang T (2005). Computing the minimum recombinant haplotype configuration
491 from incomplete genotype data on a pedigree by integer linear programming. *J*
492 *Comp Biol* **12**: 719-39.

493 Macarthur J (1944). Genetics of body size and related characters. I. Selection of small
494 and large races of the laboratory mouse. *Am Nat* **78**: 142–157.

495 MacNair MR, Parker GA (1979). Models of parent-offspring conflict. III. Intra-brood
496 conflict. *Anim Behav* **27**: 1202-1209.

497 Merilä J, Fry JD (1998). Genetic variation and causes of genotype-environment
498 interaction in the body size of blue tit (*Parus caeruleus*). *Genetics* **148**: 1233-
499 1244.

500 Mock DW, Parker GA (1997). *The evolution of sibling rivalry*. Oxford University
501 Press: Oxford.

502 Piersma T, Drent J (2003). Phenotypic plasticity and the evolution of organismal
503 design. *Trends Ecol Evol* **18**: 228-233.

504 Reading AJ (1966). Effects of parity and litter size on the birth weight of inbred mice.
505 *J Mamm* **47**: 111-114.

506 Scheiner SM (1993). Genetics and evolution of phenotypic plasticity. *Annu Rev Ecol*

507 *Syst* **24**: 35-68.

508 Snell-Rood EC, van Dyken JD, Cruickshank T, Wade MJ, Moczek AP (2010).

509 Toward a population genetic framework of developmental evolution: the costs,

510 limits and consequences of phenotypic plasticity. *Bioessays* **32**: 71-82.

511 Stearns SC (1989). The evolutionary significance of phenotypic plasticity. *Bioscience*

512 **39**: 436-445.

513 Stockley P, Parker GA (2002). Life history consequences of mammal sibling rivalry.

514 *Proc Natl Acad Sci USA* **99**: 12932-12937.

515 Trivers R (1974). Parent-offspring conflict. *Am Zool* **14**: 249-264.

516 Via, S. and Lande, R. (1985) Genotype–environment interaction and the evolution of

517 phenotypic plasticity, *Evolution* 39, 505–522

518 West-Eberhard MJ (2003). *Developmental plasticity and evolution*. Oxford University

519 Press: Oxford.

520 Wright J, Leonard ML (2002). *Begging*. Kluwer: Amsterdam.

521 Wolf JB, Cheverud JM, Roseman C, Hager R (2008). Genome-wide analysis reveals a

522 complex pattern of genomic imprinting in mice. *PLoS Genet* **4**, (6):e1000091

523 doi:10.1371/journal.pgen.1000091.

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526 **Figure legends**

527

528 **Figure 1:** Difference in average week 7 bodyweight for the homozygotes and
529 heterozygotes at *LSB16.1* shown for two different litter sizes at birth. The graph
530 illustrates that homozygotes suffer a greater reduction in weight than heterozygotes
531 for the same litter size difference. Error bars indicate the standard error of the mean.
532

533 **Table 1.** Interaction QTL with litter size at birth (*LSB*) and litter size at weaning
534 (*LSW*). The first column identifies the QTL followed by the genomic location in F₂
535 equivalent centiMorgans (cM) and the coordinates in megabases (Mb) based on
536 mouse genome build 36. This is followed by the traits affected. The column ‘Full
537 LPR’ lists the overall model LPR for the test of main effects and interactions effects.
538 Under ‘Interaction effect’ we specify which of the three effects showed an interaction
539 with the sign of effect, where ‘*a*’ refers to additive, ‘*d*’ to dominance and ‘*i*’ to
540 imprinting effects. In parentheses we give the interaction estimates. The column
541 headed ‘Test’ gives the LPR for the interaction effect. ‘Growth 13’ and ‘Growth 310’
542 refer to pre-weaning and post-weaning growth, respectively. Confidence intervals
543 (Mb) for the interaction QTL positions were determined using a one LOD drop (using
544 LPR values) following Lander & Botstein (1989). Because a locus may affect several
545 traits, the LPR for the interaction effects may be different and hence the confidence
546 intervals.
547

QTL	Location		Confidence interval	Trait	Full LPR	Interaction effect (estimate)	Test
	cM	Mb					
<i>LSB1.1</i>	42.66	93.21	87,02 – 99,36	Week 1	4.18	<i>a</i> (0.036)	1.58
			87,02 – 99,36	Week 2	6.85	<i>a</i> (0.063)	2.98
			87,02 – 99,36	Week 3	3.30	<i>a</i> (0.078)	1.98
<i>LSB4.1</i>	37.28	84.31	78,87 – 87,93	Week 4	5.11	<i>-a</i> (-0.161)	2.43
				Week 5	5.02	<i>-a</i> (-0.172)	1.85
				Week 6	6.06	<i>-a</i> (-0.195)	1.72
<i>LSB6.1</i>	29.25	65.37	105,62 – 117,19 94,76 – 134,72 94,76 – 134,72 94,76 – 134,72 94,76 – 134,72 94,76 – 134,72 94,76 – 134,72 94,76 – 134,72	Week 4	7.79	<i>-i</i> (-0.131)	1.69
				Week 5	7.44	<i>-i</i> (-0.181)	1.99
				Week 6	7.21	<i>-i</i> (-0.242)	2.43
				Week 7	6.76	<i>-i</i> (-0.302)	2.82
				Week 8	6.50	<i>-i</i> (-0.320)	2.64
				Week 9	6.97	<i>-i</i> (-0.406)	3.50
				Week 10	5.68	<i>-i</i> (-0.384)	2.86
				Growth 3-10	4.99	<i>-i</i> (-0.003.)	2.44
<i>LSW10.1</i>	49.16	111.3	99,22 – 117,08	Week 4	2.66	<i>-d</i> (-0.223)	2.62
			99,22 – 128,85	Week 5	4.59	<i>-d</i> (-0.265)	2.41
			99,22 – 117,08	Week 6	3.86	<i>-d</i> (-0.263)	1.79

			99,22 – 128,85	Week 7	3.97	<i>-d (-0.295)</i>	1.73
			99,22 – 117,08	Week 8	4.88	<i>-d (-0.370)</i>	2.12
			99,22 – 117,08	Week 9	4.68	<i>-d (-0.383)</i>	1.99
			99,22 – 117,08	Week 10	4.72	<i>-d (-0.443)</i>	2.30
LSB11.1	11.90	24.47	60,48 – 72,28	Week 4	2.37	<i>d (0.243)</i>	3.14
LSW15.1	30.19	69.47	62,91 – 76,75	Week 2	3.49	<i>d (0.074)</i>	2.71
			56,81 – 83,18	Week 3	4.63	<i>d (0.127)</i>	3.15
			62,91 – 76,75	Week 4	2.16	<i>d (0.162)</i>	1.66
LSB16.1	44.61	46.92	36,99 – 50,54	Week 5	2.16	<i>d (-0.336)</i>	3.73
			36,99 – 50,54	Week 6	2.84	<i>d (-0.454)</i>	4.64
			36,99 – 50,54	Week 7	3.32	<i>d (-0.559)</i>	5.27
			36,99 – 50,54	Week 8	2.90	<i>d (-0.613)</i>	5.14
			36,99 – 50,54	Week 9	2.72	<i>d (-0.624)</i>	4.70
			36,99 – 50,54	Week 10	2.95	<i>d (-0.713)</i>	5.36
			36,99 – 50,54	Growth 310	3.81	<i>d (-0.714)</i>	6.10

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