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ORIGINAL ARTICLE

Genetic parameters for birthweight environmental variability in mice

A. Pun[1], I. Cervantes[1], B. Nieto[1], C. Salgado[1], M.A. Pérez-Cabal[1], N. Ibáñez-Escriche[2] & J.P. Gutiérrez[1]

1 Departamento de Producción Animal, Facultad de Veterinaria, UCM, Madrid, Spain
2 Genètica i Millora Animal – Centre IRTA_Lleida, Lleida, Spain

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Correspondence
J.P. Gutiérrez, Department of Animal Production, Faculty of Veterinary, UCM, Avda. Puerta del Hierro s/n, Madrid E-28040, Spain. Tel:/Fax: +34913943767; E-mail: gutgar@vet.ucm.es

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Summary

Data from a divergent experiment for birthweight (BrW) environmental variability were used to estimate genetic parameters for BrW trait and its environmental variability by fitting both homoscedastic (HO) and heteroscedastic (HE) models. A total of 5 475 records of BrW from animals born from inbred dams, and 7 140 pedigree records were used. The heritability of BrW using the model HO was 0.27, with the litter effect much more important, 0.43. The model HE provided a genetic correlation between the trait and its environmental variability that was very high and negative, −0.97, and a high value for the additive genetic variance for environmental variability, suggesting an artefact in the model. The residual skewness was found to be essentially null. A model considering the genetic correlation null was also fitted, and used to obtain the breeding values for the selection process. Moreover, the trait was considered as maternal resulting in similar estimates under the model HO, but more reasonable for the genetic correlation between the trait and its environmental variability of 0.48 with a value of 0.25 for the additive genetic variance regarding environmental variability under the model HE. This led to the conclusion that environmental variability of BrW in mice must be selected via dams. Estimated parameters in a reduced dataset without inbred animals did not substantially change this conclusion.

Introduction

The aim of the genetic selection in animal breeding has traditionally been the increase of the mean for the productive traits. Today, some of these traits have reached an optimum level (Angel 2007) and others are delimitated by production quotas, i.e. dairy production (O'Donnell *et al.* 2011). Hence, there is an increasing interest in the homogeneity of the animal production that would decrease the cost of handling and production that ultimately would increase the profitability of the farm (Bolet *et al.* 2007). Regarding the homogeneity of the birthweight trait, this would lead to a reduction in the mortality of young animals, easiness to manage in groups, improved welfare and to create homogeneity in the final products (Damgaard *et al.* 2003) that would increase their economic value. Moreover, birthweight is a very important trait in multiparous species like rabbit (Bodin *et al.* 2010) or pig (Berard *et al.* 2008) where the homogeneity within the litter determines the competitiveness between young animals and the percentage of survival (Damgaard *et al.* 2003; Garreau *et al.* 2008). The small size and generation interval of the mouse make this laboratory mammal a good model for this kind of trait and species (Moreno *et al.* 2012). On the other hand, the existence of a genetic background affecting the variability of a trait has been demonstrated and it differs from the one controlling the trait mean, so a genetic selection can be done on the variability of a

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trait reducing it and reaching homogeneity in the trait (Scheiner & Lyman 1991) that is called canalization. SanCristobal-Gaudy *et al.* (1998) proposed a model that makes it possible to determine simultaneously the genetic parameters for the mean and for the variability with an EM-REML algorithm. Sorensen & Waagepetersen (2003) extended this method to obtain results using a Bayesian approach, and Ibáñez-Escriche *et al.* (2010) developed a freely available software called GSEVM for this approach.

The aim of this research was to estimate and discuss the genetic parameters for birthweight environmental variability in a mouse population designed for a divergent selection experiment for birthweight variability, as well as studying if birthweight environmental variability must be considered an individual or maternal trait.

Materials and methods

Experimental population

The experimental population analysed here started from a preexisting mouse population originating from a balanced genetic contribution of three inbred mice lines: BALB/c, C57BL and CBA. The three-way crossed population was maintained in panmixia during 20 generations, thus ensuring high levels of both genetic and phenotypic variabilities.

From this panmictic population, a total of 30 males and 66 females were randomly selected to be mated with BALB/c inbred females and males respectively to evaluate them. Inbred animals were considered genetically the same individual across the experiment so the genetic differences between offspring can only be associated with the parent selected from the preexisting population. Each selected male was mated with 4 inbred females and each selected female was mated once with an inbred male. During the birth period, pregnant females were checked every 24 h, and the newborns were weighed and identified within 24 h after birth. To establish the lines, the six males and twelve females with the highest and lowest additive genetic value for the environmental variability were selected to create two divergent selection lines, high variability line and low variability line, respectively. From the second generation onwards, no females were evaluated, but their Genetic Breeding Values (GBVs) were obtained as a consequence of an animal model evaluation. However, evaluation of males continued, 30 individuals were evaluated every generation using the above procedure. All the processes were repeated for five additional generations. A scheme of the experimental design is shown in Figure 1.

Animals within lines were mated following a mating design determined by simulated annealing (Fernandez & Toro 1999) that maximizes the mean GBV of the progeny without exceeding the coancestry level determined by the standard solution. Under this standard fictitious solution, each of the best six males would be mated with two females, and three and two males from the offspring of the two mates, would be selected to be evaluated in the next generation. The optimal solution is thus found by allowing the males to be mated with up to three females and a maximum of four males were selected from each mating. After carrying out the real matings, a second simulated annealing process was carried out accounting for the real number of males and females born.

Two different subsets of data can be defined as follows: the evaluation dataset, gathering the records from the evaluation periods in which all the mothers are females from the inbred line BALB/c, and the nucleus dataset, with the records belonging to the matings in the nucleus, in which all the progenitors belong to the population under selection. Notice that records from the matings of inbred males and outbred females of the first evaluation were not considered in any dataset.

The data of individual birthweight (BrW) obtained from all the litters and the pedigree that included 10 generations of the panmitic population were used to evaluate the selected progenitors. The final evaluation dataset contained a total of 5 475 records of BrW from 736 litters. The mean ± SD for the litter size (newborns) and for the BrW (g) were 7.77 ± 2.77 and

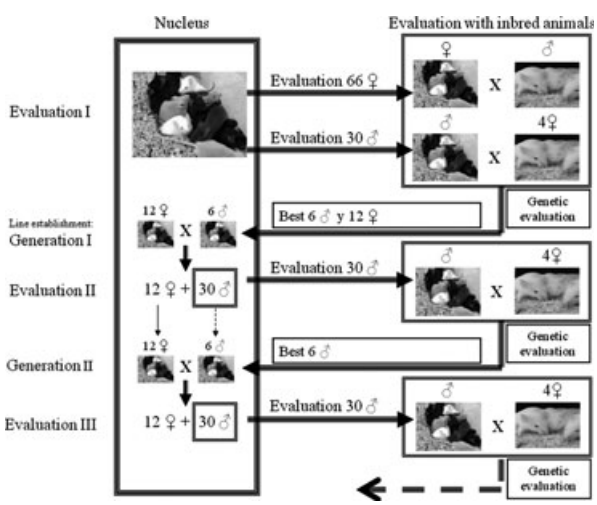

Figure 1 Scheme of the experiment.

1.51 ± 0.22 respectively. All the inbred females were considered to be the same animal in the pedigree. The total number of individuals included in the analysed pedigree was 7 140 that included 10 generations back of known pedigree in the panmitic population. The nucleus dataset contained 756 BrW records from 88 litters belonging to 84 females with a total of 2 112 individuals in the pedigree, and in this case, the mean ± SD for the litter size (newborns) and for the BrW (g) was 8.97 ± 3.02 and 1.57 ± 0.21, respectively.

Models

Two models were fitted to estimate the genetic parameters both using a Bayesian approach. The first was a homoscedastic model (HO), which is a classical additive genetic model that includes the assumption of homogeneity of the environmental variation:

$$y_i = \mathbf{x}_i\mathbf{b} + \mathbf{z}_i\mathbf{a} + \mathbf{w}_i\mathbf{c} + e_i$$

With:

$$\begin{pmatrix}\mathbf{a}\\ \mathbf{c}\end{pmatrix} \sim N\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\sigma_c^2\end{bmatrix}\right)$$

where y_i is the BrW of animal i; **b** is the vector for systematic effects; **a** is the vector of direct animal genetic effect; **c** is the vector of unknown for litter effect; $\mathbf{x}_i$, $\mathbf{z}_i$ and $\mathbf{w}_i$ are the incidence vectors for systematic, animal and litter effects respectively; e_i is the residual; **A** is the numerator relationship matrix, **I** is the identity matrix of order equal to the number of litters; σ_a^2 is the additive genetic variance, and σ_c^2 is the litter effect variance.

The second model is a heteroscedastic model (HE) developed by SanCristobal-Gaudy *et al.* (1998) which assumes that the environmental variance is heterogeneous and partially under genetic control:

$$y_i = \mathbf{x}_i\mathbf{b} + \mathbf{z}_i\mathbf{a} + \mathbf{w}_i\mathbf{c} + e^{\frac{1}{2}(\mathbf{x}_i\mathbf{b}^* + \mathbf{z}_i\mathbf{a}^* + \mathbf{w}_i\mathbf{c}^*)}\varepsilon_i$$

where * indicates the parameters associated with environmental variance; **b** and **b*** are the vectors of the systematic effects; **a** and **a*** are the vectors of the direct genetic effect; and **c** and **c*** are the vectors of the litter effect. Incidence vectors $\mathbf{x}_i$, $\mathbf{z}_i$ and $\mathbf{w}_i$ have been defined in the previous model HO. Although not relevant in the evaluation dataset in which only one mother is present, in the nucleus dataset it must be noted that **c** and **c*** are fitting the litter effect, but it is assumed that they are also fitting most of the maternal effect as observed by Ibáñez-Escriche *et al.* (2008a) when analysing litter weight trait in a similar population of mice. Correlation between direct and maternal effects was not accounted for, given that the mother is unique in the evaluation dataset, the amount of data was limited in the nucleus dataset, and there is no software available to solve such a complex model HE.

The genetic effects **a** and **a*** are distributed together and are assumed to be Gaussian:

$$\begin{pmatrix}\mathbf{a}\\ \mathbf{a}^*\end{pmatrix} \sim N\left(\begin{bmatrix}\mathbf{0}\\ \mathbf{0}\end{bmatrix}, \begin{bmatrix}\sigma_a^2 & \rho\sigma_a\sigma_{a^*}\\ \rho\sigma_a\sigma_{a^*} & \sigma_{a^*}^2\end{bmatrix} \otimes \mathbf{A}\right)$$

where **A** is the additive genetic relationship matrix; σ_a^2 is the additive genetic variance of the trait; $\sigma_{a^*}^2$ is the additive genetic variance affecting environmental variance of the trait; ρ is the coefficient of genetic correlation, and $\otimes$ denotes the Kronecker product.

The vectors **c** and **c*** are also assumed to be independent, with $\mathbf{c}^* \sim N(\mathbf{0}, \mathbf{I_c}\sigma_{c^*}^2)$and $\mathbf{c} \sim N(\mathbf{0}, \mathbf{I_c}\sigma_c^2)$ where $\mathbf{I_c}$ is the identity matrix of equal order to the number of litters and σ_c^2 and $\sigma_{c^*}^2$ are the litter effect variances affecting respectively, the BrW mean and its environmental variability (Ibáñez-Escriche *et al.* 2008a). A version of this model (model HEρ0) was also fitted with the genetic correlation null.

The model HO was solved by using the TM program (Legarra 2008) while the model HE was solved by using the GSEVM program (Ibáñez-Escriche *et al.* 2010). A version of GSEVM was developed *ad hoc* to force the genetic correlation to be null. Also REML estimations were obtained using VCE software (Neumaier & Groeneveld 1998) to estimate parameters under model HO to check the consistency of the Bayesian estimations.

All the models applied to the evaluation dataset included period of birth (6 levels), litter size (14 levels) and sex (male, female, unknown) as systematic effects, and the litter (736 levels) and additive genetic effect (7 140 levels) as random effects besides the residual effect.

The same models were also used to estimate genetic parameters in the nucleus dataset with the aim of measuring the role of maternal effect, by comparison with results obtained in the evaluation dataset in which we assume a unique mother for all records and then the absence of maternal effect. In this case, the number of levels was of 6 periods of birth, 11 litter sizes, and the same 3 for sex regarding systematic effects, and 88 litters and 2 112 additive genetic effects regarding random effects. In this subset, each individual BrW was also analysed by assuming to belong to the mother of the newborn to check if the birthweight environmental variability could be considered as a maternal trait. In this case, the mother

effect would comprise half the additive effect of the newborn and the whole maternal effect. Then, the variance of this effect would comprise a quarter of the additive genetic variance under model HO plus the maternal additive variance and the covariance between direct and maternal effects. Therefore, it should be possible to assess the existence of maternal determination of the trait by comparing the additive variance under this model with a quarter of the additive variance under model HO. Both the homoscedastic model HOm and heteroscedastic model HEm above were fitted. In this case, the number of individuals in the pedigree was 1 425 and only 756 records were available.

All the priors chosen for the different parameters estimated were plane except for model HEm where the little amount of data and the little size of the pedigree did not make it possible to use it, so a scaled-inversed-chi-squared distribution with 4 degrees of freedom and a scale parameter of 0.036 was chosen as a prior for the variances affecting the mean, while the same kind of distribution with 4 degrees of freedom and a scale parameter of 0.6 was chosen for variances affecting the variability of the trait. The prior for the correlation was plane between −1 and 1. All estimations were carried out checking different priors to ensure that estimations were independent of them.

To inspect the goodness of the models, the phenotypes of the last generation were excluded from the analysis and were used for validation (Efron & Tibshirani 1993).

When the trait is measured at an inadequate scale, the estimation of genetic parameters is erroneous (Yang *et al.* 2011). A study of the skewness of the residuals of the model was necessary to establish whether this artefact of the model was not influencing our results. To check if the model fitted the observed data $\mathbf{y}$, a comparison between the observed data and simulated values $\mathbf{y}_{rep}$ obtained from the marginal posterior predictive distribution of replicate data was carried out (*Gelman et al.* 2004). A discrepancy measure T($\mathbf{y}$, θ) was considered to determine systematic differences between the observed and the simulated data and, therefore, a possible failing of the model. T corresponds to the skewness coefficient of the distribution of the standardized estimate of the residuals and depends on the data and maybe on θ, an unknown vector of parameters of the model under evaluation (Yang *et al.* 2011). Given $\boldsymbol{\theta}^{(j)}$ for the jth iteration, the residual standardized z_i for each animal i was estimated and the skewness $T(\mathbf{z}, \theta^{(j)})$ of the distribution of these estimates was calculated as:

$$T\left(\mathbf{z}, \boldsymbol{\theta}^{(j)}\right) = \frac{\frac{1}{n}\sum_{i=1}^{n}(z_i)^3}{\left(\frac{1}{n}\sum_{i=1}^{n}(z_i)^2 - \left(\frac{1}{n}\sum_{i=1}^{n} z_i\right)^2\right)^{3/2}}$$

Besides each iteration, the $\mathbf{y}_{rep}$ data for each animal were simulated and its standardised residual $z_{rep,i}$ was calculated; therefore, it was directly simulated from a standard normal distribution. The skewness T ($\mathbf{z}_{rep}$, $\theta^{(j)}$) of the distribution of these estimates was also calculated. The distribution of the difference T ($\mathbf{z}$, θ) − T ($\mathbf{z}_{rep}$, θ) should be centred on zero to determine that the model fits the data correctly.

Results

The estimates of the genetic parameters under the three models in the evaluation dataset are given in Table 1. The mean of the marginal posterior distribution for the heritability (h^2) of BrW using model HO was found to be 0.27, with the litter component (c^2) more important, 0.43. Regarding σ^2_a, the HE models gave similar estimates (11.00×10^{-3} for HE and 10.96 for HEρ0) to model HO (12.30×10^{-3}). The estimates for σ^2_c were also very similar across all the three models, between 19.80×10^{-3} for model HE and 17.73×10^{-3} for model HEρ0. All the models provided larger estimates for σ^2_c than for σ^2_a with a maximum for σ^2_c, almost twice σ^2_a for model HE.

The parameters affecting environmental variance of the trait were estimated only by the HE models. In the evaluation data set (Table 1), the estimate for $\sigma^2_{a^*}$ was 1.13 for model HE and slightly increased for model HEρ0 reaching 1.29. Regarding the estimate for $\sigma^2_{c^*}$ it was higher for model HEρ0 (0.52) than for model HE (0.45), even though they were similar too. In both models, the estimate for $\sigma^2_{a^*}$ was abnormally large and higher than for $\sigma^2_{c^*}$ (2.5 times higher for both model HE and model HEρ0).

The mean of the marginal posterior distribution for the genetic correlation between the trait and its variability for model HE was extremely high and negative, −0.97. This value was unexpected given that the correlation between the variance of the birthweight and the litter weight was 0.00 in the evaluation dataset and 0.20 in the nucleus dataset. A check was carried out to inspect the relationship between birthweight variability and litter size. Variance of BrW within litter averaged, 0.0224, 0.0210 and 0.0189, for respectively litter sizes between 2 and 4, between 5 and 9, and between 10 and 14. Although higher litter sizes seem to have a lower variance, differences between litter size groups were very low.

Table 1 Mean and SD (in brackets) of the marginal posterior distribution for the BrW genetic parameters estimated with only the records belonging to matings in the evaluation periods under the homoscedastic (HO), heteroscedastic (HE) and heteroscedastic assuming the genetic correlation null between genetic effect (HEρ0) models

Model	$\sigma^2_a(\times 10^3)$	$\sigma^2_c(\times 10^3)$	$\sigma^2_{a^*}$	$\sigma^2_{c^*}$	ρ_{a,a^*}	h^2	c^2
HO	12.30 (5.161)	18.97 (1.447)	–	–	–	0.27 (0.101)	0.43 (0.036)
HE	11.00 (0.323)	19.80 (1.108)	1.13 (0.096)	0.45 (0.067)	−0.97 (0.019)	–	–
HEρ0	10.96 (1.238)	17.73 (1.263)	1.29) (0.256)	0.52 (0.116)	0	–	–

σ^2_a and $\sigma^2_{a^*}$ are the additive genetic variance affecting, respectively, the BrW mean and its variation; σ^2_c and $\sigma^2_{c^*}$ are the litter effect variances affecting, respectively, the BrW mean and its variation; ρ_{a,a^*} is the coefficient of genetic correlation; h^2 is the estimate for the heritability of the trait mean; c^2 is the estimate for the ratio of the permanent environmental variance to phenotypic variance.

To assess the influence of the maternal effect, the same parameters estimated in the evaluation dataset were also estimated in the nucleus dataset and are shown in Table 2. Given that the total number of records for this subset of the data base may not be enough to estimate correctly all the parameters, these results have to be considered with caution. Discrepancies between REML and Bayesian estimates ranged from 0% (c^2 for model HO in the nucleus dataset) to 60% (h^2 for model HOm in the nucleus data set), and were realistic on the light of the approximated standard error (REML) and standard deviations of posterior distributions (Bayesian). Obviously, discrepancies were higher when working in the nucleus data set and particularly under the maternal model. As a consequence, only general and rough conclusions will be drawn from the analyses concerning the nucleus dataset. In this case, the estimates of the variances and genetic correlation did not converge for model HEρ0, and therefore, these results are not presented in Table 2. Regarding the additive genetic variance of the mean of the trait for model HE, its estimate of 18.87×10^{-3} was around twice the estimates for the model HO, 10.18×10^{-3}, and for the models when the evaluation dataset was analysed. This model HE was the only model where σ^2_a had a higher estimate than the one for σ^2_c. The estimate of the litter variance for model HE was 14.92×10^{-3}, nearly the same than for model HO, 17.04×10^{-3}. The estimate of the additive genetic variance for the variability $\sigma^2_{a^*}$ reached its maximum value for model HE (1.53) together with the highest estimate of the litter variance for the variability $\sigma^2_{c^*}$ (1.27) that was around 2.5 times higher than for models with the evaluation dataset. As before, the value for $\sigma^2_{a^*}$ is higher than the one for $\sigma^2_{c^*}$. The estimate of the genetic correlation was −0.72 therefore negative but less extreme than before. Note, however, that the limited size of the dataset advises for only focusing on the trends in the change in the parameters, and in this sense, no important differences were seen between datasets.

When the trait was considered as maternal, the estimate for the variances affecting the mean for models HOm and HEm were roughly the same and their values were around 12.5×10^{-3}. According to the above criteria of focusing only on striking changes, it must be pointed out that the estimates for $\sigma^2_{a^*}$ and for $\sigma^2_{c^*}$ were very similar and had a more reasonable magnitude (0.25 for $\sigma^2_{a^*}$ and 0.24 for $\sigma^2_{c^*}$) than for the rest of HE models, and the genetic correlation when the trait is assigned to the mother becomes positive and with a non extreme value of 0.48.

Heritabilities and the litter component were in this case lower (0.23 and 0.38 under model HO and 0.25

Table 2 Mean and SD (in brackets) of the marginal posterior distribution for the BrW genetic parameters estimated with only the records belonging to matings in the nucleus under the homoscedastic (HO) and heteroscedastic (HE) models. Each individual BrW was also analysed by assuming that it belonged to the mother of the newborn (HOm and HEm models)

Model	$\sigma^2_a(\times 10^3)$	$\sigma^2_c(\times 10^3)$	$\sigma^2_{a^*}$	$\sigma^2_{c^*}$	ρ_{a,a^*}	h^2	c^2
HO	10.18 (5.934)	17.04 (4.527)	–	–	–	0.23 (0.124)	0.38 (0.074)
HE	18.87 (0.453)	14.92 (2.392)	1.53 (0.480)	1.27 (0.426)	−0.72 (0.111)	–	–
HOm	12.35 (8.518)	12.35 (6.139)	–	–	–	0.25 (0.153)	0.27 (0.130)
HEm	13.73 (4.364)	12.46 (3.400)	0.25 (0.091)	0.24 (0.076)	0.48 (0.359)	–	–

σ^2_a and $\sigma^2_{a^*}$ are the additive genetic variance affecting, respectively, the BrW mean and its variation; σ^2_c and $\sigma^2_{c^*}$ are the litter effect variances affecting, respectively, the BrW mean and its variation; ρ_{a,a^*} is the coefficient of genetic correlation; h^2 is the estimate for the heritability of the trait mean; c^2 is the estimate for the ratio of the permanent environmental variance to phenotypic variance.

and 0.27 under model HOm). However, they are not comparable given that in maternal models, it is assumed that the additive effect under model HOm accounts for mother genes, which affect the BrW of its progeny and half the genes of the progeny which control its own birthweight.

Moreover, for most of the parameters, the standard deviations of the marginal posterior distributions were large as a consequence of a small data set, and no fair conclusions can be derived from the comparisons between homoscedastic models.

The reasons to estimate genetic parameters under a forced null genetic correlation between the trait and its environmental variability are as follows: Figure 2 shows the GBV of all the animals for the trait mean versus the GBV concerning the environmental variance estimated with model HE and model HEρ0 using a dataset obtained as the mix of the two subpopulations seeking to maximize the available information. The shape of these graphs reflects the value of genetic correlation ρ for the mixed dataset, -0.95, hence the graph corresponding to model HE described a line of dots with a negative slope and the one corresponding to model HEρ0 described a spot cloud. The six animals of the last generation with the highest and lowest GBV for the environmental variance estimated with model HE were highlighted on both graphs. On the graph corresponding to model HE, these animals were clearly separated between lines and had extreme GBVs for both trait mean and its genetic environmental variance, although their values were not the most extreme regarding the whole population. When these animals were highlighted on the model HEρ0 graph their GBVs for the environmental variance were not extreme anymore, but it can be appreciated that animals with extreme positive (negative) additive GBV for environmental variance still had quite high (low) GBV for the trait mean. Under this model HEρ0, coefficients of variation of litters belonging to the selected males were 0.11 and 0.08 respectively for high and low variability lines, showing that the model provides reasonable solutions.

After solving model HO using the evaluation dataset, the residuals were estimated and their distribution was drawn (Figure 3), showing a roughly Gaussian pattern suggesting absence of skewness. Figure 4 shows the histogram of posterior realization of the discrepancy measure designed to test the residual skewness of the data. The mean and median of the marginal posterior distribution of the residual skewness using the evaluation dataset were roughly zero, which indicates that the conditional distribution of the data was symmetric.

Discussion

In this article, we present genetic parameters for BrW and its environmental variance in mice. The analyses

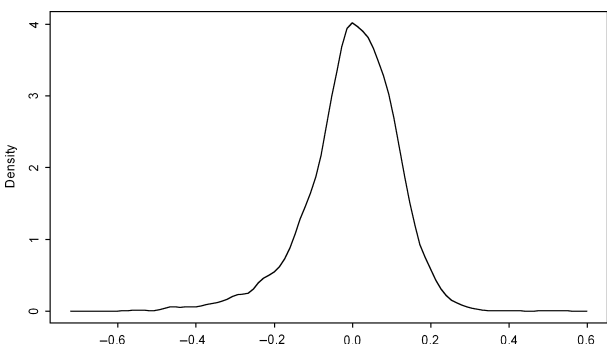

Figure 3 Distribution of estimated residuals based on the homoscedastic model.

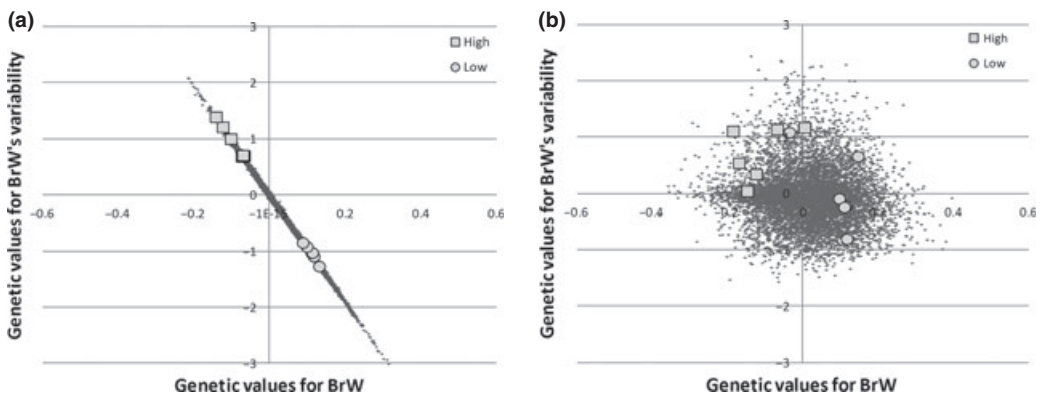

Figure 2 Genetic values for BrW and its variability using heterogeneous variance model considering null (right 1b) or not (left 1a) the genetic correlation. Square (round) spots correspond to the genetic value of the selected animals for the last generation for highest (lowest) variability if the genetic correlation is not considered null.

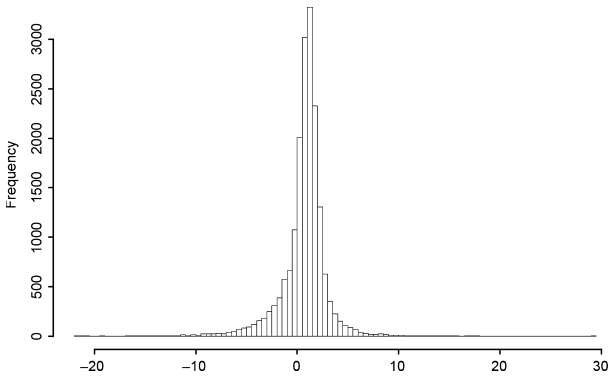

Figure 4 Histogram of the posterior predictive realization of T ($\mathbf{z}$, θ) $-$ T ($\mathbf{z}_{rep}$, θ) designed to test residual skewness of the data.

were carried out in an experimental population especially designed for divergent selection for the environmental variance of this trait. To carry out the artificial selection process, the trait was assigned to the individual owner of the performance and, aiming at as larger data set as possible, model HE by SanCristobal-Gaudy *et al.* (1998) was fitted to the whole dataset to carry out the prediction of the GBVs, and to perform with them the genetic evaluation.

Evaluation and nucleus populations should be considered as different populations, first because of the genetic background and second because of the different pedigree structure. Data from the evaluation population belong to progeny of inbred females considered as a unique mother, while data from the nucleus have a classical pedigree structure. Given the little number of data in the nucleus, the only sensitive models are the ones analysing the evaluation population data. Therefore, although genetic evaluation was made in the whole mixed dataset, as it belongs to two different populations, the genetic parameters have been obtained using two different datasets, the evaluation dataset and the nucleus dataset. Also, alternative procedures have been essayed in the search for explanations to some results.

Regarding the magnitude of the genetic parameters, under model HO for the evaluation dataset, the heritability for the BrW mean (0.27) was found to be higher than that reported previously (0.03) by Gutierrez *et al.* (2006) for the mean individual birthweight of the litter, which is related but obviously different as it was assigned to the mother unlike here. Furthermore, it is a lower variable trait given that the analyses were performed on the mean and not on the individual birthweight. Overall, genetic direct effects estimated in a model free of all maternal effects are not null, are heritable and could be selected. Even when the additive genetic variance of 12.03×10^{-3} for the BrW seems to be low, the corresponding heritability makes a genetic response possible if a heritability of this magnitude was selected (Moreno *et al.* 2012). Under model HE, the genetic parameters were found to be generally different also from those reported previously (Gutierrez *et al.* 2006) for the mean individual birthweight. These estimates are in fact incomparable, given that working on mean birthweight leads to study the environmental variability between litters, whereas when this model is applied for individual birthweight, what the analysis is studying is the within litter variability. Thus, the additive genetic variances for the mean (11.00×10^{-3}) was lower, and for its variability (1.13) was higher, than respectively 19×10^{-3} and 0.90, values previously reported by Gutierrez *et al.* (2006) for the less variable mean individual birthweight. The litter effect variances for the mean (19.80×10^{-3}) and for its variability (0.45) were also higher than 13.60×10^{-3} and 0.23 as previously reported by Gutierrez *et al.* (2006).

Gutierrez *et al.* (2006) reported that the estimated additive genetic variance was maintained and additional random environmental variance decreased when model HE was fitted and compared with the model HO, whereas Ibáñez-Escriche *et al.* (2008a), working on weight gain in mice, noted that model HE showed an important increase in the additive genetic variance when compared with model HO, accompanied by a much less important decrease in the variance of the additional random component that is exactly what is observed in the present study in the nucleus dataset. In this study, the estimation of the additive genetic variance was maintained around a value of 11×10^{-3} for model HO and HE as for Gutierrez *et al.* (2006) where it was maintained at 0.02. Regarding the litter effect variance, its estimate was also maintained when model HE was considered which, is not the case for Gutierrez *et al.* (2006), where it decreased considerably (from 0.06 to 0.01) nor for Ibáñez-Escriche *et al.* (2008a), where it decreased slightly. Even so, in all cases, model HE changed the ratio between the additive genetic variance and the litter effect variance in the direction of favouring genetic selection for BrW mean.

Regarding the estimated genetic correlation between the trait and its environmental variability, the obtained extreme value forced us to rethink the way the selection was going to be carried out. When the genetic correlation was extreme in the current genetic selection experiment for environmental variability (as happened here in both dataset), the selection would be on the GBV for the trait mean because

it was estimated better than the one for its variability. This was the reason why we decided to fit the model that sets the genetic correlation to null value (model HEρ0). In Figure 2a, it can be appreciated what would happen if the model HE would have been chosen for selection. If the animals were selected using the model HE, what would be selected is certainly their GBVs for the mean trait, but maybe not the one for their environmental variability. This fact is reflected in Figure 2b, where it can be seen that the animals selected based on their extreme environmental variability GBV (using model HE), once the correlation is set to a null value, they only maintained their extreme GBV for the mean, but not the one for the variability that are spread out in all the range of values. So, given that the high value of the correlation for model HE (Table 1) would turn the experiment meaningless, the model HEρ0 considering the genetic correlation null was carried out. Surprisingly, the other genetic parameters obtained using model HEρ0 were not really affected by the extreme change in the genetic correlation as seen when compared with the ones obtained using model HE (Table 1). Moreover, cross-validation test provided very similar correlations between the real and the predicted data of the next generation, with model HO being the best (0.44), model HEρ0 the worst (0.38) and model HE intermediate (0.40). Therefore, the genetic selection in this experiment was based on the GBVs for environmental variability obtained using the model considering the genetic correlation null.

Zhang *et al.* (2005) reported the existence of the whole range of genetic correlations between traits and their variability. Sorensen & Waagepetersen (2003), working on litter size in pigs, found a strong negative genetic correlation of −0.62; Ros *et al.* (2004), working on adult weight of snails, reported a value of about 0.80 for the same parameter postulating that skewness of residual distribution provided information about the genetic correlation between the traits and their variability, which was reaffirmed by Gutierrez *et al.* (2006). However, Rowe *et al.* (2005), analysing 35-day body weight of broiler chickens, calculated the value of a similar parameter to be about −0.10, similar to that estimated by Ibáñez-Escriche *et al.* (2008b) for weight at slaughter in pigs. Gavrilets & Hastings (1994) and Hill (2002) postulated that high genetic correlations between traits and their variability suppose that, due to pleiotropic effects, most alleles of genes controlling the mean can also act on the variance. Gutierrez *et al.* (2006) found an extreme positive genetic correlation when the trait was the mean individual birthweight which, in that case, was a trait related to the litter and consequently assigned to the mother owner of the litter. In that case, as commented before, the model HE is considering the variability between litters instead of within litters. The extreme value found here for the genetic correlation is, in any case, rare and it is widely the most extreme one published in the literature for this kind of trait (Hill & Mulder 2010). It seems that it would only appear when the genes affecting the phenotypes are strongly linked or are the same if no artefact is affecting the estimation. In this case, the correlation means that high GBVs for BrW mean would be associated with low GBVs for their environmental variability and *vice versa*. But this explanation did not seem to be consistent and perhaps this extreme value is simply the consequence of an artefact in the model.

Yang *et al.* (2011) described in litter size data for pigs and rabbits that an inadequate scale of the trait would lead to spurious estimations of genetic parameters and among them the genetic correlation between the additive genetic values for the traits and its environmental variability. Therefore, under negative skewness, those individuals away from the mean value will be in the left-hand side of the distribution and the model would provide a negative genetic correlation, such as those distributions drawn by Gutierrez *et al.* (2006) for litter size and litter weight in mice, but they would only be a consequence of the scale the trait was measured. Likewise, under positive skewness, such as for mean individual birthweight in mice (Gutierrez *et al.* 2006) or that for fibre diameter in alpaca (Gutiérrez *et al.* 2011), the distribution would provide a false-positive genetic correlation. This artefact of the model would be reflected when the distribution of residual skewness is not centred on zero. In this case, the distribution of residuals seemed to be rather symmetric (Figure 3), and the mean and the median of the residual skewness distribution drawn at Figure 4 were roughly zero, which indicates that there was normality at the level of the conditional distribution of the data. Then the genetic correlation value for model HE (−0.97, Table 1) cannot be explained by an inadequate scale of the trait and no Box-Cox transformation (Box & Cox 1964) is needed to induce normality and linearity in the conditional distribution of data. In fact, the best Box-Cox transformation of the trait in terms of null skewness was that providing the most extreme genetic correlation. The fact that this value cannot be explained by this hypothesis does not mean that this value has a biological explanation.

Another surprising result is the enormous magnitude of the additive genetic component regarding

environmental variability under models HE and HEρ0. Genetic coefficient of variation of the environmental variability can be approximated by the squared root of $\sigma^2_{a^*}$ (Hill & Mulder 2010). This parameter was 1.13 under model HE and 1.29 under model HEρ0, which were far above the maximum value reported in the review by Hill & Mulder (2010). Genetic variance components both for the trait and for the environmental variability could have been overestimated as a consequence of the extreme genetic correlation found between them. However, and surprisingly, these components were roughly the same when the genetic correlation was forced to meet a null value.

Another issue that required clarification was the possible influence of animals from inbred lines that appear in the data set as being a unique individual. This is the first time that these animals are present in this kind of selection experiments and this could be interfering in the model providing an irregular structure of data, or perhaps the assumption of there always being the same animal is far from reality. To compare with a dataset under maternal influence, analyses were carried out in the nucleus dataset. Unfortunately, this subset accounted for a scarce number of records, and results have to be interpreted with extreme caution in the light of the discrepancies found when parameters were compared with REML estimates. For example, no results are shown regarding the model HEρ0, given that it did not converge, and also standard deviation for all the parameter increased. In this scenario, some conclusions are far from being considered definitive, but some interpretations can be made. For example, the estimate of σ^2_a considerably increased for model HE (18.87×10^{-3}) probably because of a more important genetic variation in the registered progenies although no increase in this sense was observed for model HO. Another interesting point is that estimates of σ^2_c were not so robust and they decreased when moving from model HO (17.04×10^{-3}) to model HE (14.92×10^{-3}) together with the simultaneous, but stronger increase in the estimates of σ^2_a, in the sense of favouring artificial selection processes complementing previous studies (Gutierrez *et al.* 2006; Ibáñez-Escriche *et al.* 2008a). In this case, $\sigma^2_{c^*}$ seems to be overestimated with a value of 1.27. Again estimates of $\sigma^2_{a^*}$ (a high value of 1.53) and ρ (an extreme negative value of -0.72) under model HE were difficult to interpret and were in the direction of the same parameters obtained with the evaluation data set. Therefore, and this is one of the conclusions provided by analyses in the nucleus dataset, it does not seem that inbred animals were responsible for the most anomalous results. However, until this issue is solved, it would be better not to include inbred lines in such an experiment because the use of inbred females is not suitable for analysis of maternal trait. Even if the use of an inbred line to remove some parasite effects in the genetic evaluation can procure very interesting results and is a good way to estimate direct genetic effects free of maternal effects, it should only be done after ensuring that the trait is correctly chosen and assigned.

In order to find an explanation for these results, the trait was considered as a maternal trait even when the mice experiment was designed to evaluate males. As previously considered, environmental variability of the birthweight trait should be related, for instance, to the foetus ability to overcome the different uterine stiffness achieving the body size determined by its genotype. As maternal trait the environmental variability of birthweight should be regarding uterine conformation aspects leading to similar or different rooms at different points of the uterus. If the trait was uniquely determined by the additive effect of the newborn, then the estimate of σ^2_a under model HOm would be a quarter the estimate of σ^2_a under model HO. But if it was also determined by the maternal effect, then the estimate of σ^2_a under model HOm would comprise a quarter the estimate of σ^2_a under model HO, plus the variance of the genetic maternal effect, plus the covariance between both genetic effects. As this estimate under model HOm was largely higher than a quarter the estimate of σ^2_a, the trait seems to be at least partially dependent on the mother. Therefore, as BrW is not only under genetic control of the dam, but also under control of direct effects, it can be expected that BrW environmental variability would also be similarly controlled by direct and maternal effects and not only maternal.

There is more evidence of the advantage of maternal selection when considered the environmental variability of the trait. Big changes arose when comparing model HEm with the previously mentioned results under model HE, by only keeping the estimate for the σ^2_c component, but resulting in a much more reasonable estimate of $\sigma^2_{a^*}$ (0.25) if it is assumed that it approximates the square of the genetic coefficient of variation of the environmental variability (Hill & Mulder 2010). Moreover, the extreme negative genetic correlation between the trait and its variability did not appear in this case, but a positive high with no extreme genetic correlation between them of 0.48, accompanied by an estimate of σ^2_a of 13.73×10^{-3} under model HEm, less important than that of 18.87×10^{-3} under model HE. As noted before,

Gutierrez *et al.* (2006) already obtained a positive genetic correlation of 0.97 when the trait was the mean individual birthweight, which was assigned to the mother as here under model HEm. The estimate of $\sigma^2_{c^*}$ was reduced from 1.27 under model HE to 0.24 under model HEm, which is more believable according to the corresponding estimates of the other component $\sigma^2_{a^*}$. On the other hand, a null genetic additive variance would have been obtained in the evaluation dataset if the trait was entirely maternal, but this was not the case. The trait seems to be partially under individual genetic control and further but not totally under maternal control. Unfortunately, we do not have software that can solve the model by SanCristobal-Gaudy *et al.* (1998) with a genetic maternal effect affecting the mean and the variability of the trait. Thus, alternatively, the genetic background of the BrW and its variability seem to be partially and preferably maternal, and the artificial selection could be carried out by assigning the BrW value to the mother considering the trait as entirely maternal and having several records for each female. That was successfully done for rabbit birthweight by Garreau *et al.* (2008) where young rabbit weights were considered as a repeated trait of the female. This analysis has provided some clear conclusions but they have to be concretized. Unfortunately, the design of the experiment was thought to select individuals instead of mothers, leading to having only one mother in the whole evaluation dataset, from the inbred line, with thousands of offspring. The nucleus dataset only provided rough information as it contains only 84 mothers having basically one litter each. Note also that changes in σ^2_a were not between models HO and HOm, but between models HE and HEm, suggesting that if BrW could be assigned either to the individual or its mother, the environmental variability of BrW would have to be assigned definitively to the mother. Validation provided very low correlations in the nucleus dataset, giving the best fitness to the model HEm, but with a correlation of 0.14 between predicted and real values, which should be considered meaningless. However, HEm provided reasonable estimates for all the components showing that a design such as that by Garreau *et al.* (2008) in rabbits assuming the trait as maternal should be better if artificial selection for environmental variability is of interest.

Finally, assigning BrW to the mother solves how to proceed when the goal is the selection for modifying its environmental variability. However the reasons for the anomalous results when considering the trait as belonging to the individual is still not clarified. For example, the model by SanCristobal-Gaudy *et al.* (1998) could be questioned and maybe others could help shed light on this, such as the additive model (Hill & Zhang 2004), the standard deviation model (García *et al.* 2009) or the reaction norm model (Gavrilets & Hastings 1994). Further research seems to be needed.

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