

Differential sensitivity of climate conditions on birth weight genetic values in mice divergently selected for birth weight residual variance

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Abstract

After 32 generations of a divergent selection experiment for residual variance of birth weight in mice, two divergent lines were thus obtained: the heterogeneous line (H-line) and the homogeneous line (L-line). Throughout the generations, differences were observed between the two lines in traits such as litter size, survival at weaning, and birth weight variability caused by unidentified environmental conditions. The L-line exhibited advantages in terms of higher survival rates, larger litter sizes, and less sensitivity to changes in food intake. The study is an examination of the effects of climate as an environmental factor on the performance of these animals. Climate factors including maximum, minimum, and mean temperature (T), humidity (H), and TH index; at three stages (the fecundation, a week before the parturition and the parturition), were linked to a birth weight dataset consisting of 22,614 records distributed as follows: 8,853 corresponding to the H-line, 12,649 to the L-line, and 1,112 to the initial population. Out of the 27 analyzed climatic variables, the maximum temperature 1 wk before parturition (MXTW) was identified as the most influential when comparing heteroscedastic models with the deviance information criterion. The order of Legendre polynomial to apply in the following random regression model was tested by a cross-validation using homoscedastic models. Finally, MXTW was compared on how it affected the two divergent lines by analyzing predicted breeding values (PBV) obtained from a random regression heteroscedastic model. The mean PBV of the H-line in the first generation showed a range of 0.070 g with a negative slope, which was 35 times higher than the range obtained for the L-line, which varied within 0.002 g. In the last generation of selection, the H-line exhibited greater instability of PBV across temperatures, with a difference of 0.101 g between the maximum and minimum mean PBV, compared to 0.017 g for the L-line. The standard deviations of the slopes in the H-line were more dispersed than in the L-line. Unlike the H-line, the L-line had slopes that were not significantly different from 0 throughout the generations of selection, indicating greater stability in response to MXTW variations. The H-line exhibited a higher sensitivity to changes in MXTW, particularly in birth weight, with the L-line being more stable. The selection for uniformity of birth weight could lead to less sensitive animals under environmental changes.

Lay Summary

Two mice lines obtained by divergent selection for birth weight residual variance were used to determine whether environmental factors could differently affect the homogeneous and heterogeneous lines. The maximum temperature 1 wk before parturition (MXTW) had the higher impact on the birth weight of the animals. A random regression model showed the individual trajectory of birth weight throughout the changes in MXTW. It was evident that the homogeneous line is less susceptible to changes in climate. This result, therefore, supports the hypothesis that the selection for homogeneity in production animals is more advantageous. More robust animals are obtained that can better cope with changes in climate without compromising their productive traits.

Key words: climate effects, homogeneity, quantitative genetics, random regression, robustness, welfare

Abbreviations: DIC, deviance information criterion; F, fecundation; H-line, high variability line; HPD, highest posterior density interval; L-line, low variability line; P, parturition; PBV, predicted breeding values; RH, relative humidity; T, temperature; TH, temperature humidity index; W, 1 wk before parturition

Introduction

Robustness is becoming an interesting selection criterion in breeding programs (Herrero-Medrano et al. 2015) especially in the current scenario of extreme weather events becoming more common. This trait is defined as less sensitivity to environmental changes, thus allowing for the same expression of an animal's production potential under wide environmental conditions. The reduction of environmental variability in some productive traits was key at increasing animal robustness and

welfare (Mormede and Terenina, 2012) while decreasing the costs of handling and production, creating a positive relationship between animal welfare and farm benefits.

A successful divergent selection experiment was conducted to modify the residual variance of birth weight in mice (*Mus musculus*), resulting in two genetic divergent lines: a heterogeneous line with higher birth weight residual variance (H-line) and a homogeneous line with lower birth weight residual variance (L-line). In the present experiment, the trait was

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assigned to the mother, thus the selection was being done via the dam (Formoso-Rafferty et al. 2016a).

Subsequent studies on this mice population showed positive correlated responses of homogeneity for some interesting traits such as higher litter size and higher survival (Formoso-Rafferty et al. 2016b) without additional energy costs. These studies also showed higher feed and reproductive efficiency (Formoso-Rafferty et al., 2018, 2019) showing the L-line having a better performance compared with the H-line. Moreover, the L-line females showed a superior reproductive longevity (Formoso-Rafferty et al., 2022) with higher embryo and fetal survival (Formoso-Rafferty et al., 2023) demonstrating a higher robustness of the homogeneous line over the heterogeneous one. Furthermore, rabbits with higher within-litter homogeneity of birth weight exhibited higher productivity associated with larger uterine horns (Bolet et al., 2007). Due to the selection experiment, the L-line showed uniform pup birth weights within litters and across litters (El-Ouazizi El-Kahia et al., 2022; Gutiérrez et al., 2022). All these differences between the two divergent lines could be because of a distinct response to unidentified environmental factors. To further characterize the advantages of homogeneity, an analysis was done of the response of the populations obtained from the selection experiment to identified environmental factors, such as the climatic variables. Since the environment effect exerted on each individual is different, the climatic variable should be considered and studied by fitting a random regression model.

Random regression reaction norm models based on Legendre polynomials are commonly used to analyze the genetic variance and covariance components for a given trait over the whole trajectory of an environmental continuous covariate. This approach is widely used to determine the effect of heat stress in dairy milk production (Brüegemann et al. 2011; Carabaño et al., 2014) and to improve in local sheep breeds thermotolerance (Carabaño et al., 2021).

As mentioned above, the two mice lines obtained from the divergent selection experiment for residual variance of birth weight assume a heterogeneous residual variance associated with environmental factors not yet identified. This leads to an analysis of the genetic breeding value for birth weight across the range of a specific environmental factor such as climatic variables related to temperature and humidity. The aim of the present study was to apply random regression models with a heterogeneous residual variance, but including the climatic variable to prove that homogeneous individuals tended to maintain their birth weight genetic value throughout all the range of a climatic variable.

Materials and Methods

The dataset for this study came from two mice lines, high and low variability lines, resulting from a divergent selection experiment conducted to modify the residual variance of birth weight. Both divergent lines were started from an initial population originating from a balanced genetic contribution of three inbred mice lines (BALB/c, C57BL, and CBA) maintained in panmixia (Formoso-Rafferty et al., 2016a). The animals used were housed at the experimental facility of the Department of Animal Production of the Veterinary Faculty of the Complutense University of Madrid. The housing and management conditions of the animals were in accordance

with the Spanish legislation RD 53/2013 on the basic rules for the protection of animals used in experiments and other scientific purposes (Boletín Oficial del Estado 2013) and approved by the Animal Experimentation Committee (PROEX 224/18). During 32 generations (the experiment is still running at the time of writing), the genetic evaluation of the mice population was performed using a heteroscedastic model developed by SanCristobal-Gaudy et al. (1998), where the environmental variance was assumed to be heterogeneous and partially under genetic control.

Temperature in degree Celsius and humidity as a percent were automatically registered every 4 h using ESCORT Console 2.12.07 from Escort Data Loggers Inc. The temperature-humidity index taken every 4 h (THI) was also calculated according to the equation described by Hahn et al. (2009): $THI = 0.8T + RH(T - 14.4) + 46.4$, where T is the temperature expressed in degree Celsius and RH the relative humidity expressed between 0 and 1. To obtain the variables under study, we calculated the maximum (MX), minimum (MN), and mean (MD) values of T , H , and THI at three different stages: the fecundation (F) at 21 d before the birth date, a week before the parturition (W), and the parturition date (P). A total of 27 climatic variables were analyzed, combining the three values for the three climate parameters and the three different stages when they were measured. A model without any climatic variables was also analyzed.

This study was conducted on the 24th generation of the ongoing selection experiment (using all previous generations since the initial one). However, in the instances where the climatic variables were unavailable for certain generations, the corresponding birth weight records were excluded from this study, thus losing the data from six generations (generations: 9, 14, 15, 18, 21, and 22). The remaining climatic variables at the three different stages of the animal development were associated with the birth weight of 22,614 individuals, 8,853 corresponding to the H-Line, 12,649 to the L-Line, and 1,112 to the initial population having a maximum of two litters each female. After data filtering, the information registered corresponded to 18 generations of selection. The pedigree data set contained 29,737 individuals including 18 generations of selected animals and five generations traced back from the initial population.

First, to analyze the climatic variable with the most impact on birth weight, the heteroscedastic model applied during the selection process as previously defined but sequentially fitting each of the climatic variables defined above. The model equation was:

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

where y_i is the birth weight of the individual i , $*$ indicates the parameters associated with residual variance; $\mathbf{b}$ and $\mathbf{b}^*$ are the vectors of the systematic effects that include generation (18 levels), litter size (from 2 to 17, 16 levels), sex (male, female, or unknown, 3 levels), parity number (2 levels), and the climatic variable (one for the 27 climatic variables of the study as covariate); $\mathbf{m}$ and $\mathbf{m}^*$ are the vectors of the maternal genetic effect; $\mathbf{c}$ and $\mathbf{c}^*$ are the vectors of the litter effect as random effects; $\mathbf{x}_i$, $\mathbf{z}_i$, and $\mathbf{w}_i$ are the corresponding incidence vectors and finally $\varepsilon_i \sim N(0, 1)$. This model accounts for the maternal genetic correlation between the trait and its variability (ρ_{mm^*}).

The genetic effects $\mathbf{m}$ and $\mathbf{m}^*$ are distributed together and are assumed to be Gaussian:

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

where A is the additive genetic relationship matrix; σ_m^2 is the additive genetic variance of the trait; $\sigma_{m^*}^2$ 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 $\mathbf{c}$ and $\mathbf{c}^*$ are also assumed to be independent, with $\mathbf{c} \sim N(0, I_c\sigma_c^2)$ and $\mathbf{c}^* \sim N(0, I_{c^*}\sigma_{c^*}^2)$, where 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 the BW mean and its environmental variability.

At this step no random regression was applied, 27 models were analyzed, one for each climatic variable with its minimum, maximum, and mean value and 3 time points. To compare models and select the specific climatic variable which most influenced the individual birth weight from the 27, the deviance information criterion (DIC) was used; with the lower DIC, being the better model (Spiegelhalter et al., 2002). Since this first analysis was solved with a Bayesian approach, to determine the relevance of the climatic variable, the high posterior density interval (95%) of the marginal posterior distribution was computed to check whether this interval included 0 or not to declare b as relevant or not.

The second step was to determine the order of Legendre polynomial that will be applied later on to the heterogeneous random regression model. At this step, a homoscedastic model was fitted to avoid the over parameterization in the heteroscedastic model. A cross-validation approach was applied to evaluate the prediction ability of the different models (Tibshirani and Efron, 1993; Browne, 2000). The models used in this case were homoscedastic, by applying random regression and the Legendre polynomials order that varied in each case:

$$y_i = \mathbf{x}_i\mathbf{b} + r(\mathbf{v}, \text{leg}^v) + r(\mathbf{m}, \text{leg}^m) + r(\mathbf{c}, \text{leg}^c) + e_i \quad (2)$$

where y_i is the birth weight of the individual i ; $\mathbf{b}$ is the vector of the systematic effects (sex, generation, litter size, and parity number); $\mathbf{x}_i$ is the incidence vector; r is a polynomial of random regression applied over; $\mathbf{v}$ is the vector containing the values of the selected climatic variable and with a Legendre polynomial (leg^v); $\mathbf{m}$ is the vector of the maternal genetic effect with its Legendre polynomial (leg^m); and $\mathbf{c}$ is the vector of the litter effect also with its Legendre polynomial (leg^c). First and second order of Legendre polynomials were assayed for leg^v , leg^m , and leg^c with a total of eight combinations.

For each model, the entire data set was divided into a training data set containing the first 17 generations with 21,612 birth weight records (95.6%) to estimate the parameters and fit each model, and a validation data set which correspond to the last generation with 1,002 birth weight records (4.4%). The last generation was used as the validation set because the focus was on predicting birth weight in the next generation of selection imitating the selection process for select parents based on progeny performance. The predicted birth weights of the validation data set were reconstructed using the solutions for the model effects previously obtained with the training data sets (climate variable, sex, parity number,

litter size, and breeding values for each animal). Climate variable was modelled as covariate random regressed, using the corresponding Legendre polynomial coefficient to reconstruct the data. Regarding generation effect, the average of different levels of generation effect was used since it was not possible to estimate the value for the validation set (the same for each animal then it is not affecting the results). No litter random effect was summed up because the mean of the expected distribution was zero. Finally, the breeding value decomposed by the random regression was included and scaled by the corresponding Legendre polynomial coefficient. To compare real and predicted performances of the validation set, the Pearson's correlations (ρ) between real and predicted data were calculated. The model with the highest correlation value was used to determine the most appropriate order of Legendre polynomial.

Finally, after having compiled the information that was obtained from the previous analyses (i.e., equations (1) and (2)) that defined the most influencing climatic variable (1) and the order of Legendre polynomials which fitted the model better (2), this information was then fitted to a random regression heteroscedastic model, including the same effects previously listed, to study how the climatic variable selected in the previous analysis (1) affected the breeding values of each line of selection:

$$y_i = \mathbf{x}_i\mathbf{b} + r(\mathbf{v}, \text{leg}^v) + r(\mathbf{m}, \text{leg}^m) + r(\mathbf{c}, \text{leg}^c) + e^{1/2(\mathbf{x}_i\mathbf{b}^* + \mathbf{z}_i\mathbf{m}^*)} \varepsilon_i \quad (3)$$

All the parameters were denoted in the previous model equations (1) and (2).

Statistical analyses were later performed to compare the mean regression coefficients of the Legendre polynomial between lines in the former and the latter generations.

The heteroscedastic model to select the most influencing climatic variable in the birth weight was analyzed in a Bayesian scenario using GSEVM software (Ibáñez-Escriche et al. 2010). To determine the order of Legendre polynomial with homoscedastic models, VCE was used (Groeneveld 2010). Finally, analyses with the random regression heteroscedastic model was performed using ASReml software (Gilmour et al., 2015).

Results

There were no differences in the genetic parameters estimated across the 27 models assaying each climatic variable. The model without any climatic variable had a genetic variance for the birth weight of 0.0056 (± 0.0007) and 0.0490 (± 0.0090) for birth weight residual variance. The genetic correlation between the birth weight and its environmental variability was 0.4563 (± 0.1211). Table 1 shows the regression coefficients affecting the birth weight and its environmental variability corresponding to each 27 climatic covariates and the DIC value obtained under each model. The lowest DIC was obtained under the model including the MXTW and its regression coefficient for the mean trait (b) showed that as the MXTW increased by 1 °C, the mean birth weight decreased by 0.0106 g. Regarding relevance, 10 out of the 27 climatic variables that were studied appeared to have the highest posterior density interval (HPDI) of the marginal posterior distribution that does not include 0 on the mean birth weight. Six of them corresponded to climatic variables measured 1 wk

Table 1. Deviance information criterion (DIC) obtained from the 28 models analyzed and its regression coefficients for the mean birth weight and its variability

Model	DIC	DIC-DICmin	b (HPD95)	b*(HPD95)
Non climate variable	-68,658	66.99		
<i>Climate variables obtained the fecundation day</i>				
MNTF	-68,669	55.63	-0.0021 (-0.0055, 0.0016)	-0.0106 (-0.0333, 0.0120)
MNHF	-68,647	77.69	0.0000 (-0.0010, 0.0009)	0.0005 (-0.0046, 0.0036)
MXTF	-68,644	81.49	-0.0013 (-0.0052, 0.0016)	-0.0075 (-0.0308, 0.0167)
MXHF	-68,651	73.69	-0.0001 (-0.0011, 0.0007)	0.0047 (-0.0005, 0.0103)
MDTF	-68,661	64.34	-0.0021 (-0.0057, 0.0017)	0.0062 (-0.0196, 0.0190)
MDHF	-68,643	81.58	0.0000 (-0.0010, 0.0009)	0.0019 (-0.0052, 0.0096)
MDIF	-68,641	83.54	-0.0006 (-0.0010, 0.0009)	-0.0024 (-0.0115, 0.0135)
MNIF	-68,650	75	-0.0025 (-0.0051, 0.0005)	-0.0123 (-0.0205, -0.0045)
MXIF	-68,641	84.29	-0.0022 (-0.0055, 0.0010)	-0.0011 (-0.0099, 0.0068)
<i>Climate variables obtained 1 wk before parturition</i>				
MNTW	-68,656	69.06	-0.0092 (-0.0121, -0.0056)	-0.0053 (-0.0260, 0.0107)
MNHW	-68,651	74.36	-0.0006 (-0.0015, 0.0001)	-0.0043 (-0.0089, 0.0015)
MXTW	-68,725	0	-0.0106 (-0.0145, -0.0069)	-0.0170 (-0.0393, 0.0062)
MXHW	-68,644	80.59	0.0003 (-0.0005, 0.0011)	0.0007 (-0.0035, 0.0046)
MDTW	-68,651	73.85	-0.0110 (-0.0153, -0.0063)	-0.0228 (-0.0518, 0.0038)
MDHW	-68,640	84.71	-0.0002 (-0.0011, 0.0008)	-0.0012 (-0.0080, 0.0058)
MDIW	-68,660	65.1	-0.0084 (-0.0118, -0.0057)	-0.0142 (-0.0325, -0.0019)
MNIW	-68,659	66.2	-0.0069 (-0.0098, -0.0034)	-0.0078 (-0.0142, -0.0021)
MXIW	-68,646	78.92	-0.0076 (-0.0095, -0.0055)	-0.0108 (-0.0205, 0.0031)
<i>Climate variables obtained at the parturition day</i>				
MNTP	-68,651	74.29	-0.0045 (-0.0075, -0.0013)	-0.0031 (-0.0218, 0.0257)
MNHP	-68,640	84.96	0.0007 (-0.0002, 0.0016)	-0.0021 (-0.0086, 0.0073)
MXTP	-68,655	70.44	-0.0022 (-0.0056, 0.0018)	-0.0035 (-0.0307, 0.0211)
MXHP	-68,690	34.99	0.0009 (0.0001, 0.0018)	-0.0028 (-0.0099, 0.0035)
MDTP	-68,640	84.75	-0.0051 (-0.0092, -0.0014)	0.0056 (-0.0339, 0.0332)
MDHP	-68,642	82.95	0.0010 (-0.0001, 0.0020)	-0.0024 (-0.0087, 0.0031)
MDIP	-68,640	85.12	-0.0027 (-0.0050, -0.0010)	-0.0130 (-0.0291, 0.0040)
MNIP	-68,657	67.53	-0.0024 (-0.0056, 0.0002)	-0.0031 (-0.0304, 0.0195)
MXIP	-68,648	76.69	0.0007 (-0.0014, 0.0023)	-0.0041 (-0.0138, 0.0039)

The lowest DIC value is in bold. DIC: deviance information criterion, b (HPD95): regression coefficient for the mean (highest posterior density interval of their marginal posterior distribution), b*(HPD95): regression coefficient for the variance (highest posterior density interval of their marginal posterior distribution), climatic covariate was denoted as MD, MN, or MX meaning, respectively, mean, minimum, or maximum, followed by T for temperature (in °C), H for humidity (in %), or I for THI, and ending by P, W, or F denoting, respectively, the parturition date, 1 wk before or fecundation date.

before parturition, specifically the temperature and the temperature-humidity index: MNTW, MXTW, MDTW, MDIW, MNIW, and MXIW, and four were related to the parturition date: MNTP, MXHP, MDTP, and MDIP.

Nine out of the 10 models that had an impact on the birth weight provided a negative regression coefficient, which suggests that as the climatic variables increase, birth weight decreases. MXTW, as the variable that most affected the pup birth weight, was assayed under the random regression homoscedastic models to find the best Legendre polynomials to fit it. Table 2 shows the Pearson correlations between real and predicted birth weight after performing the cross-validation analyses for the eight models analyzed with the different combinations of the two orders of Legendre polynomials tested. It is remarkable that the correlations obtained were all very similar. The model with the highest correlation (0.7794) was that included an order 2 of the Legendre polynomial in the climatic variable and an order 1 both in the maternal genetic and litter effects.

Table 2. Pearson correlations between the real and the predicted birth weight obtained from the random regression models with different Legendre polynomials

Models	leg ^v	leg ^m	leg ^c	Pearson correlations
L(1,1,1)	1	1	1	0.7772
L(1,1,2)	1	1	2	0.7717
L(1,2,1)	1	2	1	0.7771
L(1,2,2)	1	2	2	0.7745
L(2,1,1)	2	1	1	0.7794
L(2,1,2)	2	1	2	0.7746
L(2,2,1)	2	2	1	0.7793
L(2,2,2)	2	2	2	0.7774

The model with the highest correlation is in bold. leg^v: order of Legendre polynomial applied to the climatic variable, leg^m: order of Legendre polynomial applied to the maternal genetic effect and leg^c: order of Legendre polynomial applied to the litter effect.

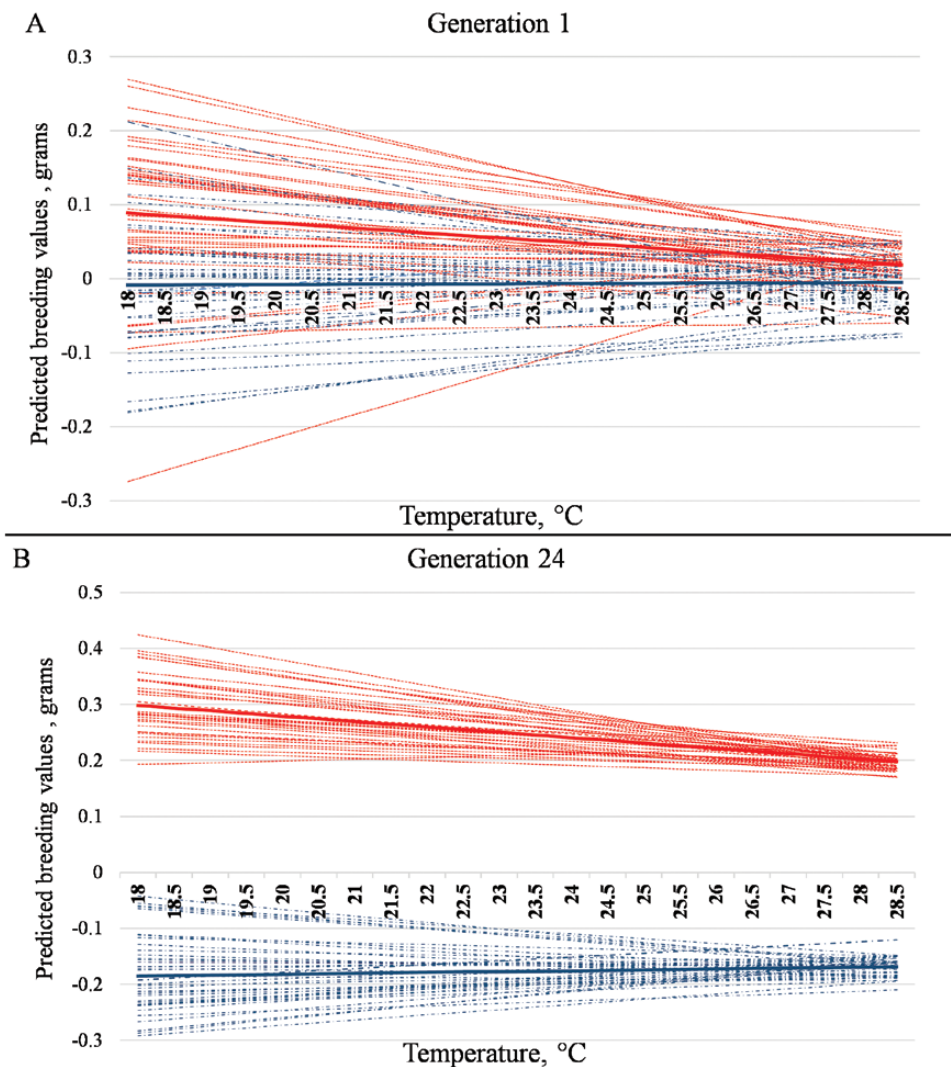


Figure 1. Individual predicted breeding values (grams) of the mothers selected (broken lines) at generations 1 (A) and 24 (B). Mean genetic trend within line is continuous in bold. Low variability line in blue and high variability line in red.

Once the best Legendre polynomials for the random regression model for MXTW was selected, a complete heteroscedastic random regression model was applied. The estimated genetic parameters were not different from those obtained in previous analyses and are not of concern in this study. However, there was a notable differential influence of the climatic variable between lines. **Figure 1** shows the individual and mean predicted breeding values (PBV) obtained for the selected mothers at the first and last generations of the selection experiment across the range of maximum temperature 1 wk before the parturition. In the first generation of selection, there was a single group of animals with higher dispersion of the PBV at lower temperatures than at higher temperatures. The L-line had slight negative values in the first selection generation while the H-line had a more positive value trend in the PBV, but most individuals in both lines had a PBV closer to zero (**Figure 1A**). However, because of the response to selection in the 24th generation, there were two clearly different PBV groups depending on the line (**Figure 1B**).

The mean PBV for each selected line showed that the H-line for the first generation varied in a range between 0.070 (from 0.019 and 0.089 g) with a negative slope, while the L-line varied within a range of 0.002 g (from -0.008 to -0.005 g)

which is 35 times lower than the H-line. In the last generation of selection: PBV were more unstable across temperatures at the H-line with a difference of 0.101 between the maximum and the minimum mean PBV compared to 0.017 for the L-line.

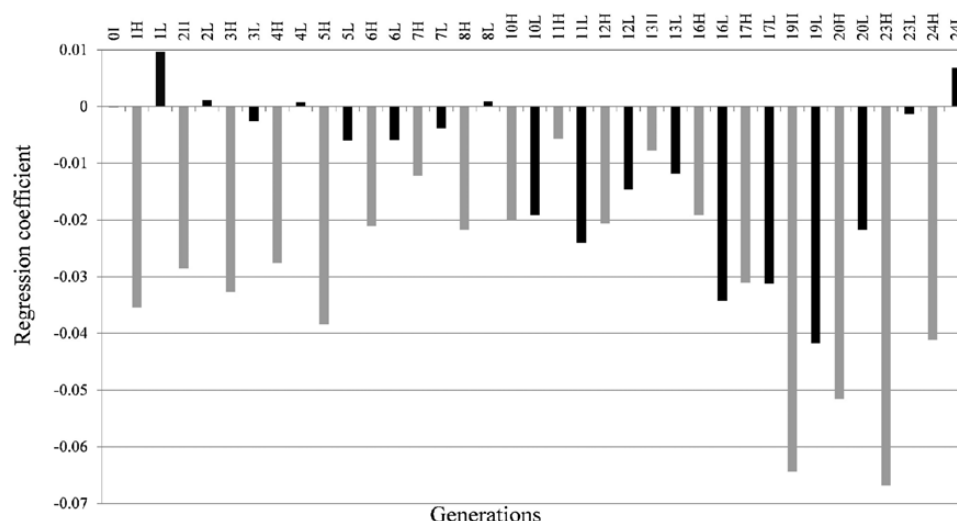
The first-order coefficient of the Legendre polynomials for each animal was averaged within line and generation to study the difference in the slopes between lines within generation (**Table 3**). From the beginning of the experiment, the H-line slopes significantly differed from 0, showing that there was variability in birth weight breeding values caused by changes in MXTW. This instability across temperatures in the H-line was in contrast to the L-line, which from the beginning of the experiment showed no significant slopes, revealing mainly no changes in birth weight breeding values through the range of temperatures. A higher dispersion was also observed in the standard deviations of the slopes in the H-line when compared with the L-line.

The slopes of these mean regression coefficients of the Legendre polynomial are worth showing in graph form and further explored (**Figure 2**). Higher absolute values were observed for the H-line in 14 out of the 18 analyzed generations while the L-line showed higher absolute values only in

Table 3. Mean regression coefficients, standard deviation, and standard error of the mean regression coefficients of the Legendre polynomial

Generation	High variability line					Low variability line				
	<i>n</i>	Mean	StdDev	StdErr	<i>P</i>	<i>n</i>	Mean	StdDev	StdErr	<i>P</i>
1	42	−0.0354	0.0405	0.0063	***	41	0.0096	0.0335	0.0052	NS
2	40	−0.0285	0.0413	0.0065	***	39	0.0012	0.0302	0.0048	NS
3	40	−0.0285	0.0413	0.0065	***	43	−0.0026	0.0219	0.0033	NS
4	42	−0.0276	0.0375	0.0058	***	42	0.0007	0.0209	0.0032	NS
5	38	−0.0384	0.0381	0.0062	***	43	−0.0060	0.0174	0.0027	*
6	42	−0.0211	0.0291	0.0045	***	40	−0.0059	0.0167	0.0026	*
7	40	−0.0122	0.0296	0.0047	**	42	−0.0038	0.0303	0.0047	NS
8	43	−0.0218	0.0324	0.0049	***	41	0.0008	0.0243	0.0038	NS
10	34	−0.0200	0.0393	0.0067	**	41	−0.0191	0.0229	0.0036	***
11	41	−0.0056	0.0455	0.0071	NS	42	−0.0240	0.0385	0.0059	***
12	39	−0.0206	0.0557	0.0089	*	40	−0.0146	0.0243	0.0038	***
13	41	−0.0078	0.0347	0.0054	NS	42	−0.0119	0.0247	0.0038	**
16	38	−0.0191	0.0378	0.0061	**	42	−0.0342	0.0219	0.0034	***
17	36	−0.0310	0.0444	0.0074	***	42	−0.0312	0.0295	0.0045	***
19	23	−0.0644	0.0521	0.0109	***	39	−0.0418	0.0646	0.0103	***
20	21	−0.0515	0.0238	0.0052	***	35	−0.0218	0.0191	0.0032	***
23	12	−0.0668	0.0470	0.0136	***	35	−0.0013	0.0190	0.0032	NS
24	34	−0.0412	0.0233	0.0040	***	41	0.0069	0.0283	0.0044	NS

n: number of individuals per generation; NS: no significance; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

**Figure 2.** Mean regression coefficients of the Legendre polynomial per generation and line. Low variability line in black (H) and high variability (L) line in gray. 0I: initial population.

three generations which means that the L-line individuals had slopes closer to 0 than those of the H-line. Specifically, the two highest mean slopes were obtained in the last two generations of the H-line, in contrast to no significant slopes in the L-line for those two generations.

Discussion

The two mouse lines obtained by Formoso-Rafferty et al. (2016a) through divergent selection for residual variance of birth weight led to several different characteristics between them: for example, the already expected reduction of the birth weight residual variance in the homogeneous line when

compared with the heterogeneous line. The selection performed in these two lines was based on the birth weight environmental variance within litter, but the factors that caused this difference in variance were not still studied. In this study, it was hypothesized that there were some unidentified environmental factors that might be causing birth weight variance that had different reactions depending on the different mouse lines. One of these unidentified environmental factors could be related to climatic conditions. Moreover, the climate is a fundamental factor in agriculture productivity and the change in the global climate causes an increasingly recurrent extreme weather events. Thus, it is necessary to seek livestock systems that ensure a high level of productivity despite all

the changes in the environment (Nardone et al., 2010). In this study, a climatic variable was identified as one of those environmental factors that causes the variance of the trait. Random regression models enable the analysis of repeated records from individuals across the trajectory of a particular variable as the time or as the range of a climatic variable. This methodology can be then used whenever there is a relationship between variables and time or age. This approach was successfully used to evaluate the genetic correlations between milk production traits and heat stress in dairy cattle in different countries (Ravagnolo and Misztal, 2000; Brüegemann et al., 2011; Carabaño et al., 2014; Santana et al., 2015, 2017), and to analyze genetic effects of heat stress on female fertility (Ravagnolo and Misztal 2002).

Despite the conditions in the facilities where the mice are housed are controlled to be optimal and stress free, there are some seasonal changes in the weather that could modify animal performance. Depending on the stage of the animal development when these changes occur, these conditions could affect more or less the birth weight. The present study includes an analysis of the time and the climate variables that most affected the birth weight variance, with MXTW having a major impact on the birth weight breeding value. It is also worth mentioning that at day 14 of gestation (one week before parturition) seemed to be the most sensitive period to climatic variables than the other two analyzed moments: fecundation and parturition. This gestation period is key for fetal survival since the mortality during this period was lower in the L-line than in the H-line (Formoso-Rafferty et al., 2023). The two selected lines behaved different to the MXTW. Interestingly, the first generation of selection had already a very different mean slope between lines. Formoso-Rafferty et al (2016a) reported that at the beginning of the selection experiment, there was a very high estimated genetic correlation between the birth weight and its environmental variability (0.73). In the first generation of selection, there was no genetic response to environmental variability of birth weight, but the response was to the trait (birth weight) itself. The differences observed in the mean PBV for birth weight between lines suggest that there was a genetic response to the selection in the sensitivity of the mice to MXTW in the first generation of selection, although this selection was not intense enough to be reflected in differences for birth weight variance between lines. In the last generation of the selection experiment, the L-line showed more stability across temperatures compared to the H-line. The absolute value of the mean slope in the L-line was lower than in the H-line (0.0069 vs. -0.0412). The mean slope of the L-line, also, appeared not to be significant while the mean slope of the H-line was at a maximum and significant. These results suggested that the L-line had no changes in birth weight genetic value across temperatures, while the H-line individuals were more sensitive to MXTW resulting in a decreased birth weight at higher temperatures.

The results of the present study are more interesting in the context of the selection experiment and the differences appearing between lines for many issues. The selection experiment resulted in a homogeneous line with a more stable performance while the heterogeneous line showed more sensitivity and higher instability regarding performance at changes in the environmental conditions. In addition, the low variability line was less sensitive to challenges such as feed restriction (Formoso-Rafferty et al., 2019). Initially, these environmental

conditions that caused more variability in the H-line over the L-line were unknown, but the present study has identified one possible environmental variable which is the MXTW that affects the individual birth weight.

These results added to those obtained in other studies previously mentioned on these selected mouse populations reinforce the fact that the selection for homogeneous environmental variability in some traits are directly linked to a higher robustness. This is also supported by studies made in other species such as rabbits, where the homogeneous line obtained from a selection on litter size residual variance resulted in animals with higher litter sizes (Blasco et al. 2017). Even better welfare showed less susceptibility to diseases after an environmental stressor (Beloumi et al. 2020). In addition, the homogeneity in birth weights of pigs within litters ease their management and that is both more profitable and beneficial for farms, while the heterogeneity is a problem mainly for piglets with lower birth weights which suffer from a delayed growth (Foxcroft et al., 2006). The selection performed during the present experiment has shown that homogeneous animals are less affected by climate changes that result in high temperatures. This fact could be useful when applied to prolific species breeding programs.

While robustness is in the spotlight of breeding programs, it is still a challenge to define a specific strategy for breeding for it. Moreover, there is no clear definition of what would be the criteria that correlates best for robustness. The way to address this issue is by considering a global robustness in the already selected animals by their birth weight residual variance. The present study includes an analysis on how sensitive this population is to environmental challenges posed by climatic variables. This study contributes a new step at showing the advantages of the selection for homogeneity even when differences were not very large. In conclusion, the present study supports the selection for low birth weight residual variance, which could be advantageous as a selection method to provide animals less sensitive to changes in climatic variables.

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Author Contributions

Conceptualization: IC, JPG; data curation: LEE, NFR; formal analysis: LEE, JPG, IC; funding acquisition: IC; investigation: LEE, NFR; methodology: IC, JPG; project administration: IC, JPG; resources: IC, software: JPG, LEE; supervision: IC, NFR, JPG; validation: NFR, LEE; visualization: LEE; writing-original draft: LEE; writing—review & editing: IC, NFR, JPG, LEE.

Conflict of Interest Statement

The authors declare no conflict of interest.

Literature Cited

Beloumi, D., A. Blasco, R. Muelas, M. A. Santacreu, M. D. L. L. García, and M. J. Argente. 2020. Inflammatory correlated response in two lines of rabbit selected divergently for litter size environmental variability. *Animals*. 10:1540. doi:10.3390/ani10091540

- Blasco, A., M. Martínez-Álvarez, M. L. García, N. Ibáñez-Escriche, and M. J. Argente. 2017. Selection for environmental variance of litter size in rabbits. *Genet. Sel. Evol.* 49:1–8. doi:[10.1186/s12711-017-0323-4](https://doi.org/10.1186/s12711-017-0323-4)
- Bolet, G., H. Garreau, T. Joly, M. Theau-Clement, J. Falières, J. Hurtaud, and L. Bodin. 2007. Genetic homogenisation of birth weight in rabbits: indirect selection response for uterine horn characteristics. *Livest. Sci.* 111:28–32. doi:[10.1016/j.livsci.2006.11.012](https://doi.org/10.1016/j.livsci.2006.11.012)
- Boletín Oficial del Estado. (2013). Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. *BOE*, 34:11370–11421.
- Browne, M. W. 2000. Cross-validation methods. *J. Math. Psychol.* 44:108–132. doi:[10.1006/jmps.1999.1279](https://doi.org/10.1006/jmps.1999.1279)
- Brügemann, K., E. Gernand, U. U. von Borstel, and S. König. 2011. Genetic analyses of protein yield in dairy cows applying random regression models with time-dependent and temperature x humidity-dependent covariates. *J. Dairy Sci.* 94:4129–4139. doi:[10.3168/jds.2010-4063](https://doi.org/10.3168/jds.2010-4063)
- Carabaño, M. J., K. Bachagha, M. Ramón, and C. Díaz. 2014. Modeling heat stress effect on Holstein cows under hot and dry conditions: selection tools. *J. Dairy Sci.* 97:7889–7904. doi:[10.3168/jds.2014-8023](https://doi.org/10.3168/jds.2014-8023)
- Carabaño, M. J., C. Pineda-Quiroga, E. Ugarte, C. Díaz, and M. Ramón. 2021. Genetic basis of thermotolerance in 2 local dairy sheep populations in the Iberian Peninsula. *J. Dairy Sci.* 104:5755–5767. doi:[10.3168/jds.2020-19503](https://doi.org/10.3168/jds.2020-19503)
- El-Ouazizi El-Kahia, L., I. Cervantes, J.P. Gutiérrez, and N. Formoso-Rafferty. 2022. Litter size variability in a divergent selected mice population for birth weight variability. Book of Abstracts of the 73rd Annual Meeting of the European Federation of Animal Science: Porto, Portugal, 5-8 September 2022. Wageningen Academic Publishers; p. 602.
- Formoso-Rafferty, N., I. Cervantes, N. Ibáñez-Escriche, and J. P. Gutiérrez. 2016a. Genetic control of the environmental variance for birth weight in seven generations of a divergent selection experiment in mice. *J. Anim. Breed. Genet.* 133:227–237. doi:[10.1111/jbg.12174](https://doi.org/10.1111/jbg.12174)
- Formoso-Rafferty, N., I. Cervantes, N. Ibáñez-Escriche, and J. P. Gutiérrez. 2016b. Correlated genetic trends for production and welfare traits in a mouse population divergently selected for birth weight environmental variability. *Animals*. 10:1770–1777. doi:[10.1017/S1751731116000860](https://doi.org/10.1017/S1751731116000860)
- Formoso-Rafferty, N., M. de la Flor, J. P. Gutiérrez, and I. Cervantes. 2018. Feed and reproductive efficiency differences between divergently selected lines for birth weight environmental variability in mice. *J. Anim. Breed. Genet.* 135:378–389. doi:[10.1111/jbg.12345](https://doi.org/10.1111/jbg.12345)
- Formoso-Rafferty, N., I. Cervantes, J. P. Sánchez, J. P. Gutiérrez, and L. Bodin. 2019. Effect of feed restriction on the environmental variability of birth weight in divergently selected lines of mice. *Genet. Sel. Evol.* 51:1–11. doi:[10.1186/s12711-019-0471-9](https://doi.org/10.1186/s12711-019-0471-9)
- Formoso-Rafferty, N., J. P. Gutiérrez, A. García-Álvarez, T. Pérez, and I. Cervantes. 2022. Impact of selection for birth weight variability on reproductive longevity: a mice model. *J. Anim. Breed. Genet.* 139:370–379. doi:[10.1111/jbg.12676](https://doi.org/10.1111/jbg.12676)
- Formoso-Rafferty, N., L. El-Ouazizi El-Kahia, M. Arias-Álvarez, J. P. Gutiérrez, and I. Cervantes. 2023. Embryo survival and fertility differ in lines divergently selected for birth weight homogeneity in mice. *J. Anim. Breed. Genet.* 00:1–9. doi:[10.1111/jbg.12778](https://doi.org/10.1111/jbg.12778)
- Foxcroft, G. R., W. T. Dixon, S. Novak, C. T. Putman, S. C. Town, and M. D. A. Vinsky. 2006. The biological basis for prenatal programming of postnatal performance in pigs. *J. Anim. Sci.* 84:E105–E112. doi:[10.2527/2006.8413_supple105x](https://doi.org/10.2527/2006.8413_supple105x)
- Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2015. ASReml user guide release 4.1 structural specification. Hemel Hempstead: VSN international ltd.
- Groeneveld, E., Kovac and N., Mielenz. 2010. VCE user's guide and reference manual version 6.0. Institute of Farm Animal Genetics.
- Gutiérrez, J. P., N. Formoso-Rafferty, C. Ojeda-Marín, L. El-Ouazizi El-Kahia, K. D. Arias, and I. Cervantes. 2022. Selection for birth weight environmental variability in mice as a model to improve animal welfare in livestock species. Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP). Wageningen Academic Publishers; p. 1979–1982.
- Hahn, G. L., J. B. Gaughan, T. L. Mader, and R. A. Eigenberg. 2009. Thermal indices and their applications for livestock environments in livestock energetics and thermal environment management. *Am. Soc. Agric. Biolog. Eng. St. Joseph, Mich.* p. 113–130.
- Herrero-Medrano, J. M., P. K. Mathur, J. T. Napel, H. Rashidi, P. Alexandri, E. F. Knol, and H. A. Mulder. 2015. Estimation of genetic parameters and breeding values across challenged environments to select for robust pigs. *J. Anim. Sci.* 93:1494–1502. doi:[10.2527/jas.2014-8583](https://doi.org/10.2527/jas.2014-8583)
- Ibáñez-Escriche, N., M. Garcia, and D. Sorensen. 2010. GSEVM v 2: MCMC software to analyze genetically structured environmental variance models. *J. Anim. Breed. Genet.* 127:249–251. doi:[10.1111/j.1439-0388.2009.00846.x](https://doi.org/10.1111/j.1439-0388.2009.00846.x)
- Mormede, P., and E. Terenina. 2012. Molecular genetics of the adrenocortical axis and breeding for robustness. *Domest Anim. Endocrinol.* 43:116–131. doi:[10.1016/j.domaniend.2012.05.002](https://doi.org/10.1016/j.domaniend.2012.05.002)
- Nardone, A., B. Ronchi, N. Lacetera, M. S. Ranieri, and U. Bernabucci. 2010. Effects of climate changes on animal production and sustainability of livestock systems. *Livest. Sci.* 130:57–69. doi:[10.1016/j.livsci.2010.02.011](https://doi.org/10.1016/j.livsci.2010.02.011)
- Ravagnolo, O., and I. Misztal. 2000. Genetic component of heat stress in dairy cattle, parameter estimation. *J. Dairy Sci.* 83:2126–2130. doi:[10.3168/jds.S0022-0302\(00\)75095-8](https://doi.org/10.3168/jds.S0022-0302(00)75095-8)
- Ravagnolo, O., and I. Misztal. 2002. Effect of heat stress on nonreturn rate in Holstein cows: genetic analyses. *J. Dairy Sci.* 85:3092–3100. doi:[10.3168/jds.S0022-0302\(02\)74396-8](https://doi.org/10.3168/jds.S0022-0302(02)74396-8)
- SanCristobal-Gaudy, M., J. M. Elsen, L. Bodin, and C. Chevalet. 1998. Prediction of the response to a selection for canalisation of a continuous trait in animal breeding. *Genet. Sel. Evol.* 30:423–451. doi:[10.1186/1297-9686-30-5-423](https://doi.org/10.1186/1297-9686-30-5-423)
- Santana, M. L. Jr, R. J. Pereira, A. B. Bignardi, A. E. V. Filho, A. Menéndez-Buxadera, and L. El Faro. 2015. Detrimental effect of selection for milk yield on genetic tolerance to heat stress in purebred Zebu cattle: genetic parameters and trends. *J. Dairy Sci.* 98:9035–9043. doi:[10.3168/jds.2015-9817](https://doi.org/10.3168/jds.2015-9817)
- Santana, M. L. Jr, A. B. Bignardi, R. J. Pereira, G. Stefani, and L. El Faro. 2017. Genetics of heat tolerance for milk yield and quality in Holsteins. *Animals*. 11:4–14. doi:[10.1017/S1751731116001725](https://doi.org/10.1017/S1751731116001725)
- Spiegelhalter, D. J., N. G. Best, B. P. Carlin, and A. van der Linde. 2002. Bayesian measures of model complexity and fit. *J. Royal Stat Soc Series B: Stat Meth*, 64, 583–639. doi:[10.1111/1467-9868.00353](https://doi.org/10.1111/1467-9868.00353)
- Tibshirani, R. J., and B. Efron. 1993. An introduction to the bootstrap. *Monographs on statistics and applied probability* 57:237–255. doi:[10.1201/9780429246593](https://doi.org/10.1201/9780429246593)