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Genetic architecture of inbreeding load for survival and body weight traits in divergently selected mouse lines

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Introduction

Inbreeding (F) is defined as the probability that two alleles at a locus are identical by descent (IBD) (Malecot, 1948) and is associated with changes in the mean and variance of quantitative traits (Falconer and Mackay 1996). Increased inbreeding raises the likelihood of negative effects in populations, commonly expressed as reductions in the mean of fitness-related traits, known as inbreeding depression. Inbreeding load refers to the proportion of mutation load attributable to recessive alleles hidden in heterozygous form and exposed through inbreeding, causing inbreeding depression (Leroy, 2014). Its magnitude may vary among individuals depending on their genotype, leading to negative, neutral or even positive effects (Perdomo-González *et al.*, 2021). Such variation has been reported in Drosophila lineages (Latter *et al.*, 1995), dairy (Miglior *et al.*, 2014) and beef cattle (Carolino and Gama, 2008). This supports the concept of an individual hidden inbreeding depression load (IL) (Casellas, 2018; Varona *et al.*, 2019), which can be considered a heritable trait expressed through the performance of inbred offspring (Fowler and Whitlock, 1999; Moorad and Wade, 2005). Consequently, individual IL can be predicted as an additive genetic value using linear models, accounting for founders and Mendelian sampling effects. Interest in environmental variability has increased due to its relevance for phenotypic stability and robustness. A long-term divergent selection experiment in mice for environmental variability of birth weight demonstrated genetic control of this trait and resulted in two lines: low (L-Line) and high variability (H-Line) (Formoso-Rafferty *et al.*, 2016a). The L-Line consistently showed superior performance in several fitness-related traits, including litter size, embryo survival, fertility, resilience to feed restriction and reproductive longevity (Formoso-Rafferty *et al.*, 2016b, 2018, 2019, 2022), as well as greater robustness to climatic fluctuations during the prepartum period (El-Ouazizi El-Kahia *et al.*, 2023). Although no differences were found in overall genomic or pedigree inbreeding (Ojeda-Marín *et al.*, 2023a, 2023b), line differences were detected in chromosomal inbreeding distribution and IBD origin (Ojeda-Marín *et al.*, 2023b; 2025b), as well as in the magnitude of inbreeding depression for litter size, which was greater in the H-Line (Ojeda-Marín *et al.*, 2025a). Differences in inbreeding depression have also been observed for other robustness-related traits. The objective of this study was to perform a genetic analysis of the inbreeding depression load for several robustness-related traits, considering partial inbreeding coefficients of ancestors in divergent lines, to assess whether the observed differences were associated with divergent selection, and to provide insight into the genetic consequences of selection for environmental variability and its implications for population management.

Materials & Methods

The pedigree dataset comprised 41,375 animals (20,886 males, 20,279 females and 210 of unknown sex). Records of survival (SUR), birth weight (BW) and weaning weight (WW) were available for animals with both parents known, totalling 40,642 records for

BW and SUR and 37,476 for WW. Data were collected for 37 generations in a divergent selection experiment for environmental variability of birth weight in mice (Formoso-Rafferty *et al.*, 2016b, 2018, 2019, 2022). The base population originated from crosses among BALB/c, C57BL and CBA inbred lines and was maintained under panmixia for more than 40 generations to preserve genetic variability. Divergent lines for high and low birth weight variability were established by selecting males and females from the best-ranked dams for each opposed criterion, with an approximate selection pressure of 25%. One-to-one matings avoiding shared grandparents were used to control relatedness. Selection efficiency was further improved using an optimization procedure to maximize mean breeding value while constraining coancestry. The experiment was initiated with 64 males and 64 females and continued with 43 males and 43 females per generation and line.

Genetic parameters for each trait (**y**) were estimated using a Bayesian linear model including standard breeding values and individual inbreeding depression load (IL) attributable to ancestors as random effects. The model was $\mathbf{y} = \mathbf{fc} + \mathbf{Xb} + \mathbf{Wl} + \mathbf{Rm} + \mathbf{Zu} + \mathbf{Ki} + \mathbf{e}$, where **f** is the total inbreeding coefficient, **c** the overall inbreeding depression effect, **b** the vector of fixed effects, **u** the infinitesimal additive genetic effect, **i** the individual IL, and **e** the residual. Litter (**l**) and maternal (**m**) permanent environment effects were also included. The prior distributions assumed for the additive genetic (u) and inbreeding depression load (i) effects followed a joint multivariate normal distribution with a zero mean vector. The variance–covariance structure was defined by G $\otimes$ A, where is $\mathbf{G} = \begin{pmatrix} \sigma_u{}^2 & \sigma_{ui} \\ \sigma_{ui} & \sigma_i{}^2 \end{pmatrix}$ and **A** is the numerator relationship matrix. The parameters σ^2_u and σ^2_i corresponded to the variance components associated with the additive genetic and inbreeding depression load effects, respectively. Litter, maternal and residual effects were assumed to follow normal distributions with variances σ_l^2, σ_m^2 and σ_e^2. **X**, **W**, **R** and **Z** are incidence matrices, and $\mathbf{K} = \mathbf{T}(\mathbf{I} - \mathbf{P})$, where **T** is a lower triangular matrix of partial inbreeding coefficients (F_{ij}) linking phenotypes to inbreeding ancestors. For computational reasons, F_{ij} values were multiplied by 10, corresponding to a 10% inbreeding coefficient. **P** is a projection matrix linking individuals to their sire and dam. Fixed effects included sex (except for SUR), generation, litter and litter size. Resolution methods were implemented in FORTRAN90. Convergence was assessed by trace and running mean plots after burn-in. A single chain of 100,000 iterations, with a burn-in of 20,000, was used for each trait. Inbreeding depression load ratios were subsequently calculated as the proportion of phenotypic variance attributable to variation in inbreeding depression effects, assuming a theoretical population with a single ancestral source of partial inbreeding.

Results & Discussion

99.03% of the animals included in the pedigree were inbred. Of these, 11.74% had an F lower than 0.05, and 76.97% had an F value higher than 0.1. The average F value in the whole population is 0.235. The average F values for those animals belonging to the most recent generation were 0.47 and 0.46 for the H-Line and the L-Line, respectively. The Mendelian decomposition of inbreeding generated a total of 20,172,730 F_{ij} coefficients belonging to 1,777 ancestors (873 males and 904 females), 686 from H-Line, 716 from L-Line and 329 from the base population. Figure 1 shows the number of times an animal appeared as an ancestor generating inbreeding ($F_{ij}>0$). H-line ancestors transmitted F_{ij} to 9–11,834 descendants, while L-line ancestors transmitted F_{ij} to 36–21,713 descendants. The mean F_{ij} (SD) was $2.36\text{x}10^{-5}$ ($4.393\text{x}10^{-4}$) while the maximum value was 0.0625 and 87.39% of the F_{ij} were lower than 0.001, indicating marginal contributions from most ancestors. This pattern contrasts with the findings of Antonios *et al.* (2025) in three dairy sheep breeds, where most ancestors contributed to inbreeding only sporadically, and only

a few ancestors were responsible for transmitting inbreeding to many descendants. This may also explain why they reported higher mean F_{ij} values than those found in this study.

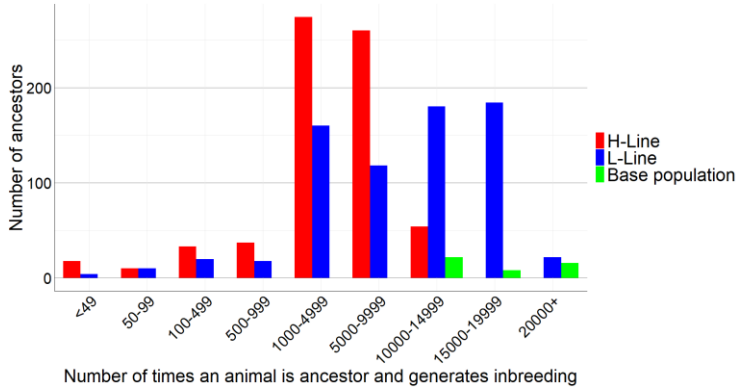

Figure 1. Distribution of the number of times that an animal is ancestor and generates inbreeding depending on the mice line.

Genetic parameters are presented in Table 1. Estimates for SUR were highly imprecise due to the complexity of the model and the nature of the trait; therefore, these results are not shown. Conversely, the obtained estimates of σ_i^2 indicate variability of inbreeding load for WW and BW among ancestors in these populations and a greater variability for WW than for BW. The inbreeding load genetic variance was larger than the additive genetic variance, which is understood as the effect expressed on the phenotype of an individual descendant with 10% of inbreeding coming from that specific ancestor. Rescaling σ_i^2 for a 1% of inbreeding, variance correspond to 0.05% and 0.07% of the phenotypic variance for BW and WW, respectively.

Table 1. Parameter estimates (SE) for birth weight (BW) and weaning weight (WW) in a divergent selection experiment for environmental variability of birth weight in mice.

Trait	σ_u^2	σ_i^2	$\sigma_{u,i}$	$r_{(u,i)}$	σ_l^2	σ_m^2	σ_e^2	ƀ
BW	0.01	0.26	-0.05	-0.13	0.012	0.01	0.02	0.15
	(0.001)	(0.077)	(0.005)	(0.132)	(0.001)	(0.001)	(0.001)	(0.016)
WW	1.09	27.28	-1.47	-0.29	1.53	0.39	0.73	1.05
	(0.078)	(11.82)	(0.801)	(0.154)	(0.060)	(0.061)	(0.030)	(0.051)

BW: birth weight, WW: weaning weight, σ_u^2:additive genetic variance, σ_i^2: inbreeding load variance, $\sigma_{u,i}$: covariance between additive genetic and inbreeding load effects, $r_{(u,i)}$: correlation between additive genetic and inbreeding load effects, σ_l^2: litter effect, σ_m^2: maternal effect, σ_e^2: residual variance, ƀ: inbreeding depression expressed by inbred descendants with a 10% of inbreeding.

The genetic correlations between additive genetic and inbreeding load effects ($r_{(u,i)}$) were negative, as expected, with a stronger negative effect observed for WW, although these estimates were associated with a large standard error. Small and negative genetic correlations were reported in milk yield of sheep (Antonios *et al.*, 2025) and fertility traits on Brown Swiss cattle (Martinez-Castillero *et al.,* 2021). Overall effect of inbreeding (β), estimated for a 10% increase in inbreeding, indicates that such an increase would be associated with gains of 1.05 g in WW and 0.15 g in BW. In one line, body weight decreases, whereas in the other it increases. Importantly, the increase in body weight observed in the H-line should not be interpreted as a beneficial outcome. Excessive body weight is associated with greater phenotypic variability and reduced survival. It is likely that, within this line, survival is biased towards heavier individuals, reinforcing this pattern. In contrast, in the L-line, body weight has decreased and appears closer to an optimal level favouring survival, despite similar overall inbreeding levels; this likely reflects divergent selection resulting in different inbreeding load effects on the traits.

Conclusions

The low values of the genetic correlation between inbreeding load and breeding values imply that selection based on predicted IL for WW and BW will not substantially increase inbreeding depression for these traits in inbred animals. Selecting individuals based on predicted IL for WW and BW would help remove deleterious recessive alleles, reducing their frequency in homozygous carriers. However, in a highly selected population such as this, it remains challenging to disentangle the effects of selection from those of inbreeding, highlighting the need for further research.

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